

FINAL

**Report on Carcinogens
Background Document for**

Aristolochic Acids

September 2, 2008



U.S. Department of Health and Human Services
Public Health Services
National Toxicology Program
Research Triangle Park, NC 27709

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FOREWORD

1 The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public
2 Health Service Act as amended. The RoC contains a list of identified substances (i) that
3 either are known to be human carcinogens or are reasonably be anticipated to be human
4 carcinogens and (ii) to which a significant number of persons residing in the United
5 States are exposed. The Secretary, Department of Health and Human Services (HHS), has
6 delegated responsibility for preparation of the RoC to the National Toxicology Program
7 (NTP), which prepares the report with assistance from other Federal health and
8 regulatory agencies and nongovernmental institutions.

9 Nominations for (1) listing a new substance, (2) reclassifying the listing status for a
10 substance already listed, or (3) removing a substance already listed in the RoC are
11 reviewed in a multi-step, scientific review process with multiple opportunities for public
12 comment. The scientific peer-review groups evaluate and make independent
13 recommendations for each nomination according to specific RoC listing criteria. This
14 background document was prepared to assist in the review of aristolochic acids. The
15 scientific information used to prepare Sections 3 through 5 of this document must come
16 from publicly available, peer-reviewed sources. Information in Sections 1 and 2,
17 including chemical and physical properties, analytical methods, production, use, and
18 occurrence may come from published and/or unpublished sources. For each study cited in
19 the background document from the peer-reviewed literature, information on funding
20 sources (if available) and the authors' affiliations are provided in the reference section.
21 The draft background document was peer reviewed in a public forum by an *ad hoc* expert
22 panel of scientists from the public and private sectors with relevant expertise and
23 knowledge selected by the NTP in accordance with the Federal Advisory Committee Act
24 and HHS guidelines and regulations. This document has been finalized based on the peer-
25 review recommendations of the expert panel and public comments received on the draft
26 document. Any interpretive conclusions, comments, or statistical calculations made by
27 the authors or peer reviewers of this document that are not contained in the original
28 citation are identified in brackets [].

1 A detailed description of the RoC nomination review process and a list of all substances
2 under consideration for listing in or delisting from the RoC can be obtained by accessing
3 the 12th RoC at <http://ntp.niehs.nih.gov/go/9732>. The most recent RoC, the 11th Edition
4 (2004), is available at <http://ntp.niehs.nih.gov/go/19914>.

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PEER-REVIEW

The draft background document on Riddelliine was peer reviewed by the Report on Carcinogens (RoC) expert panel for Riddelliine and Aristolochic Acid. The panel met in a public forum at the Sheraton Chapel Hill Hotel, Chapel Hill, NC on January 24 – 25, 2008. Members of the expert panel are as follows:

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services

National Toxicology Program

The criteria for listing an agent, substance, mixture, or exposure circumstance in the RoC are as follows:

Known To Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans^{*}, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated To Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans^{*}, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded,

or

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,

or

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

^{*} This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question that can be useful for evaluating whether a relevant cancer mechanism is operating in people.

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Executive Summary

1 Introduction

2 Aristolochic acids are a family of nitrophenanthrene carboxylic acids that occurs
3 naturally in plants in the Aristolochiaceae family, primarily of the genera *Aristolochia*
4 and *Asarum*. Botanical products from plants containing aristolochic acids are used in
5 traditional folk medicines, particularly in Chinese herbal medicine, and have been used
6 inadvertently as part of a weight-loss regimen.

7 “Aristolochic acids” were nominated by the National Institute of Environmental Health
8 Sciences (NIEHS) for possible listing in the *Report on Carcinogens* based on the
9 International Agency for Research on Cancer (IARC) classification that herbal remedies
10 containing plant species of the genus *Aristolochia* are *carcinogenic to humans* (Group 1)
11 and that naturally occurring mixtures of aristolochic acid are *probably carcinogenic to*
12 *humans* (Group 2A).

13 Human Exposure

14 The risk of human exposure to aristolochic acids remains a global problem. *Aristolochia*
15 and related plants have been used since ancient times in traditional herbal medicines for
16 obstetrics treatment and for treatment of snakebite, scorpion stings, fever, infection,
17 diarrhea, and inflammation. In contemporary medicine, *Aristolochia* plant products have
18 been used in therapies for arthritis, gout, rheumatism, and festering wounds. Herbal
19 preparations containing aristolochic acids have also been used inadvertently as part of a
20 weight-loss regimen. Individuals may potentially be exposed to aristolochic acids by
21 ingesting plants and botanical products made from plants that contain these compounds
22 or by ingesting herbal products contaminated with aristolochic acids. In one well-
23 documented occurrence, between 1,500 and 2,000 individuals were exposed to
24 aristolochic acids at weight-loss clinics in Belgium in the 1990s. In addition, exposure to
25 aristolochic acids has been proposed to result from contamination of wheat flour by seeds
26 of *A. clematitis* growing in wheat fields in the Balkan states. Exposure to aristolochic
27 acids has also been reported for other countries, including the United States; two cases of
28 renal failure in the United States have been linked to ingestion of herbal products

1 containing aristolochic acids. The use of botanical products in the United States has
2 increased dramatically since the early 1990s, with 10% of adults in the United States
3 reportedly ingesting herbal medicines in 1999 and a total of \$4.2 billion spent on herbs
4 and other botanical remedies in 2001.

5 More than 100 suppliers of botanical products that potentially contain aristolochic acids
6 have been identified in recent years. In 2001, the FDA issued warnings to consumers,
7 health care professionals, and industry associations concerning herbal products
8 containing aristolochic acids. Other countries, including the United Kingdom, Germany,
9 Canada, and Australia, have banned these herbs. Nevertheless, botanical products
10 potentially containing aristolochic acids are still available legally in other countries and
11 can be bought via the Internet.

12 **Human Cancer Studies**

13 The available literature evaluating the association between exposure to aristolochic acids
14 and cancer in humans consists of case reports, prevalence studies, and clinical studies
15 among individuals with kidney disease. The relationship between aristolochic acids and
16 urothelial cancer was first reported in a Belgian population with a kidney disease known
17 as Chinese herbal nephropathy (CHN). The subjects had consumed Chinese herbs as part
18 of a weight-loss regimen. The weight-loss clinics had changed the weight-loss regimen to
19 include powders from *Magnolia officinalis* and *Stephania tetrandra*, which was
20 subsequently found to contained aristolochic acids but not tetrandrine. Botanical products
21 containing aristolochic acids were suspected as the cause of herbal medicine nephropathy
22 because: (1) the nephropathy developed immediately after ingestion of the herbs, and in
23 some cases, it was reversible after the patient discontinued the herbs; (2) the lack of
24 exposure (in most cases) to agents known to be risk factors for nephropathy; (3) the
25 identification of aristolochic acid in the herbal products; and (4) the identification of
26 aristolochic acid–DNA (AA-DNA) adducts in tissues (usually kidney or urothelial tissue)
27 in some of the cases. The identification of aristolochic acids as the cause of the renal
28 disease led to the introduction of the term aristolochic acid nephropathy (AAN) to
29 describe those cases in which the herbs are proven to contain aristolochic acid. More than
30 100 cases of AAN in Belgian and greater than 170 cases of AAN have been reported in

1 other geographical location including other western nations such as the United States, and
2 Great Britain, and Asian countries such as Japan, Taiwan, and China. In contrast with the
3 Belgian cases, cases in other countries have involved use of the Chinese herbs containing
4 aristolochic acids for many different purposes, including weight loss, nutritional
5 supplementation, health promotion, and treatment of a variety of diseases or conditions.

6 After the publication of several case reports of urothelial cancer occurring among AAN
7 patients, two prevalence studies were conducted among the Belgian patients. Both studies
8 reported a high prevalence [40% (4/10) in the Cliniques Universitaires St.-Luc study, and
9 46% (18/39) in the Hospital Erasme Study] of urothelial cancer among women receiving
10 renal transplants as a result of AAN. Both studies identified aristolochic acids in the
11 botanical products consumed by the patients and detected AA-DNA adducts in kidney
12 tissue from the patients, demonstrating that the patients were exposed to aristolochic
13 acids. The study of patients from the Hospital Erasme reported that the prevalence of
14 urothelial cancer was higher among patients who consumed a higher dose of the
15 aristolochic acid-containing plant *Aristolochia fangchi*, but that AAN patients with and
16 without urothelial cancer did not differ significantly with respect to other risk factors for
17 urothelial cancer, such as the use of non-steroidal anti-inflammatory drugs, analgesics,
18 etc. Neither study had an unexposed comparison group.

19 In 2002, an IARC working group reviewed the available literature (which consisted
20 mainly of the two prevalence studies, and the case reports of AAN and urothelial cancer)
21 and concluded that there was sufficient evidence in humans for the carcinogenicity of
22 herbal remedies containing plant species of the genus *Aristolochia* and limited evidence
23 in humans for the carcinogenicity of naturally occurring mixtures of aristolochic acids.
24 Since the IARC (2002) review, there have been an update of the prevalence study of
25 urothelial cancer developing in AAN patients in Belgium, additional case reports of AAN
26 and urothelial cancer developing in patients with AAN (both in Belgium and worldwide),
27 several clinical investigations of urothelial cancer among kidney-transplant or dialysis
28 patients in Taiwan or China, and studies on aristolochic acids and BEN.

1 A 15-year follow-up of the Belgian patients from the Hospital Erasme found a similar
2 prevalence rate of urothelial cancer occurring in AAN patients compared with the earlier
3 report by Nortier and colleagues. [The follow-up identified a few more cases of cancer,
4 and included most but not all the previous cancer cases.] In addition, the follow-up study
5 found an increased incidence of urinary bladder cancer among cases with urothelial
6 cancer. Similar to the earlier publications, the cumulative dose of *Aristolochia* in AAN
7 patients who developed urothelial cancer was significantly higher than the dose
8 consumed by AAN patients who did not develop cancer. A case report of urothelial
9 cancer from the Belgian epidemic was also reported in a patient who did not have severe
10 renal disease. There were also additional case reports of urothelial cancer in AAN in
11 patients outside of Belgium, which supports the role of aristolochic acids as a cause of
12 upper urothelial cancer.

13 Two clinical studies among Chinese patients with renal disease (renal-transplant or
14 dialysis patients) reported an increased incidence or prevalence of transitional-cell
15 carcinoma (TCC) among patients consuming Chinese herbs or drugs containing
16 aristolochic acids compared with non-exposed patients; OR = 37 (95% CI = 11 to 216) in
17 a study of 283 dialysis patients and RR = 5.85 ($P < 0.0001$) in a study of 1,429 renal
18 transplant patients. Two other clinical studies evaluating TCC mortality or incidence
19 among Taiwanese patients with renal disease (dialysis or kidney-transplant patients)
20 reported that consumption of TCC was a risk factor for Chinese herb use (relative hazard
21 was 5.2 among transplant patients and 6.21 among dialysis patients); however, the
22 exposure assessments were not specific for aristolochic acids intake.

23 Aristolochic acids have been proposed to be a risk factor for urothelial cancer associated
24 with Balkan endemic nephropathy (BEN). BEN is a chronic tubulointerstitial disease
25 endemic to Serbia, Bosnia, Croatia, Bulgaria, and Romania that has similar morphology
26 and clinical features to AAN. Exposure to aristolochic acids is proposed to occur from
27 consumption of wheat contaminated with seeds from *A. clematitis*. AA-DNA adducts
28 have been detected in renal tissue of BEN patients and in urothelial and renal cortical
29 tissues from BEN patients with upper urothelial cancers. One study reported that the
30 majority (78%) of *p53* mutations (in tumors with *p53* mutations) in urothelial tumors

1 from patients living in endemic areas were A:T → T:A transversions, which the authors
2 stated was a mutational signature for exposure to aristolochic acid.

3 In summary, exposure to aristolochic acids has been associated with a progressive
4 interstitial renal fibrosis in several populations (primarily in Belgium, the Balkans, and
5 China). An increased incidence or prevalence of upper urothelial tumors has been
6 detected in individuals with aristolochic acid–associated end-stage renal failure. In some
7 studies, AA-DNA adducts have been detected in urothelial tissues from the cancer
8 patients, demonstrating exposure to aristolochic acids. Studies of renal-transplant or
9 dialysis patients have reported elevated risks for urothelial cancer associated with
10 consumption of herbal products containing aristolochic acids.

11 **Studies in Experimental Animals**

12 Aristolochic acids (administered orally or by injection) induced tumors at multiple sites
13 in mice, rats, and rabbits. Most studies administered a mixture of aristolochic acids I and
14 II; however, aristolochic acid I alone was used in two studies. Many of these studies used
15 a small number of animals and were of relatively short duration; only a few included
16 statistical analyses. Female mice given aristolochic acids orally developed forestomach,
17 stomach, kidney, lung, and uterine tumors and malignant lymphomas. Oral administration
18 of aristolochic acids caused forestomach, kidney, renal pelvis, urinary bladder, ear duct,
19 thymus, small intestine, and pancreatic tumors. Single cases of hematopoietic system,
20 heart, lung, mammary gland, pituitary gland, and peritoneal tumors were also reported.
21 Male Wistar rats exposed by daily s.c. injections of aristolochic acids developed
22 urothelial carcinoma of the renal pelvis and malignant fibrohistiocytic sarcoma at the
23 injection site. Aristolochic acids, given by i.p. injections, induced kidney tumors, a
24 urinary-tract tumor, and a mesothelioma of the peritoneal cavity in female New Zealand
25 White rabbits. A single i.p. injection of aristolochic acids initiated liver carcinogenesis in
26 male F344 rats when coupled with a liver-cell–proliferative stimulus. The IARC working
27 group concluded that there was sufficient evidence in experimental animals for the
28 carcinogenicity of aristolochic acids.

1 Three studies were reviewed that investigated the carcinogenicity of extracts of
2 *Aristolochia* species (one study each for *A. manshuriensis*, *A. clematitis*, and *A. contorta*)
3 when administered orally or by injection. Tumors of the forestomach and kidney were the
4 most prevalent findings following oral administration, but one study reported tumors of
5 the mammary gland, thyroid gland, and skin. Injection-site polymorphocellular sarcomas
6 also were reported in one study. One study exposed rats of both sexes to a weight-loss
7 regimen of herbal ingredients that contained aristolochic acids, and the male rats
8 developed forestomach papillomas and squamous-cell carcinomas.

9 **Absorption, Distribution, Metabolism, and Excretion**

10 Aristolochic acids are absorbed from the gastrointestinal tract and distributed throughout
11 the body, as evidenced by observation of specific DNA adducts in kidney, urinary tract,
12 liver, lung, brain, stomach, and other tissues of humans and experimental animals. The
13 available data indicate that aristolochic acid I is metabolized by both oxidative and
14 reductive pathways, whereas aristolochic acid II is metabolized only by a reductive
15 pathway. The metabolites of aristolochic acid I in rats and mice include aristolactam I,
16 aristolactam Ia, aristolochic acid Ia, aristolic acid I, 3,4-methylenedioxy-8-hydroxy-1-
17 phenanthrenecarboxylic acid, and a decarboxylated metabolite. The metabolites of
18 aristolochic acid II include aristolactam II, aristolactam Ia, and 3,4-methylenedioxy-1-
19 phenanthrenecarboxylic acid. Only aristolactam I and II have been reported in humans,
20 although full metabolic profiles determined through sensitive techniques have not been
21 reported. Phase II metabolites include the *N*- and *O*-glucuronides of aristolactam Ia, the
22 *N*-glucuronide of aristolactam II, and the *O*-glucuronide, *O*-acetate, and *O*-sulfate esters
23 of aristolochic acid Ia. The metabolites are excreted in the urine and the feces. Reported
24 half-lives in New Zealand White rabbits for aristolochic acids I and II were 0.12 hours
25 and 0.27 hours, respectively. Studies in rats show that the metabolites of aristolochic acid
26 I are excreted within 24 hours, whereas metabolites of aristolochic acid II are still present
27 in the urine at 72 hours.

28

1 **Toxicity**

2 The kidney is the primary target organ for aristolochic acid toxicity in both animals and
3 humans. As mentioned above, consumption of botanical products containing aristolochic
4 acids has been associated with AAN, which is characterized by mild tubular proteinuria,
5 extensive interstitial fibrosis, tubular atrophy, global sclerosis of glomeruli, rapid
6 progression to renal failure, and associated anemia. AAN has been described in more
7 than 100 cases (all but 1 in women) exposed at a weight-loss clinic in Belgium and in
8 more than 100 other sporadic cases in Europe, Asia, and the United States. Another
9 clinical presentation of AAN (adult-onset Fanconi syndrome) has been described in a few
10 cases in China, Korea, Japan, and Germany, and is characterized by proximal tubular
11 dysfunction, and a generally slower progression to end-stage renal disease.

12 Aristolochic acids cause renal toxicity in rats, mice, and rabbits. Rats and mice exposed
13 to high doses (given orally or by intravenous injection) of aristolochic acids developed
14 renal failure. The primary features include tubular necrosis, elevated plasma creatinine
15 and urea levels, atrophy of the lymphatic organs, superficial ulceration of the
16 forestomach, hyperplasia and hyperkeratosis of the squamous epithelium, and renal
17 failure in rats. Interstitial fibrosis was also observed in some, but not all, studies in rats
18 and mice. Sustained intoxication of rats by aristolochic acids has been proposed to result
19 in altered regeneration of tubular epithelial cells and apoptosis leading to irreversible
20 tubular atrophy and to deposition of collagen by fibroblasts.

21 In rabbits, aristolochic acids given by i.p. injection caused renal hypocellular interstitial
22 fibrosis, which decreased from the outer to the inner cortex, fibrosis of the gastric
23 mucosa, and urothelial atypia. Species and strain differences in susceptibility are
24 apparent. The dose levels of aristolochic acids required to induce acute tubular necrosis in
25 rats and mice (20 and 30 mg/kg b.w., respectively) are higher than the dose level (around
26 1 mg/kg b.w.) needed in rabbits or humans. BALB/c and C3H/He mice were more
27 susceptible than C57BL/6 mice to the nephrotoxic effects. Most animal studies used
28 purified aristolochic acids rather than the crude extracts or relatively unprocessed
29 botanical material (e.g., ground, dried root) consumed by humans. A study comparing

1 two botanical products, with similar chemical composition except for the presence of
2 aristolochic acids, resulted in renal toxicity in rats only with the product (*A.*
3 *manshuriensis*) containing aristolochic acids.

4 Aristolochic acids and their aristolactam derivatives are cytotoxic to cells growing in
5 culture, including rat and human kidney cells and macrophages. The degree of toxicity
6 varies according to cell type and chemical structure (of the individual aristolochic acids
7 or aristolactams).

8 **Genetic Damage and Mechanistic Data**

9 Aristolochic acids are metabolically activated by reductive pathways to form reactive
10 intermediate cyclic *N*-acylnitrenium ions that form adducts (dA-AAI, dG-AAI, dA-AAII,
11 and dG-AAII) at purine bases in DNA. A number of cytosolic and microsomal enzymes
12 (CYP1A1, CYP1A2, NADPH:CYP reductase, prostaglandin H synthase, DT-diaphorase,
13 xanthine oxidase, COX, and NAD(P)H:quinone oxidoreductase) are capable of
14 bioactivating aristolochic acids to the reactive species.

15 DNA adducts have been detected *in vitro*, in experimental animals exposed to
16 aristolochic acids or botanical products containing aristolochic acids, and in human tissue
17 from AAN patients, from urothelial cancer patients exposed to botanical products
18 containing aristolochic acids, and from patients with Balkan endemic nephropathy. The
19 predominant and most persistent adduct, dA-AAI (lifelong in rats and at least 89 months
20 in humans), appears to be responsible for most of the mutagenic and carcinogenic
21 properties of aristolochic acids. Mutagenic activity studies of AA–DNA adducts found
22 that the adenine adducts have a higher mutagenic potential than the guanine adducts.

23 Aristolochic acids (purified I or II, or mixtures) are mutagenic in a variety of
24 experimental conditions, including bacteria, cultured cells, and *in vivo* studies in rodents.
25 Aristolochic acid I has been tested the most extensively. In *in vitro* assays, aristolochic
26 acids induced mutations in *Salmonella typhimurium* and in cultured cells, including *hprt*
27 mutations in rat fibroblast-like and Chinese hamster cells, forward mutations in mouse
28 lymphoma cells and *p53* DNA-binding domain mutations in two studies with human *p53*
29 knock-in (Hupki) mouse fibroblast cell cultures. Mutational analysis identified mutations

1 in the *p53* DNA-binding domain in one-third (6 of 18) to one-half (5 of 10) of the
2 established Hupki mouse fibroblast cultures; A:T → T:A transversions were
3 predominant, occurring in at least 80% of the cell lines with mutations. Aristolochic acid
4 mixtures or plant extract caused mutations in *S. typhimurium* and *Drosophila*
5 *melanogaster* (sex-linked recessive lethal), and aristolochic acid II caused mutations in *S.*
6 *typhimurium*. Studies in experimental animals showed that exposure to aristolochic acid
7 mixtures or plant extracts caused mutations in granulation tissue from Sprague-Dawley
8 rats, *lacZ* mutations in the forestomach, kidney, and colon tissue from Muta mice, and *cII*
9 mutations in liver and kidney tissue from Big Blue rats. Exposure to aristolochic acid I
10 also caused mutations in granulation tissue from Sprague-Dawley rats. A:T → T:A
11 transversions were the predominant mutation type in the Muta mice and Big Blue rat
12 studies.

13 DNA binding studies show that aristolochic acids bind to adenines in codon 61 in the *H-*
14 *ras* mouse gene and to purines in the human *p53* gene. Mutational spectra studies in
15 tumors of rodents exposed to aristolochic acids identified an A:T → T:A transversion in
16 codon 61 of the *c-Ha-ras* gene in forestomach tumors (rats and mice), lung tumors (rats
17 and mice), and ear-duct tumors (rats). No mutations were identified in rats with chronic
18 renal failure not exposed to aristolochic acids. Similar findings have been reported in
19 humans. Aristolochic acid adducts have been identified in renal and urothelial tissue as
20 well as in other tissues such as liver, pancreas, and lymph nodes of AAN patients and in
21 the renal cortex of 4 BEN patients and in tumor tissue of 3 long-term residents of
22 endemic villages who had upper urinary tract (transitional-cell) malignancies. A:T →
23 T:A transversion mutations in the *p53* gene have been identified in urothelial tumors
24 from an AAN patient and in 10 of 11 patients with urothelial cancer living in the region
25 endemic for BEN; 8 of the 9 patients with adequate tissue samples for histopathologic
26 analysis had changes in their renal cortex that were diagnostic or suggestive of BEN.
27 Another study reported that *p53* is over expressed in urinary-tract tumors collected from
28 patients with AAN and identified A → C and G → A mutations in the *p53* gene from a
29 patient with a papillary transitional-cell carcinoma of the bladder. The high frequency
30 (78%) of A:T → T:A transversions in upper urothelial tumors associated with exposure

1 to aristolochic acids is in contrast to the much lower frequency of approximately 5% seen
2 for *p53* mutations in bladder and ureter tumors with other causes, and some researchers
3 have proposed it as a signature for human exposure to aristolochic acids.

4 Aristolochic acids also caused other types of genetic damage. Aristolochic acids I and II
5 and mixtures were genotoxic in the SOS chromotest in *Escherichia coli*, and aristolochic
6 acid mixtures caused sex-chromosome loss and somatic recombination in *D.*
7 *melanogaster*. In mammalian *in vitro* studies, aristolochic acid mixtures caused
8 chromosomal aberrations, sister chromatid exchange, and micronuclei in human
9 lymphocytes, and aristolochic acid I caused chromosomal aberrations and sister
10 chromatid exchange in Chinese hamster cells. Neither aristolochic acid I nor II induced
11 DNA strand breaks in rat hepatocytes, but aristolochic acids have caused DNA damage in
12 porcine proximal tubular epithelial cells and human hepatoma cells. In mammalian *in*
13 *vivo* studies, aristolochic acids [composition not specified] did not induce unscheduled
14 DNA synthesis in the pyloric mucosa of male rats. DNA damage was reported in kidney
15 cells from male Sprague-Dawley rats administered a single oral dose of aristolochic
16 acids. One study reported that intravenous injections of aristolochic acid mixtures
17 increased the frequency of micronucleated polychromatic erythrocytes in bone marrow
18 cells from NMRI female and male mice, but another study found no increase in
19 micronucleated reticulocytes in peripheral blood from male Muta mice exposed orally to
20 a mixture of aristolochic acids I and II.

21 A possible mechanism for the dose-dependent urothelial proliferation induced in rats fed
22 an aristolochic acid mixture has been proposed based on altered expression and
23 phosphorylation of cell-cycle proteins. The aristolochic acid mixture induced expression
24 of cyclin D/cdk4 and cyclin E/cdk2, increased phosphorylation of the retinoblastoma
25 (Rb) tumor suppressor protein, and decreased Rb/E2F complexes, thus freeing E2F to
26 facilitate the promotion of cell-cycle transition from the G1 to the S phase.

Abbreviations

AA: aristolochic acid

AA I: aristolochic acid I

AA II: aristolochic acid II

AAN: aristolochic acid nephropathy

APCI: atmospheric pressure chemical ionization

AR: aristolactams

β -CD: β -cyclodextrin

BD: basal diet

BEN: Balkan endemic nephropathy

BQ: below the limit of quantitation

b.w.: body weight

CAM: complementary and alternative medicine

CE: capillary electrophoresis

CHN: Chinese herb nephropathy

CHO: Chinese hamster ovary

CI: confidence interval

CV: cyclic voltammetry

CZE: capillary zone electrophoresis

D: aristolochic acid D

dA-AAI: 7-(deoxyadenosin-N⁶-yl)-aristolactam I

dA-AAII: 7-(deoxyadenosin-N⁶-yl)-aristolactam II

dAMP: deoxyadenosine monophosphate

DAD: photodiode array detector

dCMP: deoxycytidine monophosphate

dG-AAI: 7-(deoxyguanosin-N²-yl)-aristolactam I

dG-AAII: 7-(deoxyguanosin-N²-yl)-aristolactam II

dTMP: deoxythymidine monophosphate

DSHEA: Dietary Supplement Health and Education Act

ELISA: enzyme-linked immunosorbent assay

ESI: electrospray negative ion;

FDA: Food and Drug Administration

FESI-MEKC: field-enhanced sample injection–micellar electrokinetic chromatography

FLD: fluorescence detector

GST: glutathione-S-transferase

HID: highest ineffective dose

HPLC: high-performance liquid chromatography

IARC: International Agency for Research on Cancer

IC₅₀: half maximal inhibitory concentration

i.p.: intraperitoneal

i.v.: intravenous

LC: liquid chromatography

LED: lowest ineffective dose

LIF: laser-induced fluorescence

LOD: limit of detection

MDHPC: 3,4-methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid

MDPC: 3,4-methylenedioxy-1-phenanthrenecarboxylic acid

MEEKC: microemulsion electrokinetic chromatography;

MeO: methoxy

MPT: mitochondrial permeability transition

MS: mass spectrometry

MTT: 3-(4,5-dimethylthiazole)-2,5-diphenyltetrazolium bromide

N: sample size

NA: not available

N/A: not applicable

NADPH: nicotinamide adenine dinucleotide phosphate, reduced form

NAG: *N*-acetyl- β -glucosaminidase

ND: not detected

NDT: not determined

NF: not found

NI: not identified

NIEHS: National Institute of Environmental Health Sciences

NR: not reported

NS: not specified

NT: not tested

OA: orotic acid

OH: hydroxyl

OTA: ochratoxin A

Pap: papillomas

ppm: parts per million

RH: relative hazard

SCC: squamous-cell carcinoma

SCE: sister chromatid exchange

SIR: standardized incidence ratio

SMR: standardized mortality ratio

sp.: species (singular)

spp.: species (plural)

TCC: transitional cell carcinoma

TLC: thin layer chromatography

TG: thioguanine

UV: ultraviolet

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1 Introduction

2 Aristolochic acids are nitrophenanthrene carboxylic acids found primarily in the
3 Aristolochiaceae family of plants. “Aristolochic acids” were nominated by National
4 Institute of Environmental Health Sciences (NIEHS) for possible listing in the *Report on*
5 *Carcinogens* based on the finding by the International Agency for Research on Cancer
6 (IARC) that naturally occurring mixtures of aristolochic acids are *probably carcinogenic*
7 *to humans* (Group 2A). For the purposes of this document, “aristolochic acids” is used to
8 refer to either individual aristolochic acids (e.g., aristolochic acid I or aristolochic acid II
9 that were administered as pure preparations in animal studies) or to mixtures of
10 aristolochic acids that occur naturally in botanical products.

11 Botanical products containing aristolochic acids have been used in traditional herbal
12 medicine as antirheumatics, as diuretics, in the treatment of edema, in wound healing, in
13 obstetrics (to facilitate childbirth), and for other conditions such as hemorrhoids, cough,
14 and asthma. Aristolochic acids have been detected in plants of both the *Aristolochia*
15 (notably *A. clematitis*, *A. contorta*, *A. debilis*, *A. fangchi*, *A. indica*, *A. manshuriensis*, and
16 *A. serpentaria*) and *Asarum* genera of the family Aristolochiaceae. Botanical products
17 containing aristolochic acid are described in the literature by various terms, including
18 herbal preparations, herbal remedies, Chinese herbs, Chinese herbal medicines, and
19 slimming (weight-loss) regimens including Chinese herbs.

20 1.1 Chemical identification

21 “Aristolochic acids “ is a generic name for a family of nitrophenanthrene carboxylic acids
22 that have been reported to occur in plants in the Aristolochiaceae family (EMEA 2000).
23 This family includes about 450 plants in 6 genera. Most plants reported to contain
24 aristolochic acids belong to the genus *Aristolochia* or *Asarum* (FDA 2001b). These plants
25 occur in moist woodlands of temperate and tropical regions worldwide (Starr *et al.* 2003).
26 Various *Aristolochia* and *Asarum* species have been used in herbal medicines since
27 antiquity in obstetrics and in treatment of snakebite, festering wounds, and tumors, and
28 they remain in use today, particularly in Chinese herbal medicine (IARC 2002, Kohara *et*
29 *al.* 2002). All parts of the plant are used in herbal preparations (see Table 1-1 for

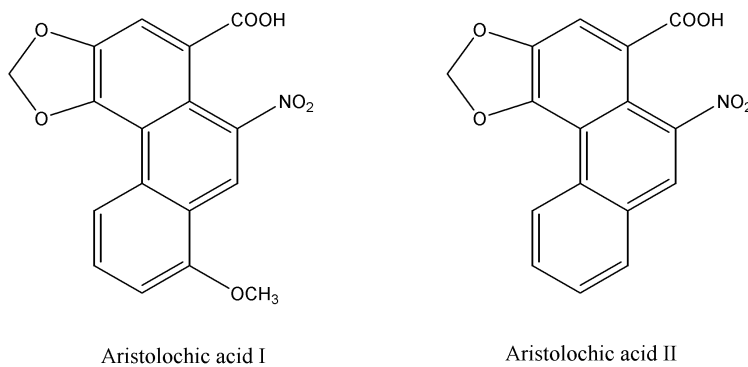
1 examples), and aristolochic acids are present in the roots, stems, leaves, and fruit (EMEA
2 2000, IARC 2002).

3 The aristolochic acid content of plants or botanical preparations varies depending on the
4 plant species, where it was grown, the time of year, and other factors. However,
5 aristolochic acid I (also called aristolochic acid A) and its demethoxylated derivative,
6 aristolochic acid II (also called aristolochic acid B), are the most widely studied; their
7 structures are shown in Figure 1-1. Other compounds found in these plants include other
8 aristolochic acids (e.g., III, IIIa, IV, IVa), aristolactams, and dioxoaporphines (Cosyns
9 2003, Kumar *et al.* 2003). Aristolochic acids I and II are the most common marker
10 compounds used to evaluate the presence of the family of aristolochic acids in plant
11 samples. Related nitrophenanthrenes, such as the aristolactam derivatives of aristolochic
12 acids, have been reported in a wider variety of plant families (Kumar *et al.* 2003). This
13 document focuses on aristolochic acids I and II because they are found in most of the
14 herbal medicines prepared from *Aristolochia* species, occur at relatively high
15 concentrations, and have been associated with toxic and carcinogenic effects. Some
16 chemical identification information for aristolochic acids I and II is listed in Tables 1-2
17 and 1-3.

Table 1-1. Examples of *Aristolochia* species used in botanical products

Aristolochia species (location)	Parts used in herbal medicine	Aristolochic acid components
<i>A. fangchi</i> (China)	root	AA I, II, IIIa
<i>A. manshuriensis</i> (China)	stem	AA I, II, IIIa, IV, IVa; aristolic acid II
<i>A. contorta</i> (China)	fruit, herb	AA I, II, IIIa, VIIa; 7-MeO-8-OH-AA; AA III methyl ester; AA IV methyl ester; aristolic acid; AA BII methyl ester
<i>A. debilis</i> (China)	fruit, herb, root	AA I, II, IIIa, IV, IVa; 7-OH-AA I; 7-MeO-AA I; 7-MeO-AA I; AA III methyl ester
<i>A. clematitis</i> (Europe)	herb, root	AA I, II, III, IIIa, IV, IVa
<i>A. indica</i> (India)	root	AA I, IVa, IVa methyl ester lactam; aristolic acid

Sources: IARC 2002, Kumar *et al.* 2003, Mix *et al.* 1982, Pailer *et al.* 1965, Yuan *et al.* 2007b.
AA = aristolochic acid; MeO = methoxy; OH = hydroxyl.

**Figure 1-1. Chemical structures of aristolochic acids I and II****Table 1-2. Chemical identification of aristolochic acid I**

Characteristic	Information
Chemical Abstracts Index name	8-methoxy-6-nitrophenanthro[3,4- <i>d</i>]-1,3-dioxole-5-carboxylic acid
CAS Registry number	313-67-7
Molecular formula	C ₁₇ H ₁₁ NO ₇
Synonyms	8-methoxy-3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid, aristolochic acid A, aristolochin, birthwort, 3,4-methylenedioxy-8-methoxy-10-nitro-1-phenanthrenecarboxylic acid

Sources: ChemIDPlus 2004a, IARC 2002.

Table 1-3. Chemical identification of aristolochic acid II

Characteristic	Information
Chemical Abstracts Index name	6-nitrophenanthro[3,4- <i>d</i>]-1,3-dioxole-5-carboxylic acid
CAS Registry number	475-80-9
Molecular formula	C ₁₆ H ₉ NO ₆
Synonyms	aristolochic acid B, 3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid, 3,4-methylenedioxy-10-nitro-1-phenanthroic acid, 3,4-methylenedioxy-10-nitro-1-phenanthrenecarboxylic acid

Sources: ChemIDPlus 2004b, IARC 2002.

1 1.2 Physical-chemical properties

2 Aristolochic acid I is a crystalline solid. Other selected physical and chemical properties
 3 of aristolochic acid I are summarized in Table 1-4 (see the Glossary for property
 4 definitions). The molar extinction coefficient (ϵ) for aristolochic acid I in ethanol is 6,500
 5 at 390 nm, 12,000 at 318 nm, and 27,000 at 250 nm (O'Neil *et al.* 2006). A solution of
 6 aristolochic acid I in acetonitrile/ethanol (1:4) was reported to be stable for 30 days when
 7 refrigerated and protected from light (Trujillo *et al.* 2006). No information was located on

1 the physical or chemical properties of aristolochic acid II other than its molecular weight
2 of 311.3 (IARC 2002).

Table 1-4. Physical and chemical properties of aristolochic acid I

Property	Information
Molecular weight	341.3
Melting point (°C)	281–286
Boiling point (°C)	NF
Density	NF
Solubility	
water	slightly soluble
acetic acid, acetone, aniline, alkalies, chloroform, diethyl ether, ethanol	soluble
benzene, carbon disulfide	practically insoluble
Octanol/water partition coefficient (log K_{ow})	3.48
Vapor pressure	NF
Vapor density	NF
Henry's law constant	NF
Critical temperature	NF
Dissociation constant (pK_a)	NF

Source: IARC 2002.

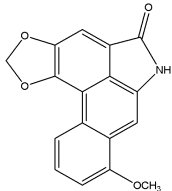
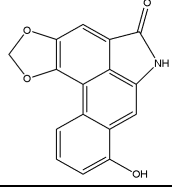
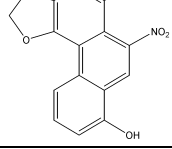
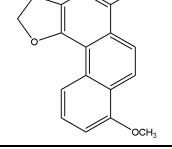
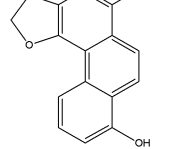
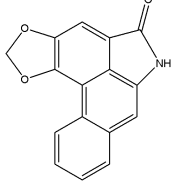
NF = not found.

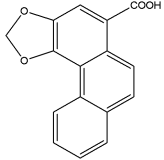
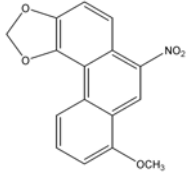
3 1.3 Metabolites

4 Krumbiegel *et al.* (1987) identified the following metabolites of aristolochic acid I in
5 rodents: aristolactam I, aristolactam Ia, aristolochic acid Ia, aristolic acid I, and 3,4-
6 methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid. The principal metabolite of
7 aristolochic acid I in rats was aristolactam Ia (46% of the dose in urine and 37% in the
8 feces). Metabolites of aristolochic acid II in rats and mice included aristolactam II,
9 aristolactam Ia, and 3,4-methylenedioxy-1-phenanthrenecarboxylic acid. These all were
10 considered minor metabolites, because the largest proportion of the dose that could be
11 accounted for in rats was as aristolactam II at only 4.6% in the urine and 8.9% in the
12 feces. In addition, Chan *et al.* (2007a) recently identified a metabolite formed from
13 decarboxylation of aristolochic acid I that is when with other Phase I metabolites of
14 aristolochic acids in Table 1-5.

1 Only aristolactam I and aristolactam II were identified in urine samples collected from 6
 2 healthy human volunteers given a mixture of aristolochic acids I and II over several days
 3 (Krumbiegel *et al.* 1987). More information on metabolites and metabolism is provided
 4 in Section 5.1.

Table 1-5. Metabolites of aristolochic acids I and II identified in rodents

Metabolite	Molecular weight	Structure
Aristolactam I	293.3	
Aristolactam Ia	279.3	
Aristolochic acid Ia	327.3	
Aristolochic acid I	296.1	
3,4-Methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid	282.1	
Aristolactam II	263.3	

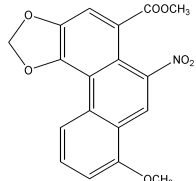
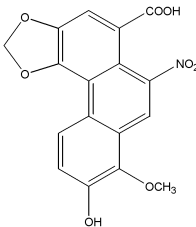
Metabolite	Molecular weight	Structure
3,4-Methylenedioxy-1-phenanthrenecarboxylic acid	266.3	
Decarboxylated metabolite	297.3	

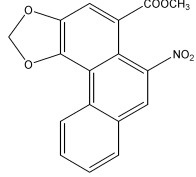
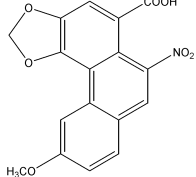
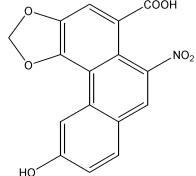
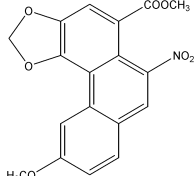
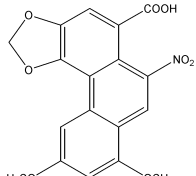
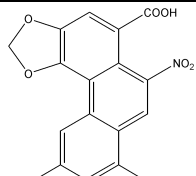
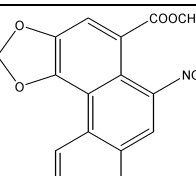
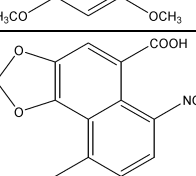
Source: Chan *et al.* 2007a, Krumbiegel *et al.* 1987.

1 1.4 Aristolochic acid analogues

2 As mentioned above, aristolochic acids are a complex mixture of nitrophenanthrene
3 carboxylic acids that are primarily found in plants in the family Aristolochiaceae. In
4 addition to aristolochic acids, other chemically related compounds found in these plants
5 include aristolactams and dioxoaporphines. The dioxoaporphines are thought to function
6 as intermediates in the biosynthesis of aristolactams, which are precursors of aristolochic
7 acids (Kumar *et al.* 2003). The structures of aristolochic acids I and II are shown in
8 Figure 1-1 (above), and examples of the structures of aristolactams are shown in Table 1-
9 5 (above). The general structure of the aristolactams is shown in Table 1-6, which also
10 shows the structures of some other aristolochic acids and the basic structure of
11 dioxoaporphines.

Table 1-6. Selected naturally occurring analogues of aristolochic acids I and II identified in plants of the family Aristolochiaceae

Compound	Molecular weight	Structure
Aristolochic acid I methyl ester	355.3	
7-Hydroxy aristolochic acid I	357.3	

Compound	Molecular weight	Structure
Aristolochic acid II methyl ester	325.3	
Aristolochic acid III	341.3	
Aristolochic acid IIIa (aristolochic acid C)	327.2	
Aristolochic acid III methyl ester	355.3	
Aristolochic acid IV	371.3	
Aristolochic acid IVa (aristolochic acid D)	357.3	
Aristolochic acid IV methyl ester	385.3	
Aristolochic acid V ^a	371.3	

Compound	Molecular weight	Structure
Aristolochic acid Va	357.3	
Aristolochic acid VIa	357.3	
Aristolochic acid VII	371.3	
Aristolochic acid VIIa	357.3	
Aristolochic acid E	357.3	
Aristolactams (numerous compounds)	Variable (depending on R groups) ^b	
Dioxoaporphines (numerous compounds)	Variable (depending on R groups) ^c	

Source: Kumar *et al.* 2003, Priestap 1985a, 2008.

^a Structure incorrectly reported in Kumar *et al.* 2003, but corrected based on Priestap 1987.

^b Where R₁ = -OH or -OCH₃, R₂ = -OCH₃, R₁ + R₂ = -OCH₂O-, and R₃ and R₄ = -H, -OH, or -OCH₃.

^c Where R₁-R₉ = -H, -OH, -OCH₃, or -CH₃ and R₂ + R₃ = -OCH₂O-.

2 Human Exposure

1 Aristolochic acids occur naturally in plants, primarily of the genera *Aristolochia* and
2 *Asarum*, that grow in temperate and tropical climates worldwide. Human exposure to
3 aristolochic acids occurs primarily through the use of these plants in traditional and folk
4 medicines. This section reviews the use (Section 2.1), production (Section 2.2),
5 measurements of exposure (Section 2.3), occurrence and exposure (Section 2.4), and
6 regulations and guidelines (Section 2.5) for aristolochic acids.

7 2.1 Use

8 As mentioned in Section 1, *Aristolochia* plants have been used since ancient times in
9 traditional herbal medicines in many parts of the world. Aristolochic acids have been
10 reported to have antibacterial, antiviral, antifungal, and antitumor effects (Kupchan and
11 Doskotch 1962, Zhang *et al.* 2004). The name *Aristolochia* (meaning the best delivery or
12 birth) is thought to be of ancient Greek origin and reflects centuries of use in obstetrics
13 (Frei *et al.* 1985). Other traditional uses included treatment for snakebite, scorpion stings,
14 fever, infection, diarrhea, and inflammation (Arlt *et al.* 2002b, Jiménez-Ferrer *et al.*
15 2005). In more recent times, aristolochic acids have been tested or used in conventional
16 pharmaceuticals. For example, in the early 1960s, they were tested for antitumor effects
17 in mice (Kupchan and Doskovitch 1962) and in clinical trials, but the trials were
18 discontinued when the aristolochic acid preparation was shown to be nephrotoxic
19 (Jackson *et al.* 1964). In contemporary medicine, *Aristolochia* plant extracts have been
20 used in therapies for arthritis, gout, rheumatism, and festering wounds (Arlt *et al.* 2002b).
21 Its anti-inflammatory properties encouraged the development of pharmaceutical
22 preparations in Germany; however, uses in contemporary medicine were discontinued in
23 Germany and other countries after the carcinogenic and mutagenic properties of
24 aristolochic acids were first reported in the early 1980s. The U.S. Food and Drug
25 Administration's (FDA's) "Approved Drug Products with Therapeutic Equivalence
26 Evaluations" ("Orange Book") does not list any prescription or over-the-counter products
27 (current or discontinued) that contain or contained aristolochic acids. Some of the
28 aristolochic acid-containing plants used in traditional herbal medicines and the
29 conditions treated are shown in Table 2-1.

1 Over 100 cases of nephropathy were reported in Belgium in the 1990s among women
2 who had consumed Chinese herbs containing aristolochic acids as part of a slimming
3 (weight-loss) regimen (see Section 3.1). Aristolochic acid nephropathy (AAN) became
4 recognized as a worldwide disease after additional cases of aristolochic acid–associated
5 nephropathy and carcinoma were reported in the United States, Europe, and Asia.
6 Exposure to aristolochic acids from wheat contaminated with seeds from *Aristolochia*
7 *clematitis* is proposed to be a risk factor for endemic Balkan nephropathy (EN or BEN)
8 (see Section 3.4) (Grollman *et al.* 2007, de Jonge and Vanrenterghem 2008).

9 In the early 2000s, the FDA (2000, 2001a, 2001c) issued warnings to healthcare
10 professionals, industry associations, and consumers regarding the safety of botanical
11 products and dietary supplements containing aristolochic acids. In its warning, the FDA
12 recommended that all botanical remedies known or suspected of containing aristolochic
13 acids be discarded (see Section 2.6 for further information on regulatory actions).
14 Nevertheless, plants containing aristolochic acids continue to be used in traditional and
15 folk medicines for a number of indications and have subsequently been shown to be
16 available on the Internet (Gold and Slone 2003a, 2003b, Schaneberg and Khan 2004, see
17 also Appendix A). Aristolochic acid–containing products, including *Aristolochia* species
18 and products for which substitutions of other plants appear to have occurred have been
19 reported on the Dutch market (Martena *et al.* 2007).

20 As described above, exposure to Aristolochic acids occurs mainly from
21 Aristolochiaceae plants, especially the genera of *Aristolochia* and *Asarum*. The fruits of
22 *Aristolochia contorta* and *A. debilis* are traditionally used as Ma Dou Ling for the
23 treatment of hemorrhoids and of cough and other conditions related with lung in Chinese
24 medicine (Chen and Chen 2004b). Their roots were used as Qing Mu Xiang for distention
25 and pain of the chest and abdomen, diarrhea, and snakebite, while their stems with leaves
26 were used as Tian Xian Teng for the treatment of chest and abdominal pain, hernia pain,
27 neuralgia, liver cancer, and sexually transmitted diseases. Other Aristolochiaceae plants,
28 such as *A. manshuriensis* (Guan Mu Tong), *A. kaempferi* (Da Ye Ma Dou Ling), and *A.*
29 *moupinensis* (Huai Tong Ma Dou Ling) are used legally as Mu Tong (usually derived
30 from *Clematis armandii* or *C. montana*) or its complementary or alternative in different

1 parts of China. Chuan Mu Tong (*C. armandii* stem) is used mainly for the treatment of
2 urethritis, to relieve pain, and to promote lactation. Some Aristolochiaceae plants, such
3 as the roots of *A. fangchi* (Guang Fang Ji) and *A. heterophylla* (Han Zhong Fang Ji), are
4 also used as source plants of Fang Ji, which was originally obtained from the
5 Menispermaceous plant *Stephania tetrandra* and used for the treatment of edema. [The
6 overlapping use of different plants as one crude drug or one plant used as different crude
7 drugs can increase the risk for exposure to aristolochic acids.]

Table 2-1. Medical uses of some plants containing aristolochic acids

Plant species	Common name	Geographic growth range	Medical uses
<i>A. clematitis</i>	birthwort	E. and S.E. Europe, N.E. United States	as an abortifacient, anti-inflammatory, antipyretic, immune system stimulant, or emmenagogue; to treat colic, wounds, or ulcers
<i>A. contorta</i>	ma dou ling	E. Asia	as an antiseptic, or sedative; to treat hemorrhoids, cough, asthma, epigastric pain, arthralgia, or edema
<i>A. debilis</i>	ma dou ling	E. Asia	as an antiseptic; to treat cough, asthma, pain, arthralgia, edema, hemorrhoids, gastric disorders, hypertension, dizziness, headache, boils, snakebite, or insect bites
<i>A. elegans</i>	elegant Dutchman's pipe	South America to Mexico	as an antiseptic, antipyretic, or emmenagogue; to treat snakebite or scorpion stings
<i>A. fangchi</i>	guang fang ji	E. Asia	as a diuretic, antipyretic, or analgesic; to treat lung disorders or rheumatic arthritis
<i>A. indica</i>	Indian birthwort	S. Asia	as an emmenagogue, abortifacient, or antipyretic; to treat snakebite or diarrhea
<i>A. kaempferi</i>	yellowmouth Dutchman's pipe	E. Asia	to treat lung ailments, hemorrhoids, or ascites
<i>A. macrophylla</i>	pipevine	E. United States	as an antiseptic; to treat swelling of the feet or legs
<i>A. molissima</i>	xun gu feng	E. Asia	as a diuretic or anti-inflammatory; to treat arthralgia or pain
<i>A. manshuriensis</i>	Manchurian birthwort	E. Asia	as an anti-inflammatory, diuretic, emmenagogue, or galactagogue
<i>A. reticulata</i>	Texas Dutchman's pipe	S.W. United States	as a stimulant or to promote sweating; to treat stomach disorders,
<i>A. rotunda</i>	snakeroot	Europe	as an abortifacient, diuretic, emmenagogue, or antihelminthic; to treat cough or wounds
<i>A. serpentaria</i>	Virginia snakeroot	S.E. United States	as an anti-inflammatory, diuretic, expectorant, or antipyretic; to treat circulatory or kidney disorders, toothache, stomach pain, or snakebite
<i>Asarum canadense</i>	wild ginger	E. and N.W. United States	as a diuretic, antihelminthic, antibiotic, or contraceptive; to treat colds, flu, cough, cramps, wounds, or asthma

Sources: Dharmananda 2001, FDA 2001b, Gold and Slone 2003a, IARC 2002, Jiménez-Ferrer *et al.* 2005, PFAF 2005.

- 1 Uses other than in herbal medicines include cultivation as ornamental plants (Starr *et al.*
- 2 2003). For example, *A. littoralis* is native to Brazil but is cultivated as an ornamental vine
- 3 in Hawaii and Florida. Several *Aristolochia* species are available on the Internet from
- 4 various greenhouses and nurseries.

1 In addition to use in studies of toxicity and carcinogenicity, aristolochic acids are used in
2 biochemical studies as relatively selective inhibitors of phospholipase A₂ (see Section
3 5.2.3).

4 **2.2 Production**

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

11 No specific data on U.S. production, imports, or sales of botanical products that may
12 contain aristolochic acids were identified; however, there are many U.S. suppliers of
13 products that may contain aristolochic acids. Gold and Sloan (2003a) identified 112
14 botanical products that may contain aristolochic acids that were available for purchase
15 over the Internet (see Appendix A). Estimates for the use of one traditional Chinese herb
16 (*A. manshuriensis* or guan mu tong) in China were reported by Hu *et al.* (2004). They
17 estimated that approximately 6,400 metric tons of guan mu tong could have been
18 consumed in China during a 20-year period beginning in 1983.

19 **2.3 Measurements of Exposure**

20 This section discusses methods for analysis of aristolochic acids (2.3.1) and biological
21 indices of exposure in humans (2.3.2).

22 *2.3.1 Analysis methods*

23 A number of methods have been developed for analysis of aristolochic acids in plant
24 extracts, including thin-layer chromatography, gas-liquid chromatography (Rao *et al.*
25 1975), and nuclear magnetic resonance (Hanna 2004), but high-performance liquid
26 chromatography (HPLC) and capillary electrophoresis (CE) are the most commonly used
27 separation methods (Li *et al.* 2005a). Detection methods also have varied over time, with
28 ultraviolet (UV) light absorption being most common in the past, but mass spectrometry
29 (MS), electrochemical detection (ED), diode-array detection (DAD), laser-induced

1 fluorescence (LIF) detection, fluorescence detection, and other methods have also been
2 reported in more recent publications (Chan *et al.* 2007a,b).

3 Extraction methods may be particularly important in the analysis of aristolochic acids. An
4 early attempt to analyze the aristolochic acid content of the herbal preparation for the
5 Belgian weight-loss regimen through pre-purification extractions with chloroform,
6 methanol, and a methanol-water mixture (1:1 by volume) was unsuccessful
7 (Vanherweghem *et al.* 1993). However, Vanhaelen *et al.* (1994) later reported that these
8 pre-purification extractions might have partly destroyed aristolochic acids, and
9 Vanhaelen *et al.* were able to demonstrate with a thin-layer chromatography (TLC)
10 method that 11 of 12 samples labeled as *Stephania tetrandra* contained aristolochic acids
11 and only 2 samples contained tetrandrine, a constituent expected to be present in a
12 preparation containing *S. tetrandra*.

13 The FDA issued a Laboratory Information Bulletin for the determination of aristolochic
14 acids in traditional Chinese medicines and dietary supplements (Flurer *et al.* 2001). This
15 method was based on an extraction method used by German regulators, and the extract
16 was analyzed for aristolochic acids via HPLC with ultraviolet (UV)/visible detection at
17 390 nm. The presence of aristolochic acids was confirmed via liquid
18 chromatography/mass spectrometry (LC/MS) with either an ion-trapping mass
19 spectrometer or a triple-quadrupole mass spectrometer. Trujillo *et al.* (2006) achieved a
20 limit of quantification (LOQ) of 2 µg/g [2 ppm or 5.9×10^{-9} mol/g] by systematically
21 optimizing the FDA reference method with regard to the test sample size, the grind size
22 for the sample, and the solvent extraction. The authors also varied the injection volume
23 and detection wavelength to determine the optimal chromatographic conditions. A
24 subsequent publication by Sorenson and Sullivan (2007) reported the results of a
25 collaborative study involving 11 laboratories (only 10 complete sets of data were
26 generated, as one laboratory conducted only the LC/UV portion and another conducted
27 only the LC/MS portion) and 13 materials prepared for the study from *Aristolochia*
28 *manshuriensis* [*A. manshuriensis*] stem, *Aristolochia spp.* root, *Akebia trifoliata* stem,
29 *Clematis armandii* stem, and *Stephania tetrandra* root, either as the native material or

1 with fortification with *Aristolochia spp.* root. The method has been adopted by AOAC
2 International as Method 2007.05 (AOAC 2007).

3 Recent publications have reported improvements in sensitivity for the detection of
4 aristolochic acids I and II. Zhou *et al.* (2006) reported a method for capillary
5 electrophoresis with electrochemical detection that had a limit of detection (LOD) of 4.0
6 $\times 10^{-8}$ M for aristolochic acid I and 1.0×10^{-7} M for aristolochic acid II. They compared
7 their analysis method with five other published methods, three that used CE and UV
8 detection and two based on LC with either UV or MS detection. The LC/MS method
9 provided a similar LOD of 3.5×10^{-8} M for aristolochic acid I and a slightly higher LOD
10 of 4.8×10^{-8} M for aristolochic acid II. Zhou *et al.* also reported that the
11 electropherograms (fingerprint profiles) differed among medicinal herbs and could be
12 used to identify specific herbs.

13 An enzyme-linked immunosorbent assay (ELISA) was reported to have a LOD for
14 aristolochic acid I (0.7 ng/mL, or $\sim 2 \times 10^{-9}$ M), but its LOD for aristolochic acid II was
15 similar to the other methods (18 ng/mL, or $\sim 6 \times 10^{-8}$ M) (Yu *et al.* 2006). Shi *et al.*
16 (2007) described results for an online concentration method with micellar electrokinetic
17 chromatography (MEKC) for CE of aristolochic acids I and II that had detection limits of
18 11 ng/mL for both compounds (LOD for AA I = 3.2×10^{-8} M; LOD for AA II = 3.5×10^{-8}
19 M). A method reported by Hsieh *et al.* (2006) using CE with LIF detection achieved
20 LODs of 8.2×10^{-9} M for AA I and 5.4×10^{-9} M for AA II.

21 The LOD for the detection methods may differ for pure aristolochic acids and
22 aristolochic acids as part of a botanical mixture; the LOD generally is higher for the more
23 complex mixtures. Jong *et al.* (2003) reported a theoretical LOD of 10 ng/mL for pure
24 aristolochic acid I. The lowest reported value for an *Asarum* plant extract was 3.3 μ g/g;
25 however, no LOD was reported for aristolochic acid in the sample matrix. Kite *et al.*
26 (2002) determined the LOD within sample matrices using crude methanol extracts of
27 *Aristolochia* species and reported that the LOD for aristolochic acid I ranged from 250 pg
28 in a sample with low levels of interfering substances to 2.5 ng in a matrix with high levels
29 of interference (0.125 to 1.25 μ g/g, based on extraction from 2 mg of herbal remedy).

1 Similarly, Shi *et al.* (2007) reported a detection limit for aristolochic acids I and II added
2 to a Chinese medicine preparation (Guanxinsuhe drop-pills) of 110 ng/g, although the
3 detection limit for pure aristolochic acids I and II as reported above was an order of
4 magnitude lower at 11 ng/mL.

5 2.3.2 *Biological indices of exposure*

6 Aristolochic acid–DNA (AA-DNA) adducts have been identified in the kidneys of
7 patients with Chinese herb nephropathy using ³²P-post-labeling analysis (Arlt *et al.*
8 2001b, Arlt *et al.* 2001a, Cosyns 2003, Gillerot *et al.* 2001). These adducts are specific
9 markers of exposure to aristolochic acids I and II (Bieler *et al.* 1997). See Section 5 for
10 further discussion of AA-DNA adducts. Grollman *et al.* (2007) described the use of
11 liquid chromatography electrospray ionization/multistage mass spectrometry (LC-
12 ESI/MS/MS³) as a means of specifically confirming the chemical identity of the dA-AL-I
13 and dA-AL-II adducts using synthetic adduct standards.

14 2.4 Occurrence and Exposure

15 This section describes the occurrence of aristolochic acids in plants (2.4.1), in food or
16 animals (insects) (2.4.2), and in botanical products, including potential human exposure
17 from botanical products (2.4.3), and potential occupational exposure (2.4.4).

18 2.4.1 Occurrence in plants

19 The geographical distribution of plants containing aristolochic acids is discussed below.
20 In addition, a variety of aristolactams have been reported to occur in the Aristolochiaceae
21 and sporadically in related plant families, including a few instances in the genus *Piper*
22 (family Piperaceae) and one report each in *Stephania* (Menispermaceae) and
23 *Schefferomitra* (Annonaceae) (Kumar *et al.* 2003).

24 Geographical distribution

25 More than 30 *Aristolochia* species are native to the United States, and they are present in
26 most states (Figure 2-1) (USDA 2005). The most widely distributed native species
27 include *A. serpentaria* (Virginia snakeroot), *A. tomentosa* (wooly Dutchman's pipe), *A.*
28 *macrophylla* (pipevine), and *A. clematitis* (birthwort). In addition, some non-native
29 species are grown as ornamentals or have escaped cultivation and become naturalized
30 (Starr *et al.* 2003). Worldwide, there are an estimated 200 to 350 *Aristolochia* species,

1 and virtually all of them contain aristolochic acids (Dharmananda 2001, Starr *et al.*
 2 2003).

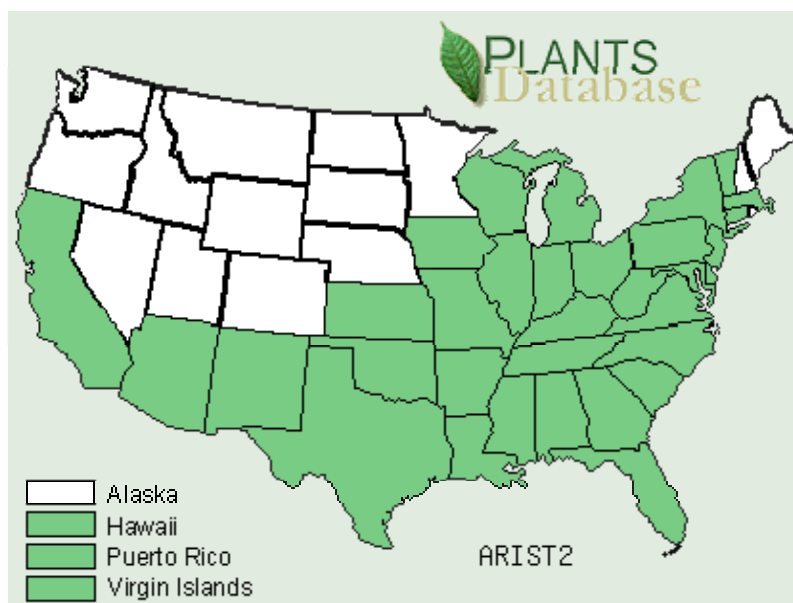


Figure 2-1. Distribution of *Aristolochia* species within the United States

Source: USDA 2005, Plants occur in states colored green.

3 Plants of the genus *Asarum* have been used by Native Americans to treat various
 4 conditions (see Table 2-1) and are still used in herbal medicines in the United States
 5 (Schaneberg *et al.* 2002, Gold and Slone 2003a). *Asarum* species (wild gingers) are
 6 widely distributed in the United States (Figure 2-2). Another genus of the family
 7 Aristolochiaceae, *Hexastylis* (plants in this genus are known as littlebrownjug or
 8 heartleaf), is a group of rare plants related to *Asarum* and endemic to the southeastern
 9 United States. Aristolochic acids were found in this species in one study (see below).

1 plants from three other genera (*Clematis*, *Stephania*, and *Akebia*) and three plant families
2 (Menispermaceae, Ranunculaceae, and Lardizabalaceae) (Zhou *et al.* 2006, Wu *et al.*
3 2007a). However, one study has reported that aristolactams, which are known both as
4 precursors of aristolochic acids in plants and as their metabolites in animals (see Table 1-
5 5), were detected in at least two other plant families (Kumar *et al.* 2003); aristolactams II
6 and BII occur in *Stephania cepharantha* (Menispermaceae family) and in *Schefferomitra*
7 *subaequalis* (Annonaceae family), but no quantitation of these molecules was provided.

8 Most studies measured aristolochic acids I and II, and in general levels of aristolochic
9 acid I were higher. In addition to the aristolochic acids I and II concentrations (see Table
10 2-2), Zhang *et al.* (2006b) also determined concentrations of three additional aristolochic
11 acids (IVa, Va, and 9-OH aristolochic acid I) and two aristolactams (I and II) for
12 medicinal parts (fruit, root, or herb, i.e., stem and leaves) of four different *Aristolochia*
13 species (*A. contorta*, *A. debilis*, *A. manshuriensis*, and *A. fangchi*). Aristolochic acids I
14 and II were the major components measured in most instances, but *A. contorta* (fruit,
15 herb, and root) contained relatively large amounts of aristolochic acid IVa [ranging from
16 79 to 3,360 ppm of crude drug], and the two aristolactams were detectable only in
17 medicinal parts from this species [ranging from 6 to 358 ppm for aristolactam I and from
18 14 to 91 ppm for aristolactam II]. Hong *et al.* (1994) identified 11 aristolochic acid
19 derivatives, including aristolactams and other compounds, in extracts from *Aristolochia*
20 *cinnabarina* roots, and Wu *et al.* (1994) identified 14 aristolochic acid derivatives in
21 extracts from stems and roots of *Aristolochia kankauensis*.

Table 2-2. Identification of aristolochic acids I and II in medicinal plants

Botanical name	Aristolochic acid content [ppm]		Reference
	AA I	AA II	
<i>Aristolochia debilis</i>	790–1,080	80–180	Hashimoto <i>et al.</i> 1999
<i>Aristolochia manshuriensis</i>	1,690–8,820	140–1,000	
<i>Aristolochia fangchi</i>	1,030–2,220	40–220	
<i>Asarum splendens</i>	trace	ND	
<i>Asarum himalaicum</i>	trace	ND	
<i>Aristolochia fangchi</i>	437–668	144–414	Lee <i>et al.</i> 2001
<i>Aristolochia contorta</i>	< 1–83	< 1–115	
<i>Asarum heterotropoides</i>	42	ND	Jong <i>et al.</i> 2003
<i>Asarum crispulatum</i>	3377	ND	
<i>Asarum forbesii</i>	106	ND	
<i>Asarum himalaicum</i>	18	ND	
<i>Asarum sieboldii</i>	3	ND	
<i>Asarum debile</i>	18	ND	
<i>Asarum maximum</i>	86	ND	
<i>Asarum ichangense</i>	53	ND	
<i>Asarum fukienense</i>	17	ND	
<i>Asarum fukienense</i> (hot MeOH extract)	12	ND	
<i>Aristolochia debilis</i> (root)	980	350	Zhou <i>et al.</i> 2006
<i>Aristolochia debilis</i> (fruit)	270	46	
<i>Aristolochia debilis</i> (stem)	ND	ND	
<i>Aristolochia manshuriensis</i> (stem)	230	53	
<i>Aristolochia contorta</i> (fruit)	687–1770	20–185	Zhang <i>et al.</i> 2006b
<i>Aristolochia debilis</i> (herb)	102–409	24–98	
<i>Aristolochia contorta</i> (herb)	33–257	ND–110	
<i>Aristolochia debilis</i> (root)	1,190–4,710	240–1,690	
<i>Aristolochia contorta</i> (root)	2,790–5,480	1,060–1,860	
<i>Aristolochia manshuriensis</i> (stem)	1,880–9,720	256–1,880	
<i>Aristolochia fangchi</i> (root)	637–4,770	60–398	
<i>Aristolochia fangchi</i> (root)	12,980	2,424	Zhai <i>et al.</i> 2006
<i>Aristolochia manshuriensis</i> (stem)	10,850	2,977	
<i>Aristolochia contorta</i> (fruit)	4,695	574	
<i>Aristolochia contorta</i> (root)	6,421	6,108	
<i>Aristolochia contorta</i> (herb)	10,460	6,325	
<i>Aristolochia contorta</i> (fruit)	1,540	350	Yuan <i>et al.</i> 2007
<i>Aristolochia manshuriensis</i> (stem)	3,380	831	
<i>Aristolochia fangchi</i> (root)	4,280	1,200	
<i>Aristolochia debilis</i> (root)	2,610	875	
<i>Aristolochia contorta</i> (herb)	168	49	
<i>Aristolochia mollissima</i> (herb)	145	38.2	
<i>Asarum heterotropoides</i> (herb)	68.2	45	
<i>Aristolochia fangchi</i> (root)	40–400	5–70	Wu <i>et al.</i> 2007a
<i>Aristolochia heterophylla</i> (root)	200–≥400	70–170	
<i>Aristolochia manshuriensis</i> (stem)	40–400	20–70	
<i>Aristolochia mollissima</i> (stem, leaf)	30–400	ND	
<i>Aristolochia tubiflora</i> (root)	40–400	≤70	
<i>Aristolochia contorta</i> (fruit)	80–800	70–700	
<i>Aristolochia heterotropoides</i> (leaf)	40–400	ND	
<i>Asarum heterotropoides</i> (leaf)	≤400	ND	
<i>Asarum sieboldii</i> (root)	≤400	ND	

ND = not detected.

1 Aristolochic acids also occur in North American species of Aristolochiaceae (Schaneberg
2 *et al.* 2002, McMillin *et al.* 2003). Results from these two studies are summarized in
3 Table 2-3. The Schaneberg *et al.* study reported what the authors described as
4 unexpectedly high levels of aristolochic acids in *Hexastylis arifolia* (common name,
5 littlebrownjug). No current medicinal uses for this plant were identified, but Schaneberg
6 *et al.* observed that this and other species of *Hexastylis* had traditional uses that did pose
7 some health hazard. However, they also noted that *Hexastylis* is probably not collected
8 today because of its scarcity.

Table 2-3. Identification of aristolochic acids I and II in North American plants

Botanical name	Aristolochic acid content [dry wt., ppm]		Reference
	AA I	AA II	
<i>Aristolochia macrophylla</i>	3,900	6,600	Schaneberg <i>et al.</i> 2002
<i>Aristolochia serpentaria</i>	1,300	97	
<i>Hexastylis arifolia</i>	2,100	6,600	
<i>Asarum canadense</i>	BQ-370	ND	
<i>Asarum caudatum</i>	BQ	ND	
<i>Asarum wagneri</i>	ND	ND	
<i>Asarum canadense</i> (dry root)	6-18.4 ^a	NR	McMillin <i>et al.</i> 2003
Essence of wild ginger	0.048 ^a	NR	

BQ = detected below the limit of quantitation; ND = not detected; NR = not reported.

^aResults were reported as aristolochic acid.

9 2.4.2 Occurrence in foods or insects

10 Extracts from *Asarum canadense* (Canadian snakeroot or wild ginger) and *Aristolochia*
11 *serpentaria* (Virginia snakeroot) are permitted for use as flavoring substances in foods or
12 beverages; however, the latter is restricted to use only in alcoholic beverages (CFR
13 2003). No information was identified on use of either *Asarum canadense* or *Aristolochia*
14 *serpentaria* in any specific food or beverage products. It has been proposed that
15 contamination of wheat flour by *Aristolochia* species growing as weeds adjacent to wheat
16 fields might be responsible for some cases of Balkan endemic nephropathy (see Section
17 3.4) (Hranjec *et al.* 2005). Aristolochic acids also occur in several species of butterflies
18 whose larvae feed on *Aristolochia* plants (IARC 2002), including species of the genera
19 *Atrophaneura*, *Battus*, *Pachliopta*, and *Troides*.

1 2.4.3 Occurrence and concentrations in botanical products

2 This section discusses first the occurrence of aristolochic acids as a contaminant in herbal
3 products and then its occurrence and concentrations in botanical preparations made from
4 plants that contain aristolochic acids.

5 Occurrence as a contaminant in herbal preparations

6 Herbal preparations can pose a number of quality-related problems, including
7 contamination with prohibited or restricted substances, substitution of ingredients,
8 contamination with toxic substances, and differences between the labeled and actual
9 product contents (MCA 2002).

10 Two herbal remedies prepared from *Aristolochia debilis* or *A. contorta*, known,
11 respectively, as Tian-Xian-Teng (herbs, including the stems and leaves of the plants) and
12 Ma-Dou-Ling (the fruits of the plants), appear in the official 2005 Chinese pharmacopeia
13 (Zhang *et al.* 2006b). However, three additional crude drugs derived from *Aristolochia*
14 species that were listed in the 2000 edition of the Chinese pharmacopeia were cancelled
15 by the Chinese State Food and Drug Administration in 2003 and 2004 because the
16 content of aristolochic acid in the drugs was high enough to cause AAN. These drugs
17 were Qingmuxiang (the roots of *A. debilis*), Guangfangji (the roots of *A. fangchi*) and
18 Guanmutong (the stems of *A. manshuriensis*).

19 The complexity of herbal nomenclature systems used in traditional medicines
20 (particularly traditional Chinese medicines) can lead to confusion and increased risk of
21 inadvertent exposures to aristolochic acids. It is sometimes a practice in traditional
22 Chinese medicine to substitute one similarly named plant species for another, and the
23 similarity of the Chinese names for *Aristolochia* species and other innocuous herbs can
24 result in unintended exposure to *Aristolochia* (Flurer *et al.* 2001).

25 Wu *et al.* (2007a) described three categories of nomenclature used in traditional Chinese
26 medicine with examples of each involving botanicals containing aristolochic acids. (1) A
27 one-to-one category describes one plant part from one plant species corresponding to one
28 herb. The herb guan mu tong refers to the stem of *Aristolochia manshuriensis*, while herb
29 mu tong is derived from *Akebia* species (bai mu tong) or *Clematis* species (chuan mu
30 tong), which do not contain aristolochic acids (EMEA 2000, IARC 2002, Zhu 2002). (2)

1 A multiple-to-one category describes multiple herbs derived from different parts of the
 2 same species of plant. The three herbs ma dou ling, qing mu xiang, and tian xian teng are
 3 derived, respectively, from the fruit, root, and stem of *A. debilis* or *A. contorta*. (3) A
 4 one-to-multiple category describes one herb that refers to multiple plant species. The herb
 5 fang ji refers to the root of either *A. fangchi* (guang fang ji), *Stephania tetrandra* (han
 6 fang ji), *Cocculus trilobus*, or *C. orbiculatus* (mu fang ji) (EMEA 2000, IARC 2002). *A.*
 7 *fangchi* belongs to the Aristolochiaceae family, while the latter three belong to the
 8 Menispermaceae family and do not contain aristolochic acids. [The first and third
 9 categories described by Wu *et al.* have the greatest potential to contribute to the
 10 unintended substitution of botanical material containing aristolochic acids for material
 11 that does not contain it.] Possible substitutions for “fang ji,” “mu tong,” “mu xiang,” and
 12 “ma dou ling” are listed in Table 2-4.

Table 2-4. Plant species supplied as “fang ji,” “mu tong,” “mu xiang,” and “ma dou ling”

Supplied as	Pinyin name	Botanical name	Part used
Fang ji	han fang ji	<i>Stephania tetrandra</i>	root
	guang fang ji	<i>Aristolochia fangchi</i>	
	mu fang ji	<i>Cocculus trilobus</i> <i>Cocculus orbiculatus</i>	
Mu tong	guan mu tong	<i>Aristolochia manshuriensis</i>	stem
	chuan mu tong	<i>Clematis armandii</i> <i>Clematis montana</i>	
	bai mu tong	<i>Akebia quinata</i> <i>Akebia trifoliata</i>	
Mu xiang	qing mu xiang	<i>Aristolochia debilis</i>	root
	mu xiang	<i>Aucklandia lappa</i>	
	guang mu xiang	<i>Saussurea lappa</i>	
	tu mu xiang	<i>Inula helenium</i> <i>Inula racemosa</i>	
	chuan mu xiang	<i>Vladimiria souliei</i> <i>Vladimiria souliei</i> var. <i>cinerea</i>	
Ma dou ling	ma dou ling	<i>Aristolochia contorta</i> <i>Aristolochia debilis</i>	fruit
	gua lou	<i>Trichosanthis kirilowii</i>	

Sources: EMEA 2000, IARC 2002, Zhu 2002.

1 Substitutions arising because of name confusion have also been reported between
 2 botanicals used in Japanese herbal medicines and botanicals with similar names used in
 3 Chinese herbal medicines. In a study of an outbreak of Chinese herb nephropathy in
 4 Japan (see Section 3.1.2), Tanaka *et al.* (2001) suggested that plant species in Japanese
 5 preparations of Chinese herbal medicines could have been substituted because similar
 6 Japanese and Chinese names refer to different plants in Japan and China (see Table 2-5).
 7 Confusion may also occur among Japanese names that are similar but refer to different
 8 herbal medicines; “sei-mokkou” refers to *Aristolochia debilis* (supplied as “qing mu
 9 xiang” in Chinese herbal medicines, see Table 2-5), while the Japanese names “mokkou”
 10 and “sen-mokkou” refer to plants of other genera (EMEA 2000).

Table 2-5. Confusion of names for botanicals in Japanese and Chinese herbal medicine preparations

Botanicals used & corresponding plant name		Chinese medicines used in Japan containing “mokutsu” or “boui”
In Japanese herbal medicine	In Chinese herbal medicine	
Mokutsu <i>Akebia quinata</i>	kan-mokutsu <i>Aristolochia manshuriensis</i>	toki-shigyaku-ka-gosyuyu-syokyo-to toki-shigyaku-to gorin-san kami-gedoku-to sho-hu-san tu-do-san ryutan-syakan-to
Boui <i>Sinomenium acutum</i>	kou-boui <i>Aristolochia fangchi</i> kanchu-boui <i>Aristolochia heterophylla</i>	boui-ougi-to boui-bukuryo-to sokei-kakketsu-to

Source: Tanaka *et al.* 2001.

11 Plant substitutions such as those described above can cause serious disease of death, as
 12 shown in Belgium in the early 1990s, where over 100 cases of irreversible nephropathy
 13 were reported after *Aristolochia fangchi* was inadvertently substituted for *Stephania*
 14 *tetrandra* to prepare diet pills (see Section 3.1.1). A follow-up investigation analyzed 46
 15 batches of powders that were labeled as *Stephania* and found that 30 contained
 16 aristolochic acids and no tetrandrine, 7 contained tetrandrine and no aristolochic acids, 5
 17 contained both, and 4 did not contain either compound (Vanherweghem 1998).
 18 Vanherweghem estimated that between 1,500 and 2,000 persons were exposed to the

1 *Stephania*-labeled powders that contained aristolochic acids ranging from below the
2 detection limit (< 0.02 mg/g) to 2.9 mg/g [2,900 ppm]. A publication by Koh *et al.* (2006)
3 suggests that substitutions of *A. fangchi* for *S. tetrandra* may still occur. Samples labeled
4 as “fang ji,” i.e., *S. tetrandra*, purchased in local medicinal shops in Singapore were
5 found to contain aristolochic acids. Of 10 samples analyzed, 9 were found to contain
6 aristolochic acids (levels not reported) with “chromatographic fingerprints” similar to *A.*
7 *fangchi*.

8 Substitution of an aristolochic acid-containing plant due to name confusion was also
9 documented in Hong Kong (Liang *et al.* 2006). *Herba Aristolochia Mollissimae* [*A.*
10 *mollissima*] and *Herba Solani Lyrati* share a common name transliterated as either “bai
11 mao teng” or “pak mo tang” (Lo *et al.* 2005). Liang *et al.* confirmed the presence of 280
12 ± 105 µg/g of aristolochic acid I in four samples of *Herba Aristolochia Mollissimae*.

13 Herbs are most often traded under their Chinese pinyin names, rather than Latin
14 taxonomic names, and different plants can have similar pinyin names. In many cases, the
15 plant compositions of herbal preparations have changed over time and may vary across
16 regions of China. This can lead to confusion, particularly for herbalists who are
17 inexperienced in traditional Chinese medicine. Once a botanical material is dried and
18 ground, it is difficult to determine its identity without sophisticated chemical analysis.
19 Wu *et al.* (2007a) recommended that the confusions among botanical products could be
20 avoided if more emphasis could be placed on the importance of the pharmaceutical name,
21 which they describe as defining “the species name, the plant part, and sometimes the
22 special process performed on the herb, including cultivating conditions.”

23 *Occurrence and concentrations in botanical preparations*

24 Several studies have reported that herbal preparations used in Belgium, China, Taiwan,
25 Japan, Australia, and Switzerland contained aristolochic acids (see Section 3 for further
26 discussion of aristolochic acids content of various herbal preparations). These data are
27 summarized in Table 2-6. Vanhaelen *et al.* (1994) analyzed samples taken from
28 *Stephania tetrandra* herb powders that were distributed in Belgian pharmacies between
29 July 1990 and August 1992. Relatively high concentrations of aristolochic acids were

1 detected in 13 of 14 batches. Aristolochic acids also were found in samples of a Chinese
2 herbal medicine taken by patients presenting with renal complications in Japan (Tanaka
3 *et al.* 2000a). Gillerot *et al.* (2001) analyzed pills from a Chinese herbal preparation
4 purchased in Shanghai, China. These pills were used by a 46-year-old woman for 6
5 months before she developed severe anemia and subacute renal failure. The aristolochic
6 acids content of the herbal pills was determined to be about 0.07%. Lee *et al.* (2001)
7 analyzed weight-loss powders and pills used in Taiwan. Five weight-loss pills and 11
8 weight-loss powders were collected directly from patients admitted to a hospital in Taipei
9 because of slight renal failure. Aristolochic acids were found in 3 of 5 pills and 9 of 11
10 powders. Samples of 42 commercial Chinese plant mixtures sold for use in weight-loss
11 regimens in Switzerland were analyzed for aristolochic acid I (Ioset *et al.* 2003). Four of
12 the preparations were confirmed to contain aristolochic acid I by TLC and
13 HPLC/UV/MS, and the presence of aristolochic acid I was suspected in two additional
14 preparations. Aristolochic acid I was quantified by UV and MS methods in two samples
15 of powder reported to consist of either a single herb (han fang ji, i.e., *Stephania tetrandra*
16 root) or a mixture of 8 herbs (ba zheng san). The single herb preparation contained
17 0.044% [440 ppm] by UV and 0.040% [400 ppm] by MS, while the mixture of 8 herbs
18 contained 0.009% [90 ppm] by UV and 0.014% [140 ppm] by MS. Over-the-counter
19 Chinese prepared medicines purchased at a local store in Taiwan between January and
20 September 2001 were analyzed for aristolochic acids I and II by Ho *et al.* (2006) using
21 HPLC and UV detection. Aristolochic acid I was quantified in 8 out of 11 and
22 aristolochic acid II in 5 out of 11 samples (neither aristolochic acid I nor II was detectable
23 in 3 of the samples).

Table 2-6. Aristolochic acid contents of herbal preparations

Location	Herbal product form	Aristolochic acid contents [ppm]			Reference
		AA I	AA II	Total	
Belgium	powder	NR	NR	< 20–1,560	Vanhaelen <i>et al.</i> 1994 ^a
	powder	NR	NR	1,800–2,900	
China	pill	700	NR	0.3 mg/pill	Gillerot <i>et al.</i> 2001 ^b
Taiwan	pill	< 1–39	< 1–124	< 1–163	Lee <i>et al.</i> 2001 ^c
	powder	< 1–598	< 1–148	< 1–694	
Japan	NS	1.1–6.7	1.3–6.7	3.1–15.1	Tanaka <i>et al.</i> 2000a ^d
Switzerland	powder	90–440	NR	NR	Ioset <i>et al.</i> 2003 ^e
Taiwan	NS	ND–19.97 nmol/g	ND–3.95 nmol/g	NR	Ho <i>et al.</i> 2006 ^f
Australia	NS	8, 40	8, 210	NR	Cheung <i>et al.</i> 2006 ^g

AA I = aristolochic acid I; AA II = aristolochic acid II; NR = not reported; NS = not specified.

^aRange of values reported from 12 (upper row) and 2 (lower row) batches of *S. tetrandra* powders distributed in Belgium from 1990 to 1992.

^bSample of a Chinese herbal preparation purchased in Shanghai for “waste discharging and youth keeping” purposes.

^cRange of values from 5 weight-loss pills and 11 weight-loss powders collected from renal-failure patients treated in Taipei.

^dSamples of the same herbal medicine collected from two patients with glycosuria.

^eRange of values from 2 weight-loss powders purchased in Switzerland.

^fRange of values from 11 kinds of over-the-counter Chinese herbal medicines known to be consumed by patients prior to hospitalization for acute renal failure.

^gValues for 2 manufactured herbal products marketed under the Chinese proprietary names “Dao Chi Pian” and “Chuan Xiong Cha Tiao San.”

1 Botanical products containing aristolochic acids also can be bought in the United States
2 and other countries via the Internet (Gold 2003, Gold and Slone 2003a,b). Schaneberg
3 and Khan (2004) analyzed 25 herbal products suspected of containing aristolochic acids;
4 of the products purchased from Internet Web sites, 9 were manufactured in the United
5 States and the rest in China. Aristolochic acids I and II were detected in 6 of the products,
6 each of which contained six or more plants in the product matrix (see Table 2-7). The
7 authors also estimated the daily doses of aristolochic acids I and II for individuals who
8 took the maximum suggested dose. Nine of the products listed *Asarum* or wild ginger as
9 an ingredient, but no aristolochic acids were detected in those products. Specific
10 instances of botanical products containing aristolochic acids being sold after the ban or
11 restrictions were in place have also been reported from Australia. Cheung *et al.* (2006)
12 reported that 2 of 7 manufactured herbal products purchased in Melbourne, Australia
13 after aristolochic acids-containing herbs and products were banned in 2003 contained

1 aristolochic acids [one sample had 8 ppm of aristolochic acids I and II, and the other
 2 sample had 40 ppm of aristolochic acid I and 210 ppm of aristolochic acid II]. No
 3 aristolochic acids were detected in 21 samples of Chinese raw herbs purchased at the
 4 same time. Recalls of products containing aristolochic acids have been reported by the
 5 U.S. Food and Drug Administration beginning in 2000 and continuing with the report of
 6 a recall of two products in 2008 (see Appendix B, Table B-4 and Appendix C, Table C-
 7 1).

Table 2-7. Aristolochic acid contents and estimated daily dose from herbal products purchased over the Internet after they were banned in many countries

Product label ingredients	Aristolochic acid I		Aristolochic acid II	
	Concentration [ppm]	Daily dose (mg/day.)	Concentration [ppm]	Daily dose (mg/day)
Long Dan Xie Gan Wan	50	0.07	ND	N/A
Long Dan Xie Gan Wan	40	0.05	ND	N/A
Lung Tan Xie Gan	110	0.48	90	0.40
Lung Tan Xie Gan Wan	90	0.40	80	0.35
Gaun Xin Su He Wan	80	0.16	30	0.06
<i>Aristolochia</i> root	280	0.64	140	0.32

Source: Schaneberg and Khan 2004.

N/A = not applicable; ND = not detected.

8 *Exposure from using botanical products*

9 Individuals who use herbal medicines that contain *Aristolochia* or *Asarum* species are the
 10 most likely to be exposed to aristolochic acids. Herbal preparations are available in
 11 several forms (e.g., capsules, extracts, teas, or dried herbs). The herbs may be ingested or
 12 applied to the skin (e.g., to treat wounds); thus, exposure may occur through ingestion or
 13 skin contact. However, no published studies of skin absorption of aristolochic acids in
 14 humans or experimental animals were found. Exposure could potentially occur through
 15 direct contact with the plants, either in their natural habitats or as cultivated ornamentals.
 16 Direct contact with *Asarum canadense* leaves has been reported to cause dermatitis
 17 (PFAF 2005).

18 No estimates were found of the number of people in the United States who are exposed to
 19 aristolochic acids in herbal medicines, but two cases of renal failure resulting from
 20 ingestion of herbal products containing aristolochic acids have been reported in the

1 United States (Meyer *et al.* 2000, CR 2004, Grollman *et al.* 2007). According to the
2 reports, one of the cases, which was reported by both Meyer *et al.* and *Consumer*
3 *Reports*, was clearly exposed to products containing aristolochic acids before the FDA
4 issued a safety warning in 2000 for botanical products containing aristolochic acids;
5 however, the second case involved exposure that might have continued even after the
6 safety warning. The use of herbal products is much greater in China, and a few estimates
7 for consumption and exposure in that country are available. IARC (2002) reported that
8 about 320 metric tons of dried stems of *A. manshuriensis* were consumed in China in
9 1983, but no data were reported for other years or other countries. However, Hu *et al.*
10 (2004) estimated from this report that approximately 6,400 metric tons of guan mu tong,
11 i.e., *A. manshuriensis*, involving an estimated 1 billion patients, could have been
12 consumed in China during a 20-year period beginning in 1983. [However, their estimates,
13 based on 6 g per day with a 10-day course, would result in potential exposure to 100
14 million rather than 1 billion patients, even assuming that each patient was treated with
15 only one course of guan mu tong.] Although no data specific for *Aristolochia* or *Asarum*
16 herbal product use in the United States were found, several reports indicate the use of
17 complementary and alternative medicine (CAM), including botanical products, has
18 increased in the 1990s and 2000s (Barnes *et al.* 2004, Bent and Ko 2004). It has been
19 reported by the Centers for Disease Control and Prevention that 29% of adults in the
20 United States used CAM in 1999, and 10% of the adults ingested herbal medicines
21 (Straus 2002). In addition the total spent for dietary supplements in the United States in
22 2001 was \$17.8 billion of which \$4.2 billion was spent on herbs and other botanical
23 remedies (Marcus and Grollman 2002).

24 Exposure to aristolochic acids from herbal medicines has also been reported in other
25 countries (see Section 3). Case reports from China indicate that renal failure has occurred
26 after ingestion of herbal medicines for 6 months or less. Gillerot *et al.* (2001) reported
27 that a 46-year-old Chinese woman developed anemia and renal failure after taking two
28 herbal pills per day for 6 months. A sample of the pill powder confirmed the presence of
29 aristolochic acids (see Table 2-6). [The estimated total intake of the herbal powder and
30 aristolochic acids (based on an average amount of herbal powder per pill of 430 mg and
31 an aristolochic acids content of 0.3 mg per pill) over 6 months (~180 days) would be

1 about 154 g of herbs and 110 mg of aristolochic acids.] Lo *et al.* (2004) reported a case of
2 acute renal failure in a 75-year-old man who had taken an herbal medicine as a tonic for
3 10 days. The total dose of *A. fangchi* was estimated to be about 100 mg.

4 **2.4.4 Occupational exposure**

5 Herbalists are potentially exposed to aristolochic acids while gathering plants and while
6 preparing or applying botanical products. Gardeners, landscapers, or nursery workers that
7 handle or transplant *Aristolochia* or *Asarum* plants could potentially be exposed to
8 aristolochic acids. However, occupational exposures to aristolochic acids have not been
9 documented.

10 **2.5 Regulations and guidelines**

11 This section summarizes regulations and guidelines applicable to botanical products
12 containing aristolochic acids in the United States (2.5.1) and other countries (2.5.2).

13 **2.5.1 United States**

14 Some botanical products are regulated as dietary supplements by the FDA under the
15 Dietary Supplement Health and Education Act (DSHEA) of 1994 (FDA 1995). Under
16 DSHEA, the manufacturer and distributor of a product are responsible for assuring the
17 safety of the product. No FDA premarket safety review is required for ingredients that
18 were marketed as food before 1994. However, manufacturers are required to record
19 adverse events and to report to the FDA serious adverse events reported to them about
20 their products. The FDA may restrict a substance if it poses a significant and
21 unreasonable risk under the conditions of use on the label or as commonly consumed, but
22 the burden of proof is with the FDA. Label requirements for dietary supplements under
23 DSHEA include the following: product name; net quantity of contents; ingredients and
24 amounts; supplement facts, including serving size, amount, and active ingredient; list of
25 other ingredients for which no daily value has been established; and the name and address
26 of the manufacturer, packer, or distributor. Product claims are limited; if claims are made,
27 the product label generally must contain a disclaimer that the product has not been
28 evaluated by the FDA and is not intended to diagnose, treat, cure, or prevent any disease.
29 Products that are intended to diagnose, treat, cure, or prevent a disease generally meet the

1 definition of a drug and must meet the safety and efficacy standards set by the FDA in
2 order to be legally marketed in the United States.

3 The FDA (2000, 2001a,c) issued warnings to health care professionals, industry
4 associations, and consumers regarding safety concerns for botanical products containing
5 aristolochic acids. This warning covered botanical products that included species of the
6 genera *Aristolochia*, *Asarum*, *Bragantia*, *Stephania*, *Clematis*, *Akebia*, *Cocculus*,
7 *Diploclisia*, *Menispermum*, or *Sinomenium*, mu tong, fang ji, guang fang ji, fang chi, kan-
8 mokutsu, or mokutsu. A complete list of the botanicals of concern identified by the FDA
9 is included in Appendix B.

10 The FDA urged practioners who prescribe botanical remedies to discard any products that
11 may contain aristolochic acids. Likewise, manufacturers and distributors were urged to
12 review their manufacturing procedures to ensure that botanical products are free of
13 aristolochic acids. An import alert also was issued to provide for the immediate detention
14 without physical examination of any botanical dietary ingredients that either are labeled
15 as *Aristolochia* or may be confused with it unless there is analytical evidence that the
16 product does not contain aristolochic acids. The consumer advisory urged consumers to
17 immediately discontinue use of any botanical products that contain or likely contain
18 aristolochic acids (FDA 2000, 2001a, 2001c).

19 Under 21 CFR Part 111 (Current Good Manufacturing Practice in Manufacturing,
20 Packaging, Labeling, or Holding Operations for Dietary Supplements), FDA requires
21 manufacturers to establish and meet specifications for the identity, purity, strength, and
22 composition of dietary supplements and for limits on contamination for dietary
23 supplements that they manufacture. Because of the critical importance of ensuring the
24 proper identity of dietary ingredients, the FDA also requires each firm that uses a dietary
25 ingredient to perform its own testing or examination for identity of each dietary
26 ingredient prior to use. The FDA has established a procedure that allows for submission
27 to, and review by, FDA of an alternative to the required 100 percent identity testing of
28 components that are dietary ingredients, provided certain conditions are met. The FDA
29 has provided information to assist in the selection of the most appropriate and reliable

1 identity test and the general principles for consideration in setting performance standards
2 for such tests.

3 2.5.2 Other countries

4 The United Kingdom banned the use of herbs that contain aristolochic acids in 1999.
5 Canada, Germany, and Australia have also banned use of these herbs (Kessler 2000).

6 Zhu (2002) noted that because of the reports of nephropathy due to *Aristolochia*
7 *manshuriensis* in China, the 2000 Chinese Pharmacopoeia for the first time listed guan
8 mu tong as toxic, and future editions are expected to reinstate *Akebia* species as the
9 official source of mu tong.

10 2.6 Summary

11 The risk of human exposure to aristolochic acids remains a global problem. Native
12 *Aristolochia spp.* have been used as herbal remedies for millennia in virtually every
13 country throughout the world, including Europe, Asia, Africa, and North and South
14 America. Many of these plants are still used in herbal medicines today even though their
15 use has been restricted or banned in the United States and other countries. Individuals
16 may potentially be exposed to aristolochic acids by ingesting plants and botanical
17 products made from plants that contain these compounds or by ingesting herbal products
18 contaminated with aristolochic acids. Between 1,500 and 2,000 people were exposed to
19 aristolochic acids at a weight-loss clinic in Belgium from May 1990 to October 1992.
20 Exposure to aristolochic acids has also been reported in other countries, including the
21 United States; two cases of renal failure in the United States were linked to ingestion of
22 herbal products containing aristolochic acids. The use of botanical products in the United
23 States has increased dramatically since the early 1990s, with 10% of adults in the United
24 States reportedly ingesting herbal medicines in 1999 and a total of \$4.2 billion spent on
25 herbs and other botanical remedies in 2001. More than 100 suppliers of botanical
26 products that potentially contain aristolochic acids have been identified in recent years. In
27 2001, the FDA issued warnings to consumers, health care professionals, and industry
28 associations concerning herbal products containing aristolochic acids. Other countries,
29 including the United Kingdom, Germany, Canada, and Australia, have banned these

- 1 herbs. Nevertheless, botanical products potentially containing aristolochic acids are still
- 2 available legally in other countries and can be bought via the Internet.

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3 Human Cancer Studies

Several *Aristolochia* species and other related plant species (such as *Asarum* species) containing aristolochic acids have been used in traditional herbal medicines to treat various conditions such as edema, urinary infections, inflammation, and pain (see Section 2.1). The inadvertent use of *Aristolochia* in weight-loss preparations in Belgium has been responsible for much of the exposure discussed in this section. An IARC working group convened in 2002 to evaluate some traditional herbal medicines concluded that there was (1) sufficient evidence in humans for the carcinogenicity of herbal remedies containing plant species of the genus *Aristolochia* and (2) limited evidence in humans for the carcinogenicity of naturally occurring mixtures of aristolochic acids. The IARC review was based largely on two case-series reports that found a high percentage of urothelial cancer in women suffering from Chinese herb nephropathy (CHN or herbal medicine nephropathy) and undergoing prophylactic nephroureterectomy because of end-stage renal failure.

Three main terms have been used in the literature to designate the renal disease due to consumption of herbs. These are CHN, aristolochic acid nephropathy (AAN), and phytotherapy-associated interstitial nephritis (PAIN). CHN is a general term that has been applied to all cases with a progressive interstitial renal fibrosis caused by consumption of Chinese herbs irrespective of the content of aristolochic acids and includes patients with AAN and PAIN. The identification of aristolochic acids as the cause of the renal disease led to the introduction of AAN to describe those cases in which the herbs are proven to contain aristolochic acid. PAIN has been used more recently to describe cases similar to CHN but without documentation of aristolochic intake (that is consumption of herbs known or proven to contain aristolochic acid) (Gillerot *et al.* 2001, Solez *et al.* 2001, Cosyns 2002a). Use of the term PAIN to describe these cases avoids the possible prejudicial use of CHN, which could imply that Chinese herbs in general cause renal impairment. The term PAIN is not yet widely used in the literature; therefore, this document will generally use the term, “herbal medicine nephropathy,” when AAN is not appropriate because exposure to aristolochic acids had not been confirmed.

1 The available literature consists of case reports, prevalence studies, and clinical studies
2 among individuals with kidney disease. Because the cancer studies involved patients with
3 herbal medicine nephropathy or AAN, the overall findings of case reports evaluating the
4 relationship between this disease and consumption of herbal remedies containing
5 aristolochic acids are summarized briefly in Section 3.1. Case reports and the prevalence
6 studies on urothelial tumors are described in Section 3.2. Clinical studies evaluating the
7 prevalence or incidence of urothelial cancer among kidney-transplant or dialysis patients
8 who consumed Chinese herbs are described in Section 3.3. Balkan endemic nephropathy
9 and its association with urothelial cancer are described briefly in Section 3.4 because of a
10 possible relationship with aristolochic acids. Section 3.5 discusses issues important to the
11 evaluation of the human studies on botanical products containing aristolochic acids, and
12 Section 3.6 summarizes the findings.

13 **3.1 Studies on herbal medicine nephropathy or AAN**

14 Case reports of herbal medicine nephropathy or AAN that have been associated with
15 consumption of herbs containing aristolochic acids are summarized in Table 3-1.

16 *3.1.1 Belgian epidemic*

17 Herbal medicine nephropathy or CHN was first reported in Belgian women who had
18 consumed Chinese herbs as part of a weight-loss regimen. Vanherweghem *et al.* (1993)
19 reported 2 cases of a rapidly progressive interstitial renal fibrosis occurring in 2 women
20 less than 50 years old who had followed the same weight-loss regimen prescribed at the
21 same Brussels-area clinic shortly before their diseases were diagnosed. Although the
22 incidence of chronic interstitial nephritis is high in Belgium, it is generally associated
23 with high intake of analgesics, and there is usually a 10- to 20-year gap between onset of
24 disease and renal failure. Because of the unique characteristics of these 2 cases and
25 because the women had normal renal function before starting the weight-loss regimen,
26 the authors conducted an epidemiological survey of women under 50 who were treated at
27 the seven principal dialysis units in Brussels from 1989 to 1992. Seven additional women
28 under age 50 were identified who had a diagnosis of interstitial nephritis and had
29 followed a weight-loss regimen from the same clinic as the 2 index cases. In 1990, the
30 clinic had changed the weight-loss regimen to include powders from *Stephania tetrandra*

1 and *Magnolia officinalis*. The Chinese name for *S. tetrandra* is “fang ji,” which is similar
2 to the name for *Aristolochia fangchi* (“guang fang ji”) (see Section 2.4.3 and Table 2-4).
3 A subsequent publication showed that most of the herb powders delivered to the Belgian
4 clinic under the name *S. tetrandra* from 1990 to 1992 contained aristolochic acids but not
5 tetrandrine, a compound expected to be present in a preparation made from *S. tetrandra*,
6 suggesting that *A. fangchi* was used in place of *S. tetrandra* (Vanhaelen *et al.* 1994).

7 Aristolochic acids are known nephrotoxic agents that cause acute renal failure and tubular
8 lesions in experimental animals and humans (as reviewed by Cosyns 2003).

9 Arlt *et al.* (2002b) reviewed case reports of renal disease and cancer and consumption of
10 aristolochic acids. They reported that 86 patients with herbal medicine nephropathy had
11 been treated at the Hospital Erasme, in Brussels (reported in publications mainly by
12 Vanherweghem and colleagues), and 18 patients with herbal medicine nephropathy had
13 been treated at the Cliniques Universitaires St.-Luc, in Brussels (reported mainly by
14 Cosyns and colleagues). All of the patients had taken a Chinese herbal remedy,
15 prescribed for weight loss, which contained *A. fangchi*, and all but one of the patients
16 were women. A number of studies published by Cosyns and coworkers or
17 Vanherweghem and coworkers have (1) detected aristolochic acids in the preparations
18 used by the patients, (2) detected aristolochic acid–DNA (AA-DNA) adducts in renal and
19 urothelial tissues from the herbal medicine nephropathy patients (Bieler *et al.* 1997,
20 Schmeiser *et al.* 1996) (in all 38 samples from Hospital Erasme and 8 from the Cliniques
21 St.-Luc) (Arlt *et al.* 2002b), (3) reported a significant correlation between the cumulative
22 consumption of *A. fangchi* (substituted for *S. tetrandra*) and renal-failure progression rate
23 (Martinez *et al.* 2002), and (4) reported correlations of the rate of renal-failure
24 progression with the duration of Chinese herb treatment and with the interval between
25 withdrawal of treatment and diagnosis of disease (Reginster *et al.* 1997). Based on these
26 studies, as well as studies in other countries (see below), it has been proposed that CHN
27 be renamed aristolochic acid nephropathy (AAN) (Arlt *et al.* 2002b).

28 Vanherweghem (1998) estimated that about 5% of the exposed population (*i.e.*, patients
29 taking the weight-loss regimen from May 1990 to October 1992) developed renal disease.
30 The mean average exposure per patient was about 900 mg of powder per day for 6 to 12

1 months. Reasons for the relatively low prevalence of renal disease may be batch-to-batch
2 variation in the amount of aristolochic acids in the herbal remedies, variation in genetic
3 (*e.g.*, metabolic enzymes) or gender susceptibility to the toxin, variation in compliance
4 with the weight-loss regimen, or variation in and possible synergy with the other agents
5 in the Chinese herbal medicines (Meyer *et al.* 2000, Chang *et al.* 2001).

6 3.1.2 Worldwide cases of herbal medicine nephropathy or AAN

7 The Arlt *et al.* (2002b) review reported that more than 170 cases of AAN had been
8 identified outside Belgium, and additional cases reports of AAN have been published
9 since that review. As of 2004, 11 additional cases had occurred in Europe outside of
10 Belgium (Arlt *et al.* 2004b). In addition, a case in Belgium not related to the weight-loss
11 regimen epidemic has been reported (Vanherweghem *et al.* 1998). Cases of AAN or
12 herbal medicine nephropathy have been reported from France (Arlt *et al.* 2002b, 2004b)¹,
13 Germany (Krumme *et al.* 2001), Spain (Pena *et al.* 1996), the United Kingdom (Lord *et*
14 *al.* 1999, 2001, Cronin *et al.* 2002), the United States (Meyer *et al.* 2000), China or Hong
15 Kong (Gillerot *et al.* 2001, Arlt *et al.* 2002b, Lo *et al.* 2004, Lo *et al.* 2005)², Japan
16 (Izumotani *et al.* 1993, Ubara *et al.* 1999, Tanaka *et al.* 2001, Arlt *et al.* 2002b)³, Korea
17 (Lee *et al.* 2004), and Taiwan (Yang *et al.* 2000, Chang *et al.* 2001, Yang *et al.* 2002b,
18 Tsai *et al.* 2005, Hong *et al.* 2006, Yang *et al.* 2006). In contrast with the Belgian cases,
19 cases in other countries have involved use of the Chinese herbs containing aristolochic
20 acids for many different purposes, including weight loss, nutritional supplementation,
21 health promotion, and treatment of a variety of diseases or conditions (see Table 3-1).
22 [The cases discussed below and summarized in Table 3-1 are limited to those that were
23 either published in English or published in another language but included in a review
24 published in English.]

25 Aristolochic acids (usually aristolochic acids I and II) were identified in most of the
26 herbal preparations used by these patients, and AA-DNA adducts were identified in the
27 patient's tissue in a few of the studies. The aristolochic acids-containing herbs that were

¹Arlt *et al.* 2002b cited the following publications in French: Pourrat *et al.* (1994) and Stengel and Jones (1998).

²Arlt *et al.* 2002b also cited the following publications in Chinese: Chen *et al.* (2001) and Li *et al.* (2001).

³Arlt *et al.* 2002b also cited the following two publications in Japanese: Tanaka *et al.* (1997a,b).

1 described as present or potentially present in the herbal preparations used in these studies
2 included the following:

- 3 • *A. fangchi*– in fang chi (Lo *et al.* 2004) and boui (Izumotani *et al.* 1993,
4 Tanaka *et al.* 2001),
- 5 • *A. manshuriensis*– in mu tong (Lord *et al.* 1999, Li *et al.* 2001, Lord *et al.*
6 2001, Arlt *et al.* 2002b, Lo *et al.* 2004, Tsai *et al.* 2005), kan-mokutsu
7 (Nishimagi *et al.* 2001, Tanaka *et al.* 2001, Kazama *et al.* 2004), and
8 longdan xieganwan (Laing *et al.* 2006),
- 9 • *A. pistolochia*– in herbal tea (Arlt *et al.* 2002b),
- 10 • *A. mollissima*– in pak mo tang (Lo *et al.* 2005),
- 11 • *A. heterophylla*– in boui (Izumotani *et al.* 1993, Tanaka *et al.* 2001),
- 12 • *Asarum* spp. (wild ginger or xi xin)– in duhuo tisheng tang (Yang *et al.*
13 2006).

14 As with the cases in Belgium, name confusion (for example, between Japanese and
15 Chinese names) may also have resulted in the substitution of Chinese herbs containing
16 aristolochic acids in the herbal remedy (see Section 2.4.3 and Table 2-5). In some cases,
17 the herbs consumed were not reported, and in other cases, *Aristolochia*-related species
18 were not listed as ingredients, but aristolochic acids were detected in the herbal remedy.

19 The review of the worldwide case reports has suggested that AAN has two clinical
20 presentations. (See also Section 5.2.2 for a discussion of the time course of the acute and
21 chronic phases of experimental AAN in Wistar rats exposed to aristolochic acids by
22 subcutaneous injections [Pozdzik *et al.* 2007]). The first presentation, which has been
23 reported mainly in women from Belgium and other Western countries, is characterized by
24 severe interstitial fibrosis and subacute renal failure with anemia. The fact that most of
25 the cases have been reported in women may be due to the association of most of the
26 Belgian cases with a weight-loss clinic, which appears to have had a predominantly
27 female clientele; the rest of the European cases occurred equally in men and women.

28 The second presentation, which manifests itself as Fanconi syndrome, has been observed
29 in men and women and is more common in Asian countries (see Table 3-1); however,
30 Chen *et al.* (2001) reported 58 cases of AAN at a hospital in Beijing that were divided
31 into three types: (1) acute AAN (N = 4), (2) tubular dysfunction AAN, which included

1 Fanconi syndrome in some cases (N = 7), and (3) chronic AAN (N = 47). Fanconi
2 syndrome is characterized by proximal tubular dysfunction and slowly progressive renal
3 dysfunction reported to be reversible when exposure to aristolochic acids ceased (Lee *et*
4 *al.* 2004). Reported cases of Fanconi syndrome (Izumotani *et al.* (1993), Ubara *et al.*
5 (1999), Krumme *et al.* (2001), Lee *et al.* (2004) and Tsai *et al.* (2005)) improved when
6 exposure to herbal medicines containing aristolochic acids was interrupted; however, this
7 improvement was temporary for some patients even though they did not resume use of
8 the herbal medicine (Lee *et al.*). [It should be noted that an acute, limited phase of
9 intoxication will not necessarily be followed by a chronic phase, but only recovery from a
10 chronic phase could be interpreted as true reversibility of AAN.] Although this
11 presentation has mostly been reported from Asian countries, the case reported by
12 Krumme *et al.* (2001) was that of a Caucasian man in Germany. Hypokalemia with
13 paralysis has been reported in 2 AAN patients with Fanconi syndrome (Yang *et al.*
14 2002a, Tsai *et al.* 2005), and cases of AAN with Fanconi syndrome that rapidly
15 progressed to renal failure have been documented (Lee *et al.* 2004, Hong *et al.* 2006).
16 Almost all of the reported cases were in adults, but the case reported by Hong *et al.*
17 (2006) occurred in a 10-year-old boy. Reasons for the slower and possibly reversible
18 progression of symptoms have been the subject of speculation (Tanaka *et al.* 2000a,
19 Tanaka *et al.* 2001), but no data have been presented to explain the differences. Tsai *et al.*
20 (2005) stated that as of 2005, 24 cases of Fanconi syndrome secondary to AAN have
21 been reported, mostly following consumption of the herb *A. manshuriensis*. In contrast,
22 the Belgian cluster of cases followed consumption of *A. fangchi*.

Table 3-1. Case reports of herbal medicine nephropathy or aristolochic acid nephropathy (AAN)^a

Country (Institution)	Reference(s)	Use	Herbal product	AA detected	AA-DNA adducts	No. of cases	Characteristics of herbal medicine nephropathy /AAN
Belgian weight loss epidemic							
Belgium (Hospital Erasme)	Arlt <i>et al.</i> 2002b, Vanhaelen <i>et al.</i> 1994, Vanherweghem <i>et al.</i> 1993, Vanherweghem 1998	weight-loss	contained <i>A. fangchi</i>	I and II	+ in 38 of 38 patients analyzed	84	end-stage renal failure (N = 50), chronic renal failure (N = 28), deceased (N = 6) hypocellular, outer cortical interstitial fibrosis
Belgium (Cliniques Universitaires St.-Luc)	Arlt <i>et al.</i> 2002b, Bieler <i>et al.</i> 1997, Cosyns <i>et al.</i> 1994a, Schmeiser <i>et al.</i> 1996, Cosyns <i>et al.</i> 1999, Kanaan <i>et al.</i> 2003	weight-loss	contained <i>A. fangchi</i>	I and II	+ in 8 of 8 patients analyzed	18	end-stage renal failure (N = 16), chronic renal failure (N = 2) hypocellular, outer cortical interstitial fibrosis
Other cases from Western countries							
France	Stengel and Jones 1998 ^b , Arlt <i>et al.</i> 2004b, Pourrat <i>et al.</i> 1994 ^b	weight-loss	“Preparation Number 28”	+	+ in 2 of 2 patients analyzed ^c	4 ^d	end-stage renal failure hypocellular, outer cortical interstitial fibrosis
Germany	Krumme <i>et al.</i> 2001	hyperuricemia and prostatism	“Akebia 14”	I and II	NDT	1	Fanconi syndrome, reversible interstitial fibrosis
Spain	Pena <i>et al.</i> 1996	pain relief	<i>A. pistolochia</i> (taken as an infusion)	NDT	NDT	1	end-stage renal failure hypocellular interstitial fibrosis
Belgium	Vanherweghem <i>et al.</i> 1998	arthralgias	<i>Labelled as Stephania, but S. tetrandra not detected by chemical analysis</i>	I and II	NDT	1	rapidly progressive renal failure interstitial fibrosis

Country (Institution)	Reference(s)	Use	Herbal product	AA detected	AA-DNA adducts	No. of cases	Characteristics of herbal medicine nephropathy /AAN
United Kingdom	Lord <i>et al.</i> 1999	eczema	mu tong (<i>A. manshuriensis</i> or species of <i>akebia</i> or <i>clematis</i>) (taken as an infusion)	I and II	+	2	rapidly progressive renal failure interstitial fibrosis
United Kingdom	Cronin <i>et al.</i> 2002	hepatitis B	NR	I and II	NDT	1	renal failure and bone marrow suppression interstitial fibrosis
United States	Meyer <i>et al.</i> 2000	pain relief	NR	+	NDT	1	renal failure and bone marrow suppression interstitial fibrosis
United States	Grollman <i>et al.</i> 2007		Herbal remedy containing <i>Aristolochia</i>	NDT	+	1	end-stage renal failure
Cases from Asian countries							
China	Gillerot <i>et al.</i> 2001	health	various roots and leaves	I, II, and AR	+	1	rapidly progressive renal failure hypocellular interstitial fibrosis
China	Chen <i>et al.</i> 2001 ^b	Chinese traditional drugs	NR	+	NDT	58	chronic AAN with chronic renal failure (N = 47), acute AAN with acute renal failure (N = 4), Fanconi syndrome (N = 7) interstitial fibrosis
China	Li <i>et al.</i> 2001		mu tong (<i>A. manshuriensis</i>)	NDT	NDT	51	AAN (tubulointerstitial nephropathy) interstitial fibrosis

Country (Institution)	Reference(s)	Use	Herbal product	AA detected	AA-DNA adducts	No. of cases	Characteristics of herbal medicine nephropathy /AAN
Hong Kong	Lo <i>et al.</i> 2004	tonic herbal remedy	mu tong (<i>A. manshuriensis</i>) and fang chi (<i>A. fangchi</i>)	I and II	NDT	1	acute renal failure (recovery) with underlying focal segmental glomerulosclerosis interstitial fibrosis
Hong Kong	Lo <i>et al.</i> 2005	Crohn's disease	pak mo tang (<i>A. mollissima</i>)	I	+	1	end-stage renal failure hypocellular interstitial fibrosis
Hong Kong	Laing <i>et al.</i> 2006	"liver enhancement"	longdan xieganwan (<i>A. manshuriensis</i>)	NDT	NDT	1	end-stage renal failure interstitial fibrosis
Japan	Izumotani <i>et al.</i> 1993	obesity	boui (<i>A. fangchi</i> and <i>A. heterophylla</i>)	NDT	NDT	1	Fanconi syndrome, somewhat reversible ^e no interstitial fibrosis
Japan	Tanaka <i>et al.</i> 1997b ^b	Chinese herbal remedy	NR	+	NDT	1	NA
Japan	Ubara <i>et al.</i> 1999	health promotion	various roots ^f	+	NDT	1	Fanconi syndrome, partly reversible hypocellular interstitial fibrosis
Japan	Nishimagi <i>et al.</i> 2001	edema	kan-mokutsu (<i>A. manshuriensis</i>) ^g	I	NDT	1	progressive renal failure interstitial fibrosis
Japan	Tanaka <i>et al.</i> 2001 also described in Tanaka <i>et al.</i> 2000a, Tanaka <i>et al.</i> 1997a	coldness of extremities, atopic dermatitis, nephrotic syndrome	kan-mokutsu (<i>A. manshuriensis</i>) and boui (<i>A. fangchi</i> and/or <i>A. heterophylla</i>) ^g	I, II and D	NDT	13 ^h	Fanconi syndrome (N = 9) hypocellular, outer cortical interstitial fibrosis
Japan	Kazama <i>et al.</i> 2004	sterility	kan-mokutsu (<i>A. manshuriensis</i>)	NDT	NDT	1	Fanconi syndrome interstitial fibrosis
Korea	Lee <i>et al.</i> 2004	weight loss	NR	I and II	NDT	1	Fanconi syndrome and subsequent renal failure

Country (Institution)	Reference(s)	Use	Herbal product	AA detected	AA-DNA adducts	No. of cases	Characteristics of herbal medicine nephropathy /AAN
Taiwan	Yang <i>et al.</i> 2000	various purposes including weight-loss, nutritional supplement	NR	NDT	NDT	12	PAIN ⁱ most cases had rapid deterioration of renal function
Taiwan	Chang <i>et al.</i> 2001	nutritional supplement, weight loss, and treatment of non-renal disease	NR	NDT	NDT	20	PAIN ⁱ rapidly progressive renal failure
Taiwan	Yang <i>et al.</i> 2002a Yang <i>et al.</i> 2001	seizure and tonic encouragement leg edema	NR	I and II	NDT	2	1 st patient: subacute renal failure, interstitial fibrosis 2 nd patient: Fanconi syndrome with hypokalemic paralysis; hypocellular interstitial fibrosis
Taiwan	Tsai <i>et al.</i> 2005	leg edema	mu tong (<i>A. manshuriensis</i>)	I	NDT		Fanconi syndrome with hypokalemic paralysis, reversible No renal biopsy
Taiwan	Hong <i>et al.</i> 2006	health improvement	NR	I and II	NDT	1 ^j	Fanconi syndrome with progressive renal failure and anemia interstitial fibrosis
Taiwan	Yang <i>et al.</i> 2006	lower back pain or nausea	duhuo tisheng tang, which contains xi xin (wild ginger, <i>Asarum</i> spp)	I and II	NDT	1	progressive deterioration in renal function, not reversible interstitial fibrosis

AA = aristolochic acids; AAN = aristolochic acid nephropathy, AR = aristolactams I and II; D = aristolochic acid D; NA = not applicable; NDT = not determined; NR = not reported; + = positive result; PAIN = phytotherapy-associated interstitial nephritis.

^aOther cases may have been reported in the non-English literature, but the studies summarized here are limited to those reported in the English literature or reviewed in the English literature.

^bAs cited by Cosyns *et al.* (1999) and Arlt *et al.* (2002b) [the original publication was not in English and was not reviewed].

^c Pfohl-Leszkowicz *et al.* 2007 did not detect AA-DNA adducts from the two “positive” by Arlt *et al.* 2004b cases (see Sections 3.4 and 3.5.1).

^d Four is the total number of non-overlapping cases reported by Stengel and Jones 1998, Pourrat *et al.* 1994), and Arlt *et al.* 2004b.

^e Reversible after first hospital admission, but the patient resumed taking the drugs, and the condition improved but was not completely reversible after the second hospital admission.

^f Some ingredients were similar to those reported in other cases; none of the herbs were *Aristolochia* species.

^g Chinese medicines that contained kan-mokutsu included toki-shigyaku-ka-gosyuyu-syo-kyo-to, tenshin-toki-shigyaku-ka-gosyuyu-syokyo-to, and ryutan-shakan-to, and the medicine consumed that contained boui was boui-ougi-to.

^h Number of cases includes cases from references in the Japanese literature (N = 8) in addition to cases discussed in the report (N = 5); the 5 cases described in this report appear to include the same cases described by Tanaka *et al.* 1997a (N = 1) and Tanaka *et al.* 2000a (N = 2).

ⁱ AA has not been identified in the herbs consumed by the patients; however, they are included in the table because they were reported in the Arlt *et al.* 2002b review.

^j A 10-year-old boy.

1 **3.2 Urothelial cancer**

2 Cases of urothelial cancer have been reported among patients with AAN. Most of these
3 cases have occurred among the Belgian patients (Cosyns *et al.* 1994b, Vanherweghem *et*
4 *al.* 1995, Reginster *et al.* 1997, Kanaan *et al.* 2003, Nortier *et al.* 2003,), but a few cases
5 have also been reported in the United Kingdom (Lord *et al.* 2001, Laing *et al.* 2006),
6 Taiwan (Chang *et al.* 2001, Yang *et al.* 2000, 2001), France (Arlt *et al.* 2004b), and Hong
7 Kong (Lo *et al.* 2005). The case reports are summarized in Table 3-2.

8 *3.2.1 Case reports of urothelial cancer related to the Belgian epidemic*

9 Cosyns *et al.* (1994a) reported mild to moderate atypia and atypical hyperplasia of the
10 urothelium in two of three women (aged 27 to 32) with severe renal failure resulting from
11 ingestion of weight-loss pills containing Chinese herbs. One of these women
12 subsequently developed transitional-cell carcinoma (TCC) of the bladder (papillary, low-
13 grade, without evidence of invasion), ureters (microscopic, low- to intermediate-grade),
14 and renal pelvis (microscopic, low-grade) (Cosyns *et al.* 1994b). A subsequent
15 publication reported the presence of AA-DNA adducts in kidney tissue from these three
16 patients (Schmeiser *et al.* 1996).

17 Shortly after the Cosyns *et al.* (1994b) publication, another case of cancer, a papillary
18 TCC of the renal pelvis, occurred among the Belgian women with herbal medicine
19 nephropathy who had followed the weight-loss regimen (Vanherweghem *et al.* 1995).
20 The 42-year-old woman had also used analgesics, which are a risk factor for renal disease
21 and urothelial malignancies. The authors stated that they thought the timing of renal
22 disease correlated better with consumption of herbal products than analgesics, and that
23 the rapid progression and histological aspects were more typical of herbal medicine
24 nephropathy than of disease caused by analgesics.

25 Reginster *et al.* (1997) identified 2 cases of urothelial cancer in a retrospective analysis of
26 15 cases of women with herbal medicine nephropathy (aged 27 to 59) who were followed
27 between 1991 and 1995. The purpose of the study was to compare the clinical pattern and
28 progression of renal function in herbal medicine nephropathy patients with that in
29 patients with interstitial nephropathies of other origins. The authors reported that one
30 woman had a papillary TCC of the urinary bladder and microinvasive urothelial

1 carcinoma of the ureter; she later developed two more papillary bladder tumors. This
2 patient is the same one whose case was reported by Cosyns *et al.* (1994b), as described
3 above. The other woman had *in situ* urothelial carcinoma of the ureter, and her case is
4 one of the cases reported in the prevalence study by Cosyns *et al.* (1999).

5 Kanaan *et al.* (2003) reported that a 53-year-old woman presenting with severe renal
6 failure developed a non-invasive papillary TCC of the urinary bladder. The patient
7 reported attending the Belgian weight-loss clinic before the addition of *A. fangchi*
8 (substituted for *S. tetrandra*) to the weight-loss regimen; however, pathological
9 examination of the kidneys showed lesions typical of AAN, and AA-DNA adducts were
10 detected in the right kidney. (This patient is one of the 7 cases, identified as of 2002, with
11 urothelial cancer from the Cliniques Universitaires St.-Luc treatment center, but is not
12 one of the 4 cases included in the prevalence study described below.)

13 All of the cases reported above were in patients with severe renal failure. However,
14 Nortier *et al.* (2003) reported a case of invasive carcinoma of the ureter in a 69-year-old
15 woman that developed without severe renal failure. The woman presented with
16 pyelonephritis [kidney infection] associated with hydronephrosis [inability of urine to
17 drain from the kidneys] and with elevated serum creatinine levels. She had taken the
18 Belgian weight-loss regimen containing *A. fangchi*, at an estimated cumulative dose of
19 189 g [it was not clear whether the cumulative dose referred to the weight-loss regimen
20 as a whole or just to the *A. fangchi*] between 1991 and 1992 and had not been exposed to
21 well-known nephrotoxic agents; however, she was an active smoker. AA-DNA adducts
22 were detected in postmortem tissues from the kidney, liver, pancreas, and lymph nodes,
23 with the highest levels occurring in the kidney (81 ± 22 per 10^9 nucleotides). Smoking-
24 related adducts were detected in the lung tissue.

25 3.2.2 Prevalence studies in the Belgian cases with herbal medicine nephropathy or 26 AAN

27 Two case-series studies (one from each of the two major treatment centers in Brussels)
28 determined the prevalence of urothelial cancer among Belgian women who had renal
29 transplants as a result of herbal medicine nephropathy. The case series associated with the
30 Cliniques Universitaires St.-Luc studied 10 patients who had received renal transplants

1 from September 1992 through August 1998 (Cosyns *et al.* 1999). These patients
2 underwent recommended nephroureterectomies during or after renal transplantation
3 because of reported cases of urothelial cancer (described above). These women had
4 followed a weight-loss regimen, prescribed at the same clinic between 1990 and 1992, for
5 an average of 20 months, and were subsequently diagnosed with CHN (herbal medicine
6 nephropathy). Renal transplantation occurred 9 to 67 months (average 34 months) after
7 the weight-loss regimen was discontinued. AA-DNA adducts had previously been
8 detected in the kidneys of 6 of the patients and were described in another publication [the
9 study evaluated only 6 patients] (Bieler *et al.* 1997). Histologic analysis was performed
10 on 19 native kidneys and ureters. High-grade TCC *in situ* of the urinary tract was found
11 in 7 samples from 4 of 10 [40%] patients (aged 27, 42, 41, and 59). One of the patients
12 had invasive TCC of the ureter and noninvasive papillary TCC. [This is the same case
13 that was reported by Cosyns *et al.* [1994b] and described in Section 3.2.1.] The urothelial
14 lesions were located in the renal pelvis (3 patients), upper ureter (4 patients), midureter (1
15 patient), and lower ureter (3 patients). All 10 patients had moderate atypia of the
16 medullary collecting ducts, renal pelvis, and ureter. Tumor suppressor protein p53 was
17 overexpressed in the pelviureteric urothelium in all patients. The authors stated that the
18 observed prevalence of urothelial cancer (40%) was greater than would be predicted on
19 clinical grounds (13%). The authors excluded smoking and the immunosuppressive
20 regimen as potential causes of cancer, because only 1 of the 4 patients with cancer was a
21 smoker, compared with 5 of the 6 patients without cancer, and because the duration of
22 immunosuppression was identical between patients who developed cancer and those who
23 did not. Arlt *et al.* (2002b) stated that the number of cases of urothelial carcinoma had
24 risen to 7 as of January 2002.

25 Nortier *et al.* (2000) and Nortier and Vanherweghem (2002) reported on the prevalence
26 of urothelial carcinoma among patients at the Hospital Erasme. At the time of their study,
27 105 patients with herbal medicine nephropathy had been treated at this center, of whom
28 43 had reached end-stage renal failure. Because of the case reports of urothelial cancer
29 occurring in herbal medicine nephropathy patients, 39 of the patients with end-stage renal
30 failure agreed to undergo the recommended prophylactic removal of their nonfunctioning
31 kidneys and ureters. The diagnosis of CHN was based on consumption of the weight-loss

1 pill containing *A. fangchi* and rapidly progressive deterioration of renal function, which
2 was confirmed by histological findings. All of the patients had consumed the pills, with
3 an average of 13.3 months of consumption, and end-stage renal failure occurred 3 to 85
4 months after the patients had stopped taking the pills. Cumulative doses (mean ingested
5 dose) of all the components in the pills were calculated for each patient from
6 prescriptions obtained from pharmacists. The intended components in the pills included
7 *S. tetrandra* (actually *A. fangchi*), *M. officinalis*, acetazolamide, fenfluramine (an appetite
8 suppressant), and diethylpropion [an appetite suppressant]. In addition, each patient was
9 interviewed for smoking status and the use of analgesics, nonsteroidal anti-inflammatory
10 drugs, and mesotherapy (injections of artichoke extracts or theophylline).

11 Urothelial cancer was found in 18 of the 39 patients (prevalence = 46%, 95% confidence
12 interval [CI] = 29% to 62%), and 77 kidneys and 78 ureters were available for histologic
13 evaluation (Nortier *et al.* 2000). One patient had a papillary bladder tumor, and the other
14 17 patients had carcinoma of the ureter, renal pelvis, or both. Mild to moderate urothelial
15 atypia was found in 19 of the 21 patients without urothelial cancer. AA-DNA adducts
16 were detected in the kidneys of the patients with herbal medicine nephropathy (samples
17 were available from 38 of the 39 patients, and total adduct levels ranged from 1.7 to 175
18 per 10⁹ nucleotides) but not in 8 patients (controls) with end-stage renal failure unrelated
19 to herbal medicine nephropathy. Adduct levels did not differ between the patients with
20 and without urothelial cancer. Tissue samples from 25 kidney specimens with a diagnosis
21 of neoplasia (12 specimens), dysplasia (7 specimens), or no abnormalities (6 specimens)
22 were also analyzed for adducts of the mycotoxin ochratoxin A (OTA) with DNA. Low
23 levels of OTA-related DNA adducts (1.3 to 6.8 per 10⁹ nucleotides) were detected in
24 tissue from 2 of 12 patients with cancer and 2 of 7 with dysplasia; no adducts were
25 detected in the control patients. The cumulative doses of *A. fangchi*, *M. officinalis*, and
26 acetazolamide were significantly higher in patients with urothelial cancer than in patients
27 without cancer; these compounds were almost always prescribed together. The
28 prevalence of urothelial cancer was significantly higher ($P = 0.05$) in the 15 patients who
29 received a total dose of *A. fangchi* greater than 201 g (10 cases) than in the 24 patients
30 who ingested less than 200 g (8 cases). Patients with and without urothelial cancer did not

1 differ significantly with respect to smoking status or the use of mesotherapy, nonsteroidal
2 anti-inflammatory drugs, or analgesics.

3 Lemy *et al.* (2008) reported on the 15-year follow-up of patients from the Hospital
4 Erasme. The subjects were selected from a cohort of 112 [6 more than reported by
5 Nortier and colleagues in the 2000 and 2002 publications] patients with AAN who were
6 seen at the Hospital Erasme from 1992 to 2007; 54 patients (11 more than the 2000 and
7 2002 publications) had developed end-stage renal disease. Patients [N = 38, which
8 included 32 of the patients reported in the 2000 and 2002 publications] were enrolled in
9 the follow-up study if they had (1) a functional kidney transplant, (2) surgical removal of
10 their kidneys and ureters and biopsy of the bladder, and (3) a history of regular
11 cystoscopies. [Seven patients from the previous study were not included in the updated
12 study because they either died before kidney transplantation or did not agree to regular
13 cystoscopy of the bladder.] Upper-tract urothelial carcinoma was found in 17 AAN
14 patients; 12 of these patients developed cancer of the urinary bladder during follow-up.
15 Urinary bladder cancer was diagnosed 68 to 169 months after cessation of aristolochic
16 acids exposure. Similar to the earlier publications, the cumulative dose of *Aristolochia*
17 ingested by patients with AAN who developed upper-tract urothelial cancer (236 ± 90.8
18 g) was significantly higher than for AAN patients who did not develop cancer ($156 \pm$
19 70.3 g). No significant relationship was found between cumulative dose of *Aristolochia*
20 and development of bladder cancer.

21 3.2.3 Case reports of urothelial cancer outside Belgium

22 Case reports of urothelial cancer in patients with AAN or herbal medicine nephropathy
23 have also been reported in Taiwan, the United Kingdom, France, and Hong Kong. Two
24 studies in Taiwan have reported 3 cases of bladder TCC among a series of patients
25 undergoing renal biopsies because of unexplained renal failure. Yang *et al.* (2000)
26 detected 2 cases of cancer [1 case not tissue proven] among 12 patients undergoing
27 biopsies from 1995 to 1998, and Chang *et al.* (2001) detected 1 bladder carcinoma among
28 20 patients undergoing biopsies from 1994 to 1998. In both studies, the patients had taken
29 Chinese herbal regimens (plant extracts, pills, or powders) for a variety of reasons, and
30 their medical histories did not reveal any known cause for deterioration of renal function.

1 The pathological lesions and clinical features were similar to those observed in herbal
2 medicine nephropathy, and most of the patients had normal renal function before using
3 the herbal preparations. Aristolochic acids were not measured in the herbal regimen, and
4 the authors of the studies stated that they could not identify the etiologic agents. [These
5 studies are reviewed here because they were included in the reviews by IARC (2002)
6 and/or Arlt *et al.* (2002b)]. Another study in Taiwan reported papillary TCC in a 57-year-
7 old woman with subacute renal failure and severe anemia. Aristolochic acids I and II
8 were detected in the Chinese herbs that she had taken for “control of seizure and tonic
9 encouragement” (Yang *et al.* 2001).

10 Lord *et al.* (2001) reported invasive TCC in the renal pelvis and ureter of a 49-year-old
11 woman who had developed end-stage renal failure after taking an herbal remedy
12 containing aristolochic acid. [This case is 1 of 2 cases of AAN that were reported in an
13 earlier publication [Lord *et al.* 1999] and are summarized in Table 3-2.] AA-DNA
14 adducts were detected in both ureteral (40 per 10⁹ nucleotides) and renal tissues (3.8 per
15 10⁹ nucleotides). The authors stated that the woman did not have any confounding factors
16 at the time of presentation with AAN; she was a nonsmoker and was not taking any other
17 medicine.

18 A case of urothelial cancer was reported in a 34-year-old French woman who had taken
19 an herbal drug, “Preparation Number 28,” as part of a weight-loss regimen. The herbal
20 drug was later shown to contain aristolochic acids (Arlt *et al.* 2004b). The woman
21 developed rapidly progressive renal failure and died in 2000. Autopsy revealed extensive
22 and severe renal interstitial fibrosis suggestive of AAN and high-grade TCC in the right
23 urinary tract, with invasive liver metastases. Higher levels of AA-DNA adducts were
24 detected in lung, spleen, adrenal gland, liver, and ureter, and lower levels were detected
25 in urinary bladder, brain, and kidney [adduct levels for a second patient reported in the
26 same publication were low for one kidney and the highest reported in the study for the
27 other kidney] (see Section 5.3.1 for adduct levels in all tissues examined). DNA adducts
28 were also detected in the small intestine and stomach. The lower level of adducts in the
29 kidney compared with the ureter differs from the Belgian studies, which reported that
30 adducts were higher in renal than in ureteral tissue.

1 Lo *et al.* (2005) reported a case of TCC in a 60-year-old man from Hong Kong who had
2 consumed an herbal remedy (pak mo tang) containing *A. molissemiae* [*A. mollissima*] for
3 chronic Crohn's disease and recently diagnosed colon cancer. *A. mollissima* was thought
4 to have been inadvertently substituted for another herb at the level of the wholesaler. The
5 man developed nephropathy, characterized by hypocellular interstitial fibrosis, 2 months
6 after taking the herbal remedy and end-stage renal failure 12 months after taking the
7 remedy. The cumulative dose of *A. mollissima* at the time of end-stage renal failure was
8 800 g [compared with 190 g for *A. fangchi* previously reported by Martinez *et al.* 2002].
9 A bladder polyp histologically compatible with a diagnosis of TCC was detected by
10 cystoscopy. Aristolochic acid I was detected in the herbs, and AA-DNA adducts were
11 detected in the renal biopsy sample. The authors stated that they could not definitely
12 prove this was a case of AAN because the patient had also taken mesalazine, which also
13 causes interstitial nephritis, but the clinical pattern appeared to be more characteristic of
14 an aristolochic acid-induced nephropathy. This was the first suspected case of AAN
15 associated with consumption of *A. mollissima*.

16 Another case report from the United Kingdom described the occurrence of TCC in the
17 urinary bladder of a 30-year-old Chinese man who had consumed the Chinese herb
18 Longdan Xieganwan for at least 5 years to "enhance" his liver (Laing *et al.* 2006). The
19 authors reported that Longdan Xieganwan contained (prior to 2002) *A. manshuriensis*
20 root (*caulis*); however, the authors did not analyze the product for aristolochic acids or
21 the patient's tissues for AA-DNA adducts. The patient presented with symptoms of renal
22 toxicity, and renal biopsy showed that he had interstitial fibrosis consistent with CHN.
23 The patient progressed to end-stage renal failure after the diagnosis of bladder cancer.
24 This case was reported after aristolochic acids had been banned in several countries.

Table 3-2. Case reports of urothelial cancer

Reference	Exposure	Study population or description of patients	Number (prevalence) of cancer cases	Effects: Tumor location and characterization or other effects	Comments
Case reports in Belgium					
Cosyns <i>et al.</i> 1994b	Consumption of weight-loss agent containing <i>A. fangchi</i> AA-DNA adducts detected in the kidney Schmeiser <i>et al.</i> 1996	27-yr-old woman with severe renal failure	1	papillary TCC of bladder and microscopic TCC of renal pelvis and ureters	First patient identified with AAN in the Belgian epidemic who developed urothelial cancer Tumor detected as a result of nephroureterectomy performed at the time of renal transplantation
Vanherweghem <i>et al.</i> 1995	Consumption of weight-loss agent containing <i>A. fangchi</i>	42-yr-old woman with rapidly progressive renal failure	1	papillary TCC of renal pelvis and multifocal <i>in situ</i> TCC of adjacent urothelial epithelium	Patient also used analgesics
Reginster <i>et al.</i> 1997	Consumption of weight-loss agent containing <i>A. fangchi</i> AA-DNA adducts were detected in the renal tissues of 5 patients (Schmeiser <i>et al.</i> 1996)	15 women aged 27–59 with CHN ^a	2	1st patient: papillary TCC of bladder and microinvasive urothelial carcinoma of ureter 2nd patient: <i>in situ</i> carcinoma of ureter	Patients not screened for tumors; tumors detected as a result of nephroureterectomies performed at the time of kidney transplants (performed on 5 patients)
Kanaan <i>et al.</i> 2003	Probably consumption of weight-loss agent AA-DNA adducts detected in the right kidney (patient 8, Table 1 in Arlt <i>et al.</i> 2001b)	53-year-old woman	1	TCC <i>in situ</i> of ureter Papillary TCC of bladder	This patient is one of the 7 cases of urothelial cancer identified from the 18 AAN patients treated at the Cliniques St. Luc (reviews by Arlt <i>et al.</i> 2002b)
Nortier <i>et al.</i> 2003	Consumption of weight-loss agent containing <i>A. fangchi</i> AA-DNA adducts detected in kidney, liver, pancreas and lymph nodes	69-yr-old woman	1	poorly differentiated tumor in left ureter with invasion of adjacent adipose tissue and lymph nodes	First cancer case reported from patient without severe renal failure Woman was an active smoker
Belgian prevalence studies					

Reference	Exposure	Study population or description of patients	Number (prevalence) of cancer cases	Effects: Tumor location and characterization or other effects	Comments																		
Cosyns <i>et al.</i> 1999 Cliniques Universitaires St.-Luc	Consumption of weight-loss agent containing <i>A. fangchi</i> ; average duration 20 mo AA-DNA adducts detected in tissues of subset (7/10) of patients	10 women aged 27–59 who received renal transplants 1992–98 and underwent nephroureterectomies	4 (40%)	Tumor types included 1 papillary bladder TCC, 1 invasive TCC of ureter, and TCC <i>in situ</i> of the renal pelvis and ureter	Includes patient described by Cosyns <i>et al.</i> 1994a and Reginster <i>et al.</i> 1997 Arlt <i>et al.</i> reported that as of 2002, 7 patients with urothelial cancer had been identified at this hospital 1 of 4 patients with cancer was a smoker, compared with 5 of 6 patients without cancer Most tumors detected as a result of nephroureterectomies performed at the time of renal transplant																		
Nortier <i>et al.</i> 2000, Nortier and Vanherweghem 2002 Hospital Erasme	Consumption of weight-loss agent containing <i>A. fangchi</i> ; average duration 13 mo AA-DNA adducts detected in kidneys of all available samples (N = 38)	Cohort: 105 patients with AAN 39 women (mean age 54) with end-stage renal failure (total = 43) and who underwent nephroureterectomies	18 (46%) 95% CI = 29–62	<i>Tumor description</i> 1 urinary bladder tumor; the rest of the tumors in renal pelvis and ureter <i>Other effects</i> comparison of cumulative dose of herbal remedy in patients with and without cancer <table border="0"> <thead> <tr> <th><u>Ingredient</u></th> <th><u>P-value</u></th> </tr> </thead> <tbody> <tr> <td><i>A. fangchi</i></td> <td>0.035</td> </tr> <tr> <td><i>M. officinalis</i></td> <td>0.026</td> </tr> <tr> <td>acetazolamide</td> <td>0.012</td> </tr> <tr> <td>fenfluramine</td> <td>0.130</td> </tr> <tr> <td>diethylpropion</td> <td>0.200</td> </tr> </tbody> </table> prevalence of urothelial cancer vs. total dose of <i>A. fangchi</i> <table border="0"> <thead> <tr> <th><u>Dose</u></th> <th><u>Prevalence</u></th> </tr> </thead> <tbody> <tr> <td>> 201 g</td> <td>66.7% (10/15)</td> </tr> <tr> <td>< 200 g</td> <td>33.3% (8/24)*</td> </tr> </tbody> </table>	<u>Ingredient</u>	<u>P-value</u>	<i>A. fangchi</i>	0.035	<i>M. officinalis</i>	0.026	acetazolamide	0.012	fenfluramine	0.130	diethylpropion	0.200	<u>Dose</u>	<u>Prevalence</u>	> 201 g	66.7% (10/15)	< 200 g	33.3% (8/24)*	Women also interviewed for smoking status and use of analgesics, nonsteroidal anti-inflammatory drugs, and mesotherapy; no significant difference was found in the use of these agents or smoking status between the patients with and without urothelial cancer Most tumors detected as a result of nephroureterectomies performed at the time of renal transplant Weight-reducing pills could contain <i>A. fangchi</i> (substituted for <i>S. tetrandra</i>), <i>M. officinalis</i> , acetazolamide, fenfluramine, and diethylpropion, the first three of which were almost always prescribed together
<u>Ingredient</u>	<u>P-value</u>																						
<i>A. fangchi</i>	0.035																						
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< 200 g	33.3% (8/24)*																						

Reference	Exposure	Study population or description of patients	Number (prevalence) of cancer cases	Effects: Tumor location and characterization or other effects	Comments
Lemy <i>et al.</i> 2008 Update (15 year follow-up after exposure) of Nortier and colleagues Hospital Erasme	Consumption of weight-loss agent containing <i>A. fangchi</i> ; average duration 13.3 mo AA-DNA adducts detected in kidneys of all available samples (N = 37)	Cohort: 112 AAN patients 38 women (of 54 with end-stage renal disease) receiving kidney transplants and followed for bladder cystoscopies	17 UCC (44.7%) 15 bladder cases (39.5% incidence)	<i>Tumor description</i> 17 cases of upper tract urothelial cancer (renal or ureter) Follow-up: 15 cases developed bladder cancer <i>Other effects</i> Mean cumulative dose (g) of ingested <i>Aristolochia</i> in AAA patients with and without cancer UCC 236 ± 90.8 No UCC 156 ± 70.3** Bladder UC 215 ± 90.3 No Bladder UC 177 ± 86.2	Includes 32 of the 39 patients reported by Nortier and colleagues. Seven of those patients were not included in this study because they either died before kidney transplation or chose not to have regular bladder follow-up. Seven patients from the previous study were not included in the updated study because they either died before kidney transplantation or did not agree to regular bladder follow-ups.
Case reports outside of Belgium					
Yang <i>et al.</i> 2000 ^a Taiwan	Consumption of Chinese herbal regimens [Herbal products and tissues not analyzed for AA or AA-DNA adducts ^a]	12 patients with CHN undergoing biopsies 1995–98 aged 28–67, mean = 46.6 11 women and 1 man cancer detected in 2 women, aged 51 and 34	2	TCC of the bladder; 1 case not tissue proven	Same study described in Table 3-1 Cancer detected after renal biopsy

Reference	Exposure	Study population or description of patients	Number (prevalence) of cancer cases	Effects: Tumor location and characterization or other effects	Comments
Chang <i>et al.</i> 2001 Taiwan	Consumption of Chinese herbal regimens [Herbal products and tissues not analyzed for AA or AA-DNA adducts ^a]	20 patients undergoing biopsies 1994–98 aged 32–57, mean = 44.3 14 women and 6 men cancer detected in 50-yr-old man	1	TCC of the urinary bladder	Same study described in Table 3-1 Cancer detected in patient with hepatitis C Cancer detected after renal biopsy
Yang <i>et al.</i> 2001 Taiwan	Consumption of Chinese herb AA I and II detected in herb product	57-yr-old woman with sub-acute renal failure	1	papillary TCC of the ureter	Same study described in Table 3-1 Cancer detected after nephroureterectomies
Lord <i>et al.</i> 2001 United Kingdom	Consumption of herbal remedy containing mu tong (<i>A. manshuriensis</i> or <i>Akebia</i> or <i>Clematis</i> spp.) AA adducts detected in ureteric and renal cancer	49-yr-old woman with end-stage renal failure	1	invasive TCC of renal pelvis and ureter	One of two AAN cases reported by Lord <i>et al.</i> 1999 (see Table 3-1)
Arlt <i>et al.</i> 2004b France	Consumption of “Preparation Number 28” AA detected in herbal remedy AA-DNA adducts detected in ureter, kidney, and tissues outside urinary tract ^b	34-yr-old woman with CHN	1	TCC of right urinary tract with invasive liver metastases	Further follow-up of 1 of the 2 CHN cases reported by Stengel and Jones 1998
Lo <i>et al.</i> 2005 Hong Kong	Consumption of pak mo tang (<i>A. mollissima</i>) AA I detected in herbal remedy AA-DNA adducts detected in renal biopsy tissue	60-yr-old man with end-stage renal failure	1	bladder polyp compatible with TCC	Same case as in Table 3-1 Patient also consumed mesalazine, which can cause interstitial nephritis (less than 1 in 500)

Reference	Exposure	Study population or description of patients	Number (prevalence) of cancer cases	Effects: Tumor location and characterization or other effects	Comments
Laing <i>et al.</i> 2006 United Kingdom	Consumption of <i>Longdan Xieganwan</i> (<i>A. manshuriensis</i> root [<i>caulis</i>]; [however, herbal product not analyzed for AA]) [Tissues not analyzed for AA-DNA adducts]	30-yr-old man with renal toxicity that developed into end-stage renal failure after cancer diagnosis.		TCC of the bladder	Same study described in Table 3-1

AA= aristolochic acid; CHN = Chinese herb nephropathy; CI = confidence interval, TCC = transitional cell carcinoma.

* $P = 0.05$, ** $P < 0.01$.

^a This study was included in the table because it was reviewed by Arlt *et al.* 2002b and/or IARC 2000.

^b Pfohl-Leskowicz *et al.* 2007 did not detect AA-DNA adducts in this patient, but did detect ochratoxin A-related DNA adducts (see Section 3.5.1 and 5.3.1).

1 **3.3 Clinical studies of urothelial cancer and consumption of Chinese herbs**

2 Four clinical studies that reported on the incidence or prevalence of TCC among renal-
3 transplant or dialysis patients who had consumed Chinese herbs were identified. Two of
4 the studies were specific for herbs containing aristolochic acids

5 Wu *et al.* (2004c) conducted a retrospective analysis of 730 kidney-transplant patients
6 (432 men and 298 women) who were followed at a hospital in central Taiwan from 1983
7 to 2003. The prevalence of TCC is high in Taiwan, especially in the endemic areas of
8 black foot disease in southern Taiwan, which is partially explained by arsenic
9 contamination of underground water. Medical records, clinical records, and outcomes
10 were reviewed retrospectively, and the mean follow-up was 72.2 ± 54.4 months. Cancer
11 developed in 63 [8.6%] of the patients, of whom 30 [4.1%] had TCC of the urinary tract.
12 The standardized mortality ratio (SMR) for TCC was 3.98 (95% CI = 2.69 to 5.70) and
13 was higher in women (SMR = 8.76, 95% CI = 5.27 to 13.66, 19 deaths) than men (SMR
14 = 1.92, 95% CI = 0.96 to 3.45, 11 cases). Multivariate analyses with the Cox proportional
15 hazard model were used to evaluate potential risk factors for TCC. A significant risk ($P <$
16 0.01) was found for Chinese herb use (relative hazard [RH] = 5.2). Significant relative
17 hazards ($P < 0.05$) were also found for age at the time of kidney transplant (RH = 1.1),
18 female sex (RH = 2.9), use of analgesics (RH = 2.6), and intake of underground water
19 (RH = 2.5). The authors stated that limitations of exposure assessment (lack of
20 information on the types of Chinese herbs consumed and retrospective collection of data)
21 prevented them from directly confirming an association between aristolochic acids and
22 TCC.

23 Another study in Taiwan reviewed the records of 1,537 chronic dialysis patients who
24 were followed from 1993 to 2002 (cumulative period of observation 5,337 patients-years)
25 (Chang *et al.* 2007a). The standardized incidence ratio (SIR) was calculated using sex-
26 specific and age-specific incidence rates from the Cancer Registry Annual Report in
27 Taiwan. Potential risk factors were evaluated using the Cox proportional hazard model.
28 The incidence of TCC among these patients was 1.69%, which was significantly higher
29 than expected from the Taiwanese population (SIR = 48.2, 95% CI = 32.8 to 70.9, 26
30 cases). TCC of the upper urinary tract was found in 14 patients. Of the 26 cases of TCC,

1 10 cases reported use of Chinese herbs, and 2 cases were diagnosed with CHN (as
2 confirmed by biopsy). The relative hazard ratio for Chinese herb use was 6.21 ($P < 0.01$).
3 The authors stated that Taiwan has the second-highest prevalence rate of end-stage renal
4 disease in the world, and that TCC is the most common carcinoma in Taiwanese dialysis
5 and kidney-transplantation patients.

6 Li *et al.* (2005b) queried the use aristolochic acids–containing drugs among 283 (118
7 men and 165 women) uremic patients undergoing dialysis in a study from China. Use of
8 aristolochic acids–containing drugs, AAN, and determination of TCC were obtained from
9 the patient’s medical history and a survey questionnaire. TCC diagnosis was confirmed
10 by tissue pathology. The authors found a higher prevalence [33.3%] of TCC among
11 individuals with a history of taking aristolochic acids–containing Chinese drugs (22/66)
12 than among individuals who did not report use of aristolochic acids–containing drugs
13 (2/198) (Li *et al.* 2005b) [OR = 37, 95% CI = 11 to 216]. (The use of aristolochic acids–
14 containing drugs was not clear in 19 patients, and no TCC cases were identified among
15 these patients). The majority of the TCC cases with a history of taking aristolochic acids–
16 containing drugs had taken Long Dan Xiegan pills. The average time between the
17 beginning of taking the aristolochic acids–containing drugs and the development of TTC
18 was 10 years. The locations of the tumors were in the bladder (N = 7), ureters (N = 4),
19 renal pelvis (N = 3), or a combination of the bladder, ureters, or pelvis (N = 4).

20 Li *et al.* (2008) conducted a retrospective review of a cohort of 1,429 Chinese renal
21 transplant patients at a single hospital center (from 1996 to 2005), and reported that the
22 TCC incidence was 1.89%. The 27 patients (21 females and 6 males) who developed
23 TCC did not have any malignancies before transplantation, and were followed up for 18
24 to 132 months (mean = 71.2 months). TCC was confirmed by pathological diagnosis and
25 locations were in the bladder (N = 18) or upper urinary tract (pelvis or ureter, N = 11);
26 two patients had tumors in both the bladder and pelvis. The patients were treated with an
27 immunosuppression regimen (including cyclosporine), and some patients were found to
28 have cyclosporine-related toxicity. Of the 27 patients with TCC, 16 had taken Chinese
29 herbs containing aristolochic acids for more than 2 months before renal transplantation.
30 The incidence of TCC was 5.42% in the group consuming aristolochic acids–containing

1 herbs and 0.97% in the group that did not consume aristolochic acids–containing herbs
2 (RR = 5.85, $P < 0.0001$). In addition to aristolochic acids exposure, the authors stated that
3 female sex and immunosuppression were associated with the development of TCC.

4 **3.4 Balkan endemic nephropathy and associated urothelial cancer**

5 Balkan endemic nephropathy (BEN), a disease endemic to Serbia, Bosnia, Croatia,
6 Bulgaria, and Romania, is discussed here because consumption of food products
7 containing aristolochic acids (*A. clematidis*) has been suggested to be an environmental
8 cause of BEN. BEN is a household chronic tubulointerstitial disease with insidious onset
9 and slow progression to terminal renal failure (Stefanovic *et al.* 2006). A review by
10 Nikolic (2006) reported that more than 20,000 patients were diagnosed with BEN in the
11 period from 1955 to 1998.

12 In part because BEN and AAN have similar morphology and clinical features, exposure
13 to aristolochic acids has been proposed to be a risk factor for BEN as well as for AAN.
14 BEN has a slower progression to end-stage renal failure, and the slow onset of the
15 nephropathy is more reminiscent of the East Asian cases of AAN with Fanconi syndrome
16 than the rapid onset seen in the Belgian cohort. However, most of the Asian cases had a
17 rapidly progressive course without Fanconi syndrome (Cosyns 2003), and some cases
18 with a more indolent evolution were found in the Belgian epidemic. The marked
19 similarity of the pathological changes in the renal cortex, as well as the similarities of the
20 overall clinical presentations, led to the earliest suggestions of a similar etiologic agent
21 (Cosyns *et al.* 1994a).

22 There is also evidence that BEN patients may have been exposed to aristolochic acids.
23 Ivic (1970) reported that flour used to bake bread, which is a dietary staple, is derived
24 from locally grown wheat and may be contaminated with seeds from *A. clematidis*. [*A.*
25 *clematidis* or birthwort is a common weed in wheat fields in the endemic area.] Hranjec *et*
26 *al.* (2005) conducted a cross-sectional study on BEN in an endemic region of Croatia.
27 The subjects included 28 cases with endemic nephropathy (meeting WHO requirement),
28 and two control groups: (1) 30 non-endemic controls who were patients with other types
29 of renal disease and (2) 30 apparently healthy residents in the endemic village. A detailed
30 questionnaire was administered that queried exposure to potentially toxic factors, medical

1 history, diet, agricultural practices, tobacco use, and alcohol consumption. The
2 questionnaire also evaluated the frequency (for the categories of observed “always” plus
3 “sometimes”) of seeing *A. clematitidis* in the fields 20 to 30 years ago. The authors
4 reported that this observation was significantly more frequent in subjects with endemic
5 nephropathy (78.2%) than in subjects with other renal disease (33.3%) or in healthy
6 controls of the endemic regions (38%). No significant differences were found between
7 the groups with respect to educational level or tobacco use; the authors did not present
8 data on other factors such as diet and alcohol consumption. Other researchers have
9 questioned whether these findings establish the exposure to aristolochic acids in these
10 regions (Long and Voice 2007, Peraica *et al.* 2008). However, Grollman *et al.* (2007) was
11 able to detect AA-DNA adducts in renal tissue from four BEN patients (whose diagnosis
12 was confirmed using the WHO criteria) from Croatia but not in five patients with
13 common forms of chronic renal disease, demonstrating that these BEN patients had been
14 exposed to aristolochic acids.

15 Both BEN and AAN are associated with an increased risk of urothelial cancer. The
16 Nikolic (2006) review stated that over 2000 cases of upper urothelial tumors were
17 diagnosed in Serbia from 1955 to 1998. Stewart *et al.* (2003) reported excess risks of
18 kidney and bladder cancer among dialysis patients with end-stage renal failure (N =
19 831,804) in the United States, Europe, Australia, and New Zealand. Most causes of
20 primary kidney disease also were associated with excess kidney and bladder cancer; the
21 standardized incidence ratios for BEN were 26.2 (95% CI = 13.1 to 46.9, 11 observed
22 cases) for kidney cancer and 18.2 (95% CI = 9.4 to 31.8, 12 observed cases) for bladder
23 cancer. Although most urinary-tract carcinoma patients from villages with high
24 prevalence of BEN have symptoms of severe renal disease, many do not (Petronic *et al.*
25 1991, Radovanovic *et al.* 1991).

26 Grollman *et al.* 2007 detected AA-DNA adducts in urothelial and renal cortical tissues
27 from 3 long-term residents of endemic villages who had upper urinary tract malignances.
28 The authors also analyzed *p53* mutations and histopathology in a study of 11 patients
29 with upper urothelial cancer who resided in the endemic villages for a minimum of 15
30 years. Histopathologic analysis was available for 9 patients, 8 of whom exhibited changes

1 in their renal cortex that were diagnostic or highly suggestive of BEN. *P53* mutations
2 were observed in all 11 patients, and tumors in which > 10% of the tumor cells stained
3 positive with a p53 antibody were used in the mutational analysis. The authors reported
4 that the majority (78%) of the mutations were A:T → T:A transversions, which they
5 stated was a mutational signature for exposure to aristolochic acids (see Section 5.3.5).

6 Other suspected environmental causes of BEN and the associated urothelial cancer are
7 the mycotoxin ochratoxin A (OTA) and long-term exposures to polycyclic aromatic
8 hydrocarbons in the water originating from Pliocene coal beds. Of these other factors,
9 OTA is probably the most studied. OTA is classified by IARC (1993) as a possible
10 human carcinogen (Group 2B) and is listed in the Report on Carcinogens as *reasonably*
11 *anticipated to be a human carcinogen* (NTP 2004) based on sufficient evidence for
12 carcinogenicity in experimental animals but inadequate evidence in humans. OTA causes
13 liver tumors in mice and renal tumors in rats and male mice. It also causes renal toxicity
14 and nephropathy in experimental animals. Some but not all studies have found higher
15 exposure (as measured by OTA in food stuff, intake of OTA, OTA levels in blood or
16 urine) in individuals from endemic areas versus non-endemic areas (as reviewed by
17 Stefanovic *et al.* 2006, Long and Voice 2007, Mally *et al.* 2007, Pfohl-Leszkowicz and
18 Manderville 2007). Some studies have also reported higher OTA blood concentrations in
19 patients with kidney disease compared with healthy individuals; however, it is not clear
20 whether accumulation of OTA is a consequence rather than the cause of impaired renal
21 function (Mally *et al.* 2007). OTA-related DNA adducts (as well as AA-DNA adducts)
22 were detected in kidney tissues from individuals with urothelial cancer or ureteral
23 stenosis living in areas where BEN is endemic and in 30% of human kidney tissue from
24 Balkan patients suffering from nephropathy and urothelial cancer (Arlt *et al.* 2002a,
25 Pfohl-Leszkowicz *et al.* 2007, Stefanovic *et al.* 2006) [see Section 5.3.1, “Studies in
26 humans with AAN or BEN”]. However, Mally *et al.* (2007) noted that OTA-induced
27 renal lesions in rats are different than those seen in BEN. The FAO/WHO Expert
28 Committee on Food Additives (EFSA 2006) concluded that the “various studies in
29 humans have associated OTA with an endemic kidney disease observed in the Balkans

1 (Balkan Endemic Nephropathy and related Urinary Tract Tumours), but convincing
2 epidemiological evidence associated with OTA exposure is currently lacking.”

3 **3.5 Discussion**

4 Two case-series studies (including a 15-year follow-up update of one of the studies)
5 (Cosyns *et al.* 1999, Nortier *et al.* 2000, Nortier and Vanherweghem 2002, Lemy *et al.*
6 2008) have reported a high prevalence of urothelial cancer among women with end-stage
7 renal failure thought to be caused by ingestion of herbal remedies containing aristolochic
8 acid; [however, these studies did not include an unexposed group of patients,
9 complicating the evaluation of causality]. Because most of the studies of urothelial cancer
10 have occurred in patients with herbal medicine nephropathy leading to end-stage renal
11 failure, it is important to consider the available data evaluating the relationship between
12 consumption of aristolochic acids and herbal medicine nephropathy and characteristics of
13 urothelial cancer in herbal medicine nephropathy patients versus patients with end-stage
14 renal failure from other causes. Another important issue is the location of the urothelial
15 tumors in humans. The strengths and weaknesses of the available studies are also
16 discussed below.

17 *3.5.1 Association between botanical products containing aristolochic acids and* 18 *nephropathy*

19 Numerous case reports or reports on clusters of patients (as described in Section 3.1 and
20 Table 3-1) have documented the development of nephropathy characterized by severe
21 interstitial fibrosis, often with renal failure and anemia, in patients who consumed
22 Chinese herbal preparations. The association between nephropathy and the consumption
23 of Chinese herbal preparations was supported by (1) the timing of exposure and disease;
24 in most cases, the nephropathy developed immediately after ingestion of the herbs, and in
25 some cases, it was reversible after the patient discontinued the herbs (usually in patients
26 with Fanconi syndrome); (2) the young age of the patients; and (3) lack of exposure (in
27 most cases) to agents known to be risk factors for nephropathy.

28 Arlt *et al.* (2001b) evaluated the role of OTA in causing herbal medicine
29 nephropathy/AAN or urothelial cancer. OTA is nephrotoxic in humans and animals and
30 is carcinogenic in rodents. It is a widespread contaminant in food and is a suspected risk

1 factor for BEN (see Section 3.4); however, it was not detected in the weight-loss regimen
2 used in Belgium. These authors reported that AA-DNA adducts were detected in urinary
3 tract tissues of 5 of 5 patients with herbal medicine nephropathy (followed at Cliniques
4 Universitaires St.-Luc), and OTA-related DNA adducts were detected in 2 kidneys and 1
5 ureter from 3 of the patients; the levels of AA-DNA adducts were about 50 times higher
6 than the levels of OTA-related DNA adducts. The detection of OTA-related DNA
7 adducts requires different chromatographic conditions from those routinely used for
8 lipophilic adducts like AA-DNA adducts; however, Artl *et al.* demonstrated that both
9 OTA-related and AA-DNA adducts were detected when analyzed under conditions
10 suitable for assaying OTA-related adducts (see Section 5.3.1). Nortier *et al.* (2000)
11 reported that low levels of OTA-related DNA adducts were found in 4 of 25 kidney
12 samples from the Belgian patients with herbal medicine nephropathy; however, the levels
13 of the major AA-DNA adduct identified in the kidneys of herbal medicine nephropathy
14 patients were about 20 times those of the OTA-related DNA adducts. The authors
15 concluded that OTA is not likely to have a key role in herbal medicine nephropathy.

16 In contrast to these findings, Pfohl-Leszkowicz *et al.* (2007) detected OTA-related DNA
17 adducts but not AA-DNA adducts in DNA samples isolated from kidney tissues from a
18 French patient (see Table 3-2) with AAN and urothelial cancer and a Belgian AAN
19 patient. Arlt *et al.* (2004b) detected AA-DNA adducts from kidney tissues from both the
20 Belgian patient [which may have been used as a positive control] and from two French
21 patients (one of which was the same as that analyzed by Pfohl-Leszkowicz *et al.*) using a
22 nuclease P1 enrichment ³²P-postlabeling method. Pfohl-Leszkowicz *et al.* (2007) used
23 chromatographic conditions that were optimized for detecting OTA-related DNA adducts
24 but presumably could detect both types of adducts. The discrepancy between the different
25 findings is unclear, and the existence of OTA-related DNA adduct formation is
26 controversial (Turesky 2005, EFSA 2006, Mally *et al.* 2007) (see also Section 5.3.1,
27 “Studies in humans with AAN or BEN” for additional discussion of methodology in
28 adduct detection and Section 5.3.5, “Mutation spectra in tumors from animals or humans”
29 for additional discussion of putative OTA-DNA adduct formation).

1 Most studies have shown that the herbal preparations to which herbal medicine
2 nephropathy patients were exposed contained aristolochic acids, and several studies have
3 detected AA-DNA adducts in tissue (usually from kidneys or ureters) from herbal
4 medicine nephropathy patients, demonstrating that the patients were exposed to
5 aristolochic acids. Few case studies have evaluated whether other ingredients in the
6 Chinese herbal preparation could be responsible for or contribute to the nephropathy, and
7 some authors have suggested that unidentified herbal ingredients may play a role in
8 causing nephropathy; this seems to be more common for the cases in Asian nations, most
9 of which have manifested as Fanconi syndrome. The data supporting an association
10 between aristolochic acids and herbal medicine nephropathy include the following:
11 (1) exposure to aristolochic acids alone causes nephropathy in experimental animals, (2)
12 intravenous administration of aristolochic acids caused renal toxicity in humans (Jackson
13 *et al.* 1964), (3) herbal medicine nephropathy has been identified in patients from
14 different countries, using botanical products for a wide variety of purposes, and using
15 complex herbal mixtures, the commonality being the presence of plant species containing
16 aristolochic acids, and (4) AA-DNA adducts occurred in patients at higher levels than
17 adducts from other suspected ingredients.

18 3.5.2 *Urothelial cancer in patients with end-stage renal failure from other causes*

19 Although the fraction of patients who developed AAN from exposure to botanical
20 products containing aristolochic acids was about 5% (see Section 3.1.1), urothelial cancer
21 occurred at a high prevalence (40% and 46% in two studies, see Table 3-2) among
22 patients in the Belgian epidemic with end-stage renal failure associated with AAN.

23 Although renal disease or dialysis is a risk factor for urothelial cancer, the prevalence of
24 cancer in AAN patients appears to be higher than that observed among patients with end-
25 stage renal disease in general. However, the prevalence studies of AAN patients were
26 very small and were conducted specifically to look for urothelial cancer. Wu *et al.*

27 (2004c) summarized the data from several large studies of kidney-transplant patients and
28 reported that the prevalence of cancer (at all sites) ranged from 4% to 18%, with an
29 average of 6%. In Western nations, the predominant cancers in transplant patients were
30 squamous-cell carcinoma of the skin and virus-related tumors. In contrast, TCC was the
31 most common cancer in the Taiwanese study, with a prevalence of 4.1% in 730 kidney-

1 transplant recipients (Wu *et al.* 2004c) (see Section 3.3). Li *et al.* (2008) reported that the
2 incidence of TCC was 1.89% among Chinese renal-transplant patients; TCC is the
3 predominant malignancy [this study also included patients consuming aristolochic acids–
4 containing Chinese herbs, see Section 3.3].

5 Marple and MacDougall (1993) reviewed the literature on the development of cancer in
6 patients with end-stage renal cancer. They reported that most studies of dialysis patients
7 have reported an excess of cancer, including urinary tract and renal cancer; cancer (at all
8 sites) occurred in approximately 1.4% to 10% of the dialysis patients in these studies.
9 They calculated a prevalence of renal cancer to be 84 cases per 100,000 (based on finding
10 67 cases of renal cancer among 79,842 end-stage renal disease patients). Acquired cystic
11 kidney disease appears to be a risk factor for renal cancer, and renal cancer is reported to
12 occur in 6% to 20% of these patients. Analgesic nephropathy is associated with an
13 increased risk of urinary-tract tumors. The prevalence of TCC among analgesic
14 nephropathy patients undergoing kidney transplants has been reported to be between 5%
15 and 24% (as cited by Cosyns *et al.* 1999). Ou *et al.* (2000) reported that the incidence of
16 TCC among dialysis patients in Taiwan was 0.89% in a study of 1,910 patients.

17 Cosyns *et al.* (1999) noted that urothelial tumors associated with exposure to aristolochic
18 acids occurred after short durations of exposure (an average of 20 months), low levels of
19 exposure (an average of 0.015 mg/kg b.w.), and short intervals between the end of
20 aristolochic acids intake and identification of the tumor (approximately 2 to 6 years). In
21 contrast, other toxin-induced urothelial tumors require longer exposure and have longer
22 induction times; for example, phenacetin abuse is associated with induction times of 22
23 years for renal pelvic cancer and 29 years for urinary bladder cancer.

24 3.5.3 Localization of the TCC tumors

25 Most of the TCC tumors reported in the Belgian epidemic studies were located in the
26 renal pelvis and/or upper ureter; Lemy *et al.* 2008 reported that all 17 tumors from the
27 Belgian cohort (Hospital Erasme) were located in the upper urothelial tract. Urothelial
28 tumors associated with BEN are predominantly upper urothelial carcinomas (Nikolic
29 2006). This is in contrast to TCC that have been associated with exposure to other
30 carcinogens. For example, upper urothelial cancers represent only 5% of all detected

1 TCC from exposure to phenacetin and arsenic (Genega and Porter 2002). [The bladder
2 tumors that were observed in the studies on aristolochic acids exposure may arise from
3 “seeding” of the upper urothelial tumors.] Lemy *et al.* (2008), in the 15-year update of the
4 Belgian cohort, reported an increased risk for the development of urinary bladder tumors
5 in patients who had upper urothelial cancers.

6 3.5.4 Strengths and weaknesses of the studies

7 [The two prevalence studies of urothelial cancer in patients with AAN and end-stage
8 renal failure (Cosyns *et al.* 1999, Nortier *et al.* 2000) are limited by the lack of an
9 unexposed control group and small sample size. However, the primary strength of both
10 studies was that exposure to aristolochic acids was demonstrated as evidenced by AA-
11 DNA adducts detected in kidney or ureteral tissues from the cancer patients. Additional
12 strengths of the study by Nortier and colleagues include (1) quantification of the
13 cumulative dose of *A. fangchi*, (2) demonstration that higher doses of *A. fangchi* were
14 associated with a higher frequency of urothelial cancer, (3) evaluation of OTA-related
15 DNA adducts in tissue from cancer patients, and (4) evaluation of potential risk factors
16 for urothelial cancer, such as smoking and the use of analgesics (see Section 3.2.2 for a
17 description of the findings).] Neither of these reports contains information concerning
18 urinary-tract carcinoma in the 95% of the population exposed to the weight-loss regimen
19 with no signs of impaired renal function. There is no published evidence that this
20 population has been observed for the development of urinary-tract carcinoma, and the
21 development of urinary-tract carcinoma in patients with little or no impairment of renal
22 function cannot be ruled out.

23 [The two clinical investigations among Chinese patients with renal transplants or dialysis
24 patients have the advantage of an unexposed group, and thus a risk estimate can be
25 calculated. However, the exposure assessment and pathology of the renal disease in these
26 studies are not as well described. Both of these studies were retrospective analyses.
27 Neither study measured aristolochic acids in the herbal products or analyzed tissues for
28 AA-DNA adducts. In the study reported by Li *et al.* 2008, it appears that information on
29 use of Chinese herbs was obtained from medical records, and there is no information on
30 the types of drugs or herbs used. The authors stated that drug or herb use had to occur

1 two months prior to transplant but did not state whether it occurred before renal disease.
2 There is no information on the pathology of the kidney disease, i.e., whether any of the
3 patients had AAN. Another limitation of this study is that the subjects were renal-
4 transplant patients who had taken immunosuppressive drugs. The study by Li *et al.*
5 (2005b) is more informative, and exposure was obtained from a questionnaire and from
6 medical records. Although the documentation is not that clear (English translation), the
7 study provides some information on the herbal remedies or *Aristolochia* species taken
8 (Longdan Xiegan or *A. manshuriensis*), and length of time the herb was taken. It also
9 stated that consumption of aristolochic acids-containing herbs occurred prior to renal
10 function injury. The study also provides more information on renal pathology and notes
11 that some of the patients (29 of 66) in the aristolochic acids group were diagnosed as
12 having AAN associated with end-stage renal failure. The clinical studies from Taiwan
13 (Wu *et al.* 2004c, Chang *et al.* 2007a) did not evaluate exposure specific for aristolochic
14 acids and thus are not as informative for the evaluation of potential carcinogenicity of
15 aristolochic acids.]

16 The literature on urothelial cancer associated with BEN is limited by the absence of an
17 analytical epidemiology study evaluating specific exposure to aristolochic acids and
18 urothelial cancer. Strengths of the literature are the detection of the AA-DNA adducts and
19 mutational analysis of *p53* mutations from urothelial tumors associated with BEN.
20 However, only a small number of subjects was evaluated, and it is unclear whether the
21 *p53* mutations and AA-DNA adducts were evaluated in the same subjects.

22 **3.6 Summary**

23 The IARC (2002) working group evaluated numerous case reports and two prevalence
24 studies of urothelial cancer that occurred in people who consumed botanical products
25 containing aristolochic acid, and concluded that there was sufficient evidence in humans
26 for the carcinogenicity of herbal remedies containing plant species of the genus
27 *Aristolochia*. Their conclusion was based on (1) the identification of AA-DNA adducts in
28 the patients with cancer, confirming that the cancer patients were exposed to aristolochic
29 acids; (2) the high percentage of urothelial cancer (an uncommon tumor) detected in
30 patients with AAN; and (3) demonstration of a dose-response relationship between

1 consumption of *A. fangchi* and the prevalence of tumors. There are no human cancer
2 studies available on exposure to aristolochic acids *per se* (that is, consumption of
3 aristolochic acids that were not part of a botanical preparation). IARC concluded that
4 there was limited evidence in humans for the carcinogenicity of naturally occurring
5 mixtures of aristolochic acids.

6 Since the IARC (2002) review, there have been an update of the prevalence study of
7 urothelial cancer developing in AAN patients in Belgium, additional case reports of AAN
8 and urothelial cancer developing in patients with AAN (both in Belgium and worldwide),
9 several clinical investigations of urothelial cancer among kidney-transplant or dialysis
10 patients in Taiwan or China, and a study on aristolochic acids and BEN.

11 The 15-year follow-up of the Belgian patients (Lemy *et al.* 2008) from the Hospital
12 Erasme found a similar prevalence rate of urothelial cancer occurring in AAN patients
13 compared with the earlier report by Nortier and colleagues. [The follow-up identified a
14 few more cases of cancer, and included most but not all the previous cancer cases.] In
15 addition, the follow-up study found an increased incidence of urinary bladder cancer
16 among cases with urothelial cancer. Similar to the earlier publications, the cumulative
17 dose of *Aristolochia* in AAN patients who developed urothelial cancer was significantly
18 higher than the dose consumed by AAN patients who did not develop cancer. A case
19 report of urothelial cancer from the Belgian epidemic was also reported in a patient who
20 did not have severe renal disease. There were also additional case reports of urothelial
21 cancer in AAN in patients outside of Belgium, which supports the role of aristolochic
22 acids as a cause of upper urothelial cancer.

23 Two clinical studies among Chinese patients with renal disease (renal-transplant or
24 dialysis patients) reported an increased incidence or prevalence of TCC among patients
25 consuming Chinese herbs or drugs containing aristolochic acids compared with non-
26 exposed patients; OR = 37 (95% CI = 11 to 216) in the study of 283 dialysis patients (Li
27 *et al.* 2005a) and RR = 5.85 ($P < 0.0001$) in the study of 1,429 renal transplant patients
28 (Li *et al.* 2008). Two other clinical studies evaluating TCC mortality or incidence among
29 Taiwanese patients with renal disease (dialysis or kidney-transplant patients) reported

1 that consumption of Chinese herbs was a risk factor for Chinese herb use (relative hazard
2 was 5.2 among transplant patients [Wu *et al.* 2004c] and 6.21 among dialysis patients
3 [Chang *et al.* 2007a]); however, the exposure assessments were not specific for
4 aristolochic acids intake.

5 Aristolochic acids have been proposed to be a risk factor for urothelial cancer associated
6 with BEN. BEN is a chronic tubulointerstitial disease endemic to Serbia, Bosnia, Croatia,
7 Bulgaria, and Romania that has similar morphology and clinical features to AAN
8 patients. Exposure to aristolochic acids is proposed to occur from consumption of wheat
9 contaminated with seeds from *A. clematitis*. AA-DNA adducts have been detected in
10 renal tissue of BEN patients and in urothelial and renal cortical tissues from BEN patients
11 with upper urothelial cancers. One study reported that the majority (78%) of *p53*
12 mutations (in tumors with *p53* mutations) in urothelial tumors from patients living in
13 endemic areas were A:T → T:A transitions, which the authors stated was a mutational
14 signature for exposure to aristolochic acids.

15 In summary, exposure to aristolochic acids has been associated with a progressive
16 interstitial renal fibrosis in several populations (primarily in Belgium, the Balkans, and
17 China). An increased incidence or prevalence of upper urothelial tumors has been
18 detected in individuals with aristolochic acids–associated end-stage renal failure. In some
19 studies, AA-DNA adducts have been detected in urothelial tissues from the cancer
20 patients, demonstrating exposure to aristolochic acids. Studies of renal-transplant or
21 dialysis patients have reported elevated risks for urothelial cancer associated with
22 consumption of herbal products containing aristolochic acids.

4 Studies of Cancer in Experimental Animals

The carcinogenic effects of aristolochic acids (administered as aristolochic acid I, a mixture of aristolochic acids I and II, or a mixture of herbal ingredients containing aristolochic acids) have been investigated in mice (oral administration), rats (oral and parenteral administration), and rabbits (parenteral administration). The IARC working group (IARC 2002) concluded that there was sufficient evidence in experimental animals of carcinogenicity of aristolochic acids.

The general toxicity of aristolochic acids in experimental animals is summarized in Section 5.2.2.

4.1 Mice

Only one study in mice given aristolochic acids (77.2% aristolochic acid I, 21.2% aristolochic acid II) was reported in the literature (Mengs 1988). The author described this as a screening study designed to provide evidence of any possible carcinogenic effect. A group of 39 female NMRI mice [age not specified] were given the mixture of aristolochic acids at a dose of 5 mg/kg b.w. daily by gavage for 3 weeks. The control group consisted of 11 mice that were given the solvent vehicle only [the authors did not identify the vehicle]. Exposed animals were sacrificed at scheduled intervals starting at the end of the exposure period and extending to 56 weeks. All organs were histologically examined, and all tumors were examined microscopically. Low-grade regenerative hyperplasia of forestomach squamous epithelium with hyperkeratosis was observed at 3 weeks but these changes improved during the next 6 weeks. Low- to middle-grade papillomatosis of the forestomach occurred in all exposed mice at 18 and 26 weeks. The first signs of forestomach malignancy were observed at 37 weeks (squamous-cell carcinoma), and by 56 weeks, all remaining mice had developed these tumors. Other neoplastic lesions (some of which were first observed at 26 weeks) included cystic papillary adenoma of the renal cortex, alveologenic lung carcinoma, uterine hemangioma, malignant lymphoma, and an adenocarcinoma of the glandular stomach. No neoplastic lesions were observed in the control group after 56 weeks. These data are summarized in Table 4-1. No statistical analyses were reported.

Table 4-1. Neoplastic lesions in female NMRI mice exposed to aristolochic acids (5 mg/kg b.w.) for 3 weeks and observed for up to 56 weeks

Group: time of sacrifice (wk)	No. of mice	Number of mice with tumors						
		Forestomach		Stomach adeno- carcinoma	Kidney adenoma	Lung carcinoma	Uterus heman- gioma	Malignant lymphoma
		Pap	SCC					
Control	11	0	0	0	0	0	0	0
Exposed								
3 ^a	10	0	0	0	0	0	0	0
9	4	0	0	0	0	0	0	0
18	4	4	0	0	0	0	0	0
26	3	3	0	0	1	0	0	1
37	5	4	1	1	1	2	0	2
48	5	4	1	0	3	3	0	3
56	8	0	8	0	6	8	3	4
Total	39	15	10	1	11	13	3	10

Source: Mengs 1988.

Statistical analysis not reported

Pap = papilloma; SCC = squamous-cell carcinoma.

^aEnd of treatment period.

1 4.2 Rats

2 The carcinogenicity of aristolochic acids in rats has been investigated following acute
3 exposure (3 days), subchronic exposure (1 to 3 months), and chronic exposure (6 to 12
4 months). In addition, one two-stage study was reviewed that tested aristolochic acids as
5 an initiator. These studies are reviewed below. No lifetime (two-year) studies were
6 identified.

7 4.2.1 Acute exposure

8 Qiu *et al.* (2000) investigated the long-term effects of acute renal injury in groups of 30
9 or 40 female Sprague-Dawley rats [age not reported] orally administered decoctions (i.e.,
10 hot water extracts) of *A. manshuriensis* at 30 or 50 g/kg per day for 7 consecutive days,
11 or 20 g/kg per day for 15 days. Renal function was assessed, and histological
12 examinations were conducted at the end of treatment and after 1, 3, and 6 months with
13 sacrifice of 6 rats per group at 0, 1, and 3 months. [The remaining animals were
14 presumably sacrificed at 6 months, but the paper is not clear on this point as the data are
15 presented as percentages of animals with tumors and do not specify the number of
16 animals examined and survival data were not provided] At the end of treatment, there
17 was evidence of acute renal injury in all dosed groups that exhibited a dose-dependent
18 pattern. Histopathological changes included acute tubular necrosis. Renal function was

1 approaching normal values by month 1, and was nearly restored at 3 to 6 months after
 2 treatment. Tubular lesions showed gradual recovery after 1 month and were nearly
 3 resolved at 3 and 6 months. However, at 6 months, renal preneoplastic lesions and
 4 extrarenal tumors were observed in all dosed groups, and renal tumors (including 4 renal
 5 mesenchymal tumors and 1 nephroblastoma) were observed with the two higher doses
 6 (Table 4-2). Extrarenal tumors included skin (appendage epithelial), thyroid gland
 7 (follicular epithelial), and mammary gland (ductal epithelial) tumors. These lesions were
 8 not observed in the control group.

Table 4-2. Neoplastic and preneoplastic lesions observed in female Sprague-Dawley rats 6 months after exposure to decoctions of *A. manshuriensis* for 7 to 15 days

Dose (g/kg)	Duration (days)	Initial No. rats	Renal preneoplastic lesions (%)	Renal tumors (%) ^a	Extrarenal tumors (%) ^b
0	15	30	0	0	0
20	15	30	100	0	12.5
30	7	30	100	25	12.5
50	7	40	100	42.8	14.4

Source: Qiu *et al.* 2000

Statistical analysis not reported?

^a Renal tumors included 4 renal mesenchymal tumors and 1 nephroblastoma.

^b Extrarenal tumor sites included skin (appendage epithelial), thyroid gland (follicular epithelial), and mammary gland (ductal epithelial); however, the number of animals with these tumors was not provided.

9 Cui *et al.* (2005) examined the carcinogenic activity of aristolochic acid I following
 10 short-term, high-dose exposure in female Sprague-Dawley rats [age not reported]. The
 11 exposed group of 24 rats was administered aristolochic acid I at a dose of 50 mg/kg b.w.
 12 in distilled water by gavage for 3 consecutive days. The control group of 20 rats was
 13 given distilled water. Survival was 100% in the exposed and control groups. Blood and
 14 urine samples for renal function tests were collected from 6 randomly selected rats on day
 15 8 and at 1, 3, and 6 months after treatment. Four rats were sacrificed on day 8, 3 rats each
 16 at 1 and 3 months, and the remaining 14 rats at 6 months, and all rats were necropsied.
 17 Samples of liver, kidney, heart, brain, and any tissue with an abnormal appearance were
 18 fixed for histological examination. At day 8, plasma urea and creatinine, urine volume,
 19 and urinary glucose, protein, and *N*-acetyl- β -glucosaminidase were significantly higher in
 20 exposed rats than in controls. However, all these parameters returned to their normal
 21 levels at 1, 3, and 6 months. No signs of preneoplastic lesions or tumors were observed

1 before 6 months; however, preneoplastic proliferation of the kidney occurred in all 14
 2 rats sacrificed at 6 months, and renal tumors (3 mesenchymal and 1 oncocytoma) were
 3 observed in 4 of 14 rats (Table 4-3). In addition, a mammary ductal carcinoma occurred
 4 in 1 rat in the exposed group. No preneoplastic lesions or tumors occurred in the control
 5 group.

Table 4-3. Neoplastic and preneoplastic lesions in female Sprague-Dawley rats exposed to aristolochic acid I (50 mg/kg b.w.) for 3 days and observed for up to 6 months

Group	No. of rats	Renal preneoplastic proliferation ((%)) ^a	Renal tumors ((%))	Mammary -ductal carcinoma ((%))
Control	10	0 (0)	0 (0)	0 (0)
Exposed	14	14 (100)**	4 (28.6) ^b	1 (7.1)

Source: Cui *et al.* 2005.

**Significantly different from the control group at $P < 0.01$ by Fisher's exact test.

^aDescribed as small nodules (2–3 mm) with white granules on the surface and varying degrees of hyperplasia.

^b[The study authors reported this as significantly greater ($P < 0.05$) than in the control group; however, the actual P -value for Fisher's exact test is 0.094.]

6 4.2.2 Subchronic to chronic exposure

7 Ivic (1970) very briefly described the development of tumors at a non-specified injection
 8 site in 10 albino rats [strain, sex, and age were not reported, and no estimate of the
 9 potential dose was provided by the authors] injected with an aqueous extract (percolate)
 10 of *Aristolochia clematitis* seeds. All 10 rats developed polymorphocellular sarcoma that
 11 grew rapidly. The author reported that the findings were confirmed in control tests, but no
 12 description of these studies was included; however, this appears to be the earliest
 13 published report of tumorigenic effects of an aristolochic acids-containing botanical
 14 product.

15 Mengs *et al.* (1982) exposed groups of 30 male and 30 female Wistar rats (10 weeks old)
 16 to aristolochic acids as the sodium salts (77.2% aristolochic acid I and 21.2% aristolochic
 17 acid II) by gavage in distilled water at a dose of 0.1, 1, or 10 mg/kg b.w. for 7 days per
 18 week for 3 or 6 months. Some rats in the low-dose group were also exposed for 12
 19 months. The control group was given distilled water by gavage. Animals were sacrificed
 20 after 3, 6, 9, 12, or 16 months. Mortality was exposure related. Samples of thyroid,
 21 thymus, lung, heart, liver, pancreas, spleen, stomach, small and large intestine, kidney,

1 adrenal gland, urinary bladder, gonads, prostate, uterus, and all tissues with abnormal
2 appearance were fixed for histological examination. Blood and urine samples also were
3 collected before and throughout the study. After 3 months, blood and urine samples gave
4 no indication of toxic effects; however, severe papillomatosis of the forestomach with
5 occasional signs of malignancy was noted in the mid- and high-dose groups. At the 6-
6 and 9-month sacrifice times for these groups, metastatic squamous-cell carcinoma of the
7 forestomach, anaplasia of the tubular epithelium, adenoma of the renal cortex, and
8 hyperplasia and papilloma or carcinoma of the renal pelvis and urinary bladder. There
9 was high treatment-related mortality in the high-dose group. Eleven males and 9 females
10 died from malignant forestomach tumors with metastases before the 9-month sacrifice.
11 One male in the mid-dose group died from a metastatic forestomach tumor after 6
12 months, and one female in the low-dose group died of a mammary carcinoma after 16
13 months. One female rat in the control group died after 12 months [cause of death was not
14 specified]. Forestomach tumors first appeared in the low-dose group at 12 months.
15 Histological examinations were not possible for 6 animals because of advanced autolysis
16 or cannibalism. Tumor incidences increased with dose and time, but no statistical
17 analyses were reported (Table 4-4). No tumors occurred in the control group.

Table 4-4. Incidence [and %] of neoplastic lesions in Wistar rats exposed to aristolochic acids for 3 to 12 months and observed up to 16 months

Exposure duration (mo)	Time of sacrifice (mo)	Dose (mg/kg b.w.)	N	Forestomach		Kidney		Renal pelvis	Urinary bladder		Total tumors
				papilloma	carcinoma	adenoma	carcinoma	carcinoma	papilloma	carcinoma	
Males											
3	3	0.0	9	0	0	0	0	0	0	0	0
		0.1	9	0	0	0	0	0	0	0	0
		1.0	9	7 [77.7]	0	0	0	0	0	0	7 [77.7]
		10.0	10	10 [100]	0	0	0	0	0	0	10 [100]
6	6	0.0	10	0	0	0	0	0	0	0	0
		0.1	10	0	0	0	0	0	0	0	0
		1.0	11	6 [54.5]	3 [27.3]	0	0	0	0	0	9 [81.8]
		10.0	18	5 [27.8]	13 [72.2]	5 [27.8]	0	8 [44.4]	3 [16.7]	3 [16.7]	18 [100]
3	9	1.0	9	3 [33.3]	6 [66.7]	1 [11.1]	0	0	0	0	9 [100]
3	12	0.0	6	0	0	0	0	0	0	0	0
		0.1	7	2 [28.6]	2 [28.6]	0	0	0	0	0	4 [57.1]
12	16	0.0	5	0	0	0	0	0	0	0	0
		0.1	4	0	4 [100]	0	0	0	0	0	4 [100]
Females											
3	3	0.0	9	0	0	0	0	0	0	0	0
		0.1	9	0	0	0	0	0	0	0	0
		1.0	9	8 [88.9]	0	0	0	0	0	0	8 [88.9]
		10.0	10	10 [100]	0	0	0	0	0	0	10 [100]
6	6	0.0	10	0	0	0	0	0	0	0	0
		0.1	10	0	0	0	0	0	0	0	0
		1.0	10	7 [70]	0	0	0	0	0	0	7 (70)
		10.0	13	5 [38.5]	8 [61.5]	0	2 [15.4]	0	1 [7.7]	1 [7.7]	13 [100]
3	9	1.0	11	7 [63.6]	2 [18.2]	0	0	0	0	0	10 [90.9] ^a
		10.0	4	0	4 [100]	4 [100]	0	0	1 [25]	0	4 [100]
3	12	0.0	7	0	0	0	0	0	0	0	0
		0.1	6	2 [33.3]	0	0	0	0	0	0	2 [33.3]
12	16	0.0	4	0	0	0	0	0	0	0	0
		0.1	5	3 [60]	1 [20]	0	0	0	0	0	5 [100] ^b

Source: Mengs *et al.* 1982.

N = number of rats examined histologically, no statistical analysis reported

^aIncludes a pituitary gland adenoma in 1 rat.^bIncludes a mammary gland carcinoma in 1 rat.

1 Mengs (1983) investigated the histopathogenesis of forestomach carcinoma caused by
2 oral administration of aristolochic acids to 8-week-old male Wistar rats (same
3 formulation and composition as Mengs *et al.* 1982). Rats in the exposed group received
4 daily doses of 10 mg/kg b.w. in distilled water by gavage for up to 6 months. The control
5 group received an equivalent volume of distilled water. Rats were sacrificed at
6 predetermined intervals starting 1 day after the first dose. In rats killed before 180 days,
7 only the stomach and esophagus were histologically examined, but in rats killed after 180
8 days, all organs and metastatic lesions were examined. Extensive necrosis of the
9 squamous epithelium was noted 2 days after the first dose. This was followed by
10 regeneration and hyperplasia, papillomatosis, and squamous-cell carcinoma. Hyperplasia
11 was pronounced by the 14th day, and papillomas were noted after 28 days. Thereafter,
12 papillomas increased in size and number, and squamous-cell carcinomas appeared after
13 90 days. Lesion progression is outlined in Table 4-5. No statistical analyses were
14 reported.

Table 4-5. Histopathogenesis of forestomach carcinoma in male Wistar rats exposed to aristolochic acids (10 mg/kg b.w.) for 1 to 180 days

Days after 1st dose	No. of rats examined	Histological findings	Lesion incidence (%)
1	5	swelling of cells and nuclei necrosis of some cells	5 (100) 3 (60)
2	5	massive epithelial necrosis	5 (100)
3	5	extensive necrosis with destruction of basal cell layer	5 (100)
4	11	necrosis, onset of regeneration	11 (100)
9	14	hyperplastic epithelium	14 (100)
14	8	marked hyperplasia and hyperkeratosis	8 (100)
28	8	more advanced hyperplastic changes small nodular papilloma	8 (100) 1 (12.5)
42	8	single papilloma up to 3 mm high	8 (100)
57	8	multiple papillomata up to 4 mm high	8 (100)
70	8	forestomach completely lined with papillomata	8 (100)
90	10	papillomatosis up to 6 mm high first signs of malignant change	10 (100) 4 (40)
180	18	papillomatosis invasive squamous-cell carcinoma metastases	5 (27.7) 13 (72.2) 8 (44.4)

Source: Mengs 1983.

1 Schmeiser *et al.* (1990) exposed 40 male Wistar rats (8 weeks old) to aristolochic acid I
2 (as the sodium salt dissolved in water) at a dose of 10 mg/kg b.w. by gavage 5 days per
3 week for 3 months. The control group (8 rats) was given water only by gavage. After the
4 end of the exposure period, the rats were killed over a 15-week period when they showed
5 weight loss or symptoms of pain or when tumors were visible or palpable in the
6 peritoneal cavity. The data are summarized in Table 4-6. A representative portion of the
7 tumors was fixed for histological examination; however, some of the tumors of the
8 pancreas and small intestine were reported to be too small for histology. All exposed
9 animals showed papillomatosis of the forestomach, and 15 of 40 (38%) showed
10 squamous-cell carcinoma. Adenocarcinoma, sarcoma, or unknown tumor type (not
11 determined morphologically due to small size) of the small intestine occurred in 23 of 40
12 (58%) and squamous-cell carcinoma of the ear duct occurred in 7 of 40 (18%). In
13 addition, adenocarcinoma of the kidney, lymphoma, and metastasis of squamous-cell
14 carcinoma in the lung and pancreas occurred in 1 rat each, and pancreatic tumors of

1 unknown type (not determined morphologically due to small size) occurred in 2
 2 additional rats. No tumors were detected in the control group. No statistical analyses were
 3 reported.

Table 4-6. Neoplastic lesions in male Wistar rats exposed to aristolochic acid I (10 mg/kg b.w) for 3 months and observed up to 7 months

Tumor location	Tumor type	Tumor incidence (%) ^a	
		Control (N = 8)	Exposed (N = 40)
Forestomach	squamous-cell carcinoma	0	15 (38)
Ear duct	squamous-cell carcinoma	0	7 (18)
Small intestine	adenocarcinoma, sarcoma, or not determined	0	23 (58)
Pancreas	not determined or squamous-cell carcinoma metastasis	0	3 (7.5) ^b
Kidney	adenocarcinoma	0	1 (2.5)
Hematopoietic system	lymphoma	0	1 (2.5)
Lung	squamous-cell carcinoma metastasis	0	1 (2.5)

Source: Schmeiser *et al.* 1990.

^aNo statistical analysis reported.

^bIncludes one metastatic tumor.

4 Hadjiolov *et al.* (1993) studied the effects of diallyl sulfide on aristolochic acids–induced
 5 tumors in male BD-6 rats [age not reported]. Aristolochic acids (10 mg/kg b.w. in
 6 distilled water) and diallyl sulfide (150 mg/kg b.w. in corn oil) were administered by
 7 gavage. [The authors did not specifically identify whether they used aristolochic acid I or
 8 a mixture of aristolochic acids I and II.] Four groups of 20 rats each were exposed for 12
 9 weeks as follows: Group 1 received aristolochic acids twice weekly; Group 2 received
 10 aristolochic acids twice weekly plus diallyl sulfide 4 hours before each dose of
 11 aristolochic acids; Group 3 received aristolochic acids twice weekly plus diallyl sulfide
 12 24 hours and 4 hours before each dose of aristolochic acids; and Group 4 received diallyl
 13 sulfide 4 times a week for 12 weeks. The study was terminated at 46 weeks, after all
 14 animals had died. Target organs included the forestomach, kidney, urinary bladder, and
 15 thymus. Tumor incidence was evaluated with the chi-square test. Survival was
 16 significantly lower in Group 1 than in the other groups. Early deaths were attributed to
 17 severe forestomach papillomatosis accompanied by hemorrhage. Incidences of
 18 hyperplastic lesions and tumors are shown in Table 4-7 for Groups 1, 2, and 3. Tumor

1 data for Group 4 were not reported. Proliferative and neoplastic lesions of the
 2 forestomach, urinary bladder, and thymus occurred in male BD-6 rats exposed to
 3 aristolochic acids. Pretreatment with diallyl sulfide significantly reduced the incidence of
 4 malignant tumors (primarily forestomach tumors) but did not affect the incidence of
 5 papillomatosis or hyperplasia. The authors concluded that pretreatment with diallyl
 6 sulfide was associated with a delay in conversion of papillomas to malignant forestomach
 7 tumors.

Table 4-7. The modifying effects of diallyl sulfide on aristolochic acids-induced hyperplastic lesions and tumors in male BD-6 rats

Organ	Lesion	N	Incidence (%)		
			AA	AA + DAS1	AA + DAS2
Forestomach	hyperplasia	20	17 (85)	14 (70)	16 (80)
	papillomatosis	20	20 (100)	19 (95)	12 (60)
	squamous-cell carcinoma or sarcoma	20	9 (45)	2 (10)**	0***
Urinary bladder	hyperplastic urothelium	20	8 (40)	5 (25)	7 (35)
	papillomatosis	20	4 (20)	2 (10)	3 (15)
	transitional-cell carcinoma	20	1 (5)	0	0
Thymus	thymoma	20	2 (10)	0	0
	total tumors ^a	20	12 (60)	2 (10)**	0***

Source: Hadjiolov *et al.* 1993.

AA = aristolochic acids (Group 1: 10 mg/kg b.w. by gavage twice weekly for 12 weeks); DAS1 = 1 dose of diallyl sulfide before each AA dose (Group 2: 150 mg/kg b.w. by gavage 4 h before); DAS2 = 2 doses of diallyl sulfide before each AA dose (Group 3: 150 mg/kg b.w. by gavage 4 and 24 h before).

** $P < 0.01$; *** $P < 0.001$ significant by the chi-square test compared with Group 1.

^aThe sum of squamous-cell carcinoma or sarcoma, transitional-cell carcinoma, and thymoma.

8 Cosyns *et al.* (1998) exposed groups of 8-week-old male and female Wistar rats to
 9 aristolochic acids (44% aristolochic acid I and 56% aristolochic acid II) or to a weight-
 10 loss regimen of herbal ingredients that contained aristolochic acids. In the first
 11 experiment, 8 male and 8 female rats were given aristolochic acids at a dose of 10 mg/kg
 12 b.w. in olive oil by gavage for 5 days a week for 3 months. The control group (6 males
 13 and 6 females) received the vehicle only. All animals were sacrificed 3 months later. In
 14 the second experiment, groups of 8 male and 8 female rats were given an herbal mixture
 15 designed to mimic the weight-loss regimen associated with the Belgian epidemic of CHN
 16 (i.e., AAN) (see Section 3.1.1). These rats received weekly intradermal injections of
 17 artichoke extract and euphyllin, and herbal pills were dispersed in olive oil and

1 administered through a gastric tube. The bulk of the herbal pill consisted of *Magnolia*
2 *officinalis* powder and powder prepared from the Chinese herb identified as *Stephania*
3 *tetrandra* but which contained aristolochic acids (91% aristolochic acid I and 9%
4 aristolochic acid II) at a concentration of 2.2 mg/g. The estimated daily dose of
5 aristolochic acids from the weight-loss regimen was 0.15 mg/kg b.w. [This was about 10
6 times the average daily intake of aristolochic acids (0.015 mg/kg b.w.) reported by
7 Cosyns *et al.* (1999) for the individuals treated at the Belgian clinic; see Section 3.1.1.]
8 Exposure lasted for 3 months, and the animals were sacrificed 11 months after exposure
9 ended. The control group (8 males and 8 females) received only the vehicle by gastric
10 tube and saline-solution injections. Mortality was not affected by exposure to aristolochic
11 acids or the herbal mixture; however, four rats exposed to aristolochic acids (2 of each
12 sex), 8 rats exposed to the herbal mixture (4 of each sex), and 4 control rats (1 male and 3
13 female) died accidentally. Tumor incidence data are shown in Table 4-8 and discussed
14 below. *P*-values were not reported for tumor incidence data

15 In the experiment with aristolochic acids, body-weight depression was observed in the
16 exposed males but not the exposed females. Male rats developed more tumors than
17 females. Tumors of the forestomach, small intestine, and kidney were the most prevalent
18 in male rats. Other tumors observed included one transitional-cell sarcoma of the bladder
19 and 1 fibrosarcoma of the heart. All male rats in the exposed and control groups
20 developed benign and malignant hyperplasia of the prostate. Forestomach papillomatosis
21 and tumors of the small intestine or kidney occurred in female rats.

22 Body weight was not affected by exposure to the herbal mixture. Forestomach papillomas
23 and squamous-cell carcinomas occurred in male rats given the weight-loss regimen but
24 not in controls. One female rat exposed to the herbal mixture developed a forestomach
25 papilloma; however, this tumor also occurred in two female rats in the control group.

Table 4-8. Tumor incidence [and %] in Wistar rats exposed to aristolochic acids or an herbal weight-loss regimen for 3 months and held up to 6 months (aristolochic acid) or 11 months (weight-loss regimen)

Exposure	Sex (N)	Dose (mg/kg b.w.)	Forestomach		Small intestine			Kidney		Urinary Bladder	Heart
			papilloma	carcinoma	leiomyo-sarcoma	angio-sarcoma	osteo-sarcoma	adenoma	malignant ^b	carci-noma	fibro-sarcoma
Aristolochic acids	M (6)	0	0	0	0	0	0	0	0	0	0
	M (6)	10	5 [83.3]	3 [50]	5[(83.3]	3 (50)	1 [16.7]	4 [66.7]	0	1 [16.7]	1 [16.7]
	F (6)	0	0	0	0	0	0	0	0	0	0
	F (6)	10	5 [83.3]	0	2 [33.3]	1 [16.7]	0	0	2 [33.3]	0	0
Weight-loss regimen ^a	M (7)	0	0	0	0	0	0	0	0	0	0
	M (4)	0.15	2 [50]	2 [50]	0	0	0	0	0	0	0
	F (5)	0	2 [40]	0	0	0	0	0	0	0	0
	F (4)	0.15	1 [25]	0	0	0	0	0	0	0	0

Source: Cosyns *et al.* 1998.

^aIncluded a mixture of various herbs and other treatments that was designed to mimic the weight-loss regimen prescribed at the Belgian clinic in the early 1990s.

^bMalignant tumor of unclear histogenesis

1 Groups of 24 male Wistar rats (4 weeks old) were given daily subcutaneous (s.c.)
2 injections of aristolochic acids (40% aristolochic acid I and 60% aristolochic acid II) at a
3 dose of 1 or 10 mg/kg b.w. in polyethylene glycol for 35 days (Debelle *et al.* 2002). The
4 control group (18 rats) was injected with a 50:50 mixture of distilled water and
5 polyethylene glycol. All rats received a single intraperitoneal (i.p.) injection of
6 furosemide at a dose of 4 mg/kg b.w. 1 week before the start of aristolochic acids
7 exposure and were maintained on a low-salt, normal protein diet. Six animals from each
8 group were killed on days 10 and 35 for renal function and histological analyses.
9 Surviving rats were observed until day 105. Kidney, lung, liver, and skin (at the injection
10 site) were fixed for histologic examination, and blood and urine samples were collected.
11 Body weight was depressed in the high-dose group. The high dose of aristolochic acids
12 was associated with nephropathy, including tubular atrophy and interstitial fibrosis.
13 Urothelial dysplasia was observed in both the low- and high-dose groups by day 10, and
14 low-grade urothelial carcinoma of the renal pelvis was detected in 3 rats in the high-dose
15 group by day 105. In addition, malignant fibrohistiocytic sarcoma developed at the
16 injection site in 2 of 6 rats in the low-dose group and in 7 of 11 rats in the high-dose
17 group that survived until the end of the study.

18 Hwang *et al.* (2006) investigated the subchronic toxicity of aristolochic acids and
19 aqueous extracts of dried fruits from *A. contorta* (described as *A. fructus* by the authors)
20 in male and female Sprague-Dawley rats (4 weeks old). Ten rats per sex per group were
21 administered daily doses of extracts of *A. fructus* at 0, 21.35, 213.5, or 2,135 mg/kg by
22 gavage for 90 days and were killed at the end of the treatment period. These doses were
23 equivalent to 0.05, 0.5, and 5 mg/kg of aristolochic acids. Other groups were dosed with a
24 mixture of aristolochic acids (44% aristolochic acid I and 56% aristolochic acid II) at 0,
25 0.05, 0.5, and 5 mg/kg for 90 days. There were significant decreases in body-weight gain
26 in the high-dose groups compared with controls. No excess mortality was reported in the
27 treatment groups, and clinical signs, hematology, and serum biochemistry in the
28 treatment groups and controls were similar. Two male rats in the *A. fructus* high-dose
29 group and one male rat in the aristolochic acids high-dose group developed carcinoma of
30 the transitional epithelium of the renal pelvis. Forestomach papillomas and carcinoma
31 occurred in both sexes in both high-dose groups. Results are summarized in Table 4-9.

Table 4-9. Tumor incidences in Sprague-Dawley rats treated with extracts of *A. fructus* or aristolochic acids for 90 days and killed at the end of the treatment period

Treatment	Sex (N)	Dose (mg/kg)	Tumor incidence [%] ^a		
			Carcinoma (Renal pelvis)	Forestomach papilloma	Forestomach Carcinoma
<i>A. fructus</i> extract	M (10)	0	0	0	0
		21.35	0	0	0
		213.5	0	0	0
		2135	2 [20]	7 [70]	3 [30]
	F (10)	0	0	0	0
		21.35	0	0	0
		213.5	0	0	0
		2135	0	8 [80]	2 [20]
Aristolochic acids	M (10)	0	0	0	0
		0.05	0	0	0
		0.5	0	0	0
		5	1 [10]	9 [90]	9 [90]
	F (10)	0	0	0	0
		0.05	0	0	0
		0.5	0	0	0
		5	0	10 [100]	1 [10]

Source: Hwang *et al.* 2006.^aStatistics not provided

1 4.3 Two-stage study

2 Rossiello *et al.* (1993) noted that aristolochic acids are known to be carcinogenic in the
3 forestomach, renal pelvis, and urinary bladder but not the liver of the rat. The authors
4 speculated that aristolochic acids were not carcinogenic in rat liver because the doses
5 tested were not necrogenic. To test whether aristolochic acids were necrogenic to rat
6 liver, male F344 rats [age not reported] were administered a single i.p. injection at a dose
7 of 10 mg/kg b.w. [the authors did not report whether they used aristolochic acid I or a
8 mixture of aristolochic acids I and II]. Control animals were injected with 0.9% saline,
9 and a positive-control group was administered carbon tetrachloride in corn oil by gavage.
10 Rats were sacrificed at 24, 48, and 72 hours after injection, and livers were processed for
11 histological examination. Another experiment was designed to test whether aristolochic
12 acids would initiate development of hepatic foci and nodules when coupled with a liver-
13 cell proliferative stimulus. Rats were given i.p. injections of aristolochic acids at 10
14 mg/kg b.w. 18 hours after undergoing a partial hepatectomy. After a 1-week recovery
15 period, the rats were divided into two groups, one maintained on the basal diet (control)

1 and the other on basal diet containing 1% orotic acid as a promoter. Rats were killed at 10
2 weeks or 10 months after exposure to aristolochic acids.

3 An i.p. dose of aristolochic acids at 10 mg/kg b.w. was not necrogenic to rat liver.
4 However, the second experiment demonstrated that the non-necrogenic dose of
5 aristolochic acids was capable of initiating hepatic foci. Glutathione-S-transferase 7-7
6 positive (GST⁺) foci were detected at 10 weeks in rats given orotic acid as a promoter. At
7 10 months, all rats in both groups exposed to aristolochic acids had GST⁺ foci, but the
8 incidence of liver nodules was higher in the promotion group (75%) than the control
9 group (14%) (Table 4-10). The authors reported that the nodules were histologically
10 similar to those generated by genotoxic carcinogens and that they exhibited significantly
11 higher incorporation of tritiated thymidine than the surrounding liver tissue; however, no
12 statistical analyses were reported.

Table 4-10. GST⁺ foci and nodules in livers of male F344 rats initiated with aristolochic acids (10 mg/kg b.w) after partial hepatectomy and promoted with 1% orotic acid^a

Exposure	No. of foci per cm ² ± SD ^b	% of rats with foci	% of rats with nodules ^c	No. of nodules per rat
AA + BD	7.8 ± 4.9	100	14	1
AA + OA	7.7 ± 4.0	100	75	4 ± 1

Source: Rossiello *et al.* 1993.

AA = aristolochic acids, BD = basal diet, OA = orotic acid.

^aRats received aristolochic acid 18 hours after 2/3 partial hepatectomy and were allowed to recover for one week before receiving diet with orotic acid (AA + OA group). Rats were killed at 10 months.

^bMeans of 5 to 6 animals.

^cThe number of animals was not reported.

13 4.4 Rabbits

14 Cosyns *et al.* (2001) noted that rats given aristolochic acids or a mixture of herbal drugs
15 in their study in Wistar rats (Cosyns *et al.* 1998) did not develop chronic nephrotoxicity,
16 despite developing digestive and urinary tract tumors. Therefore, they investigated the
17 chronic toxicity of aristolochic acids (44% aristolochic acid I and 56% aristolochic acid
18 II) in another animal model (female New Zealand White rabbits, 15 weeks old) to
19 determine whether aristolochic acids exposure would result in renal toxicity. The exposed
20 group included 12 rabbits administered i.p. injections of aristolochic acids at 0.1 mg/kg
21 b.w., 5 days per week for 17 to 21 months, and the control group included 10 rabbits

1 administered saline solution i.p. for 17 to 21 months. Blood and urine samples were
2 collected throughout the study. At sacrifice, histologic examinations were made of lung,
3 heart, liver, pancreas, spleen, stomach, intestine, kidney, adrenal gland, urinary bladder,
4 female genital tract, salivary gland, tongue, trachea, esophagus, brain, skin, skeletal
5 muscle, and any tissue with an abnormal appearance. One rabbit in the 17-month
6 exposure group died after 8 months and was not included in the analysis. All other
7 animals survived until sacrifice at 17 or 21 months. Because results were similar in the
8 17-month and 21-month exposure groups, the data were combined. Animals exposed to
9 aristolochic acids had fibrotic changes in the kidneys and stomach. Renal tumors were
10 observed in 2 rabbits (1 with renal-cell carcinoma and 1 with a tubulopapillary adenoma).
11 In addition, 1 rabbit developed a transitional-cell carcinoma of the ureter and an extensive
12 papillary malignant mesothelioma of the peritoneal cavity. No tumors occurred in the
13 control animals. The authors concluded that their study demonstrated for the first time
14 that chronic administration of aristolochic acids may induce renal fibrosis analogous to
15 the lesions observed in humans with AAN (see Section 5.2.2).

16 **4.5 Summary**

17 *4.5.1 Studies using aristolochic acids*

18 Aristolochic acids (administered orally or by injection) induced tumors at multiple sites
19 in mice, rats, and rabbits. Most studies administered a mixture of aristolochic acids I and
20 II; however, aristolochic acid I (used in two studies) also caused tumors. Many of these
21 studies used a small number of animals and were of relatively short duration; only a few
22 included statistical analyses. Moreover, the study authors did not always make it clear
23 which tumors they considered to be related to aristolochic acids exposure. [As a result of
24 these limitations, no clear difference in the spectrum of tumors induced by aristolochic
25 acid I vs. a mixture of aristolochic acids I and II was possible.] Table 4-11 summarizes
26 the results from studies that used aristolochic acids.

27 Only one study was conducted in mice. Female NMRI mice given aristolochic acids
28 orally at a dose of 5 mg/kg b.w. for 3 weeks developed forestomach, stomach, kidney,
29 lung, and uterine tumors and malignant lymphoma. The first tumors were observed at 26
30 weeks, and by week 56, all remaining mice had tumors.

1 Numerous studies were conducted in rats. Oral administration of aristolochic acids to rats
2 caused a dose- and time-dependent tumor response. Exposure to 50 mg/kg b.w. for 3 days
3 resulted in increased incidences of preneoplastic and neoplastic lesions of the kidney after
4 6 months. Rats exposed to lower doses by gavage over a longer period (1 to 10 mg/kg
5 b.w. for 3 to 6 months or 0.1 mg/kg b.w. for 12 months) developed a variety of tumors,
6 including those of the forestomach, kidney, renal pelvis, urinary bladder, ear duct,
7 thymus, small intestine, and pancreas. Single cases of hematopoietic system, heart, lung,
8 mammary gland, pituitary, and peritoneal tumors were reported. Male Wistar rats given
9 daily s.c. injections of aristolochic acids at 1 to 10 mg/kg b.w. for 35 days developed
10 urothelial carcinoma of the renal pelvis and malignant fibrohistiocytic sarcoma at the
11 injection site. A single i.p. injection of aristolochic acids at 10 mg/kg b.w. initiated liver
12 carcinogenesis in male F344 rats when coupled with a liver-cell-proliferative stimulus. In
13 12 female New Zealand White rabbits given i.p. injections of aristolochic acids at 0.1
14 mg/kg b.w. for 17 to 21 months, neoplastic lesions included 2 kidney tumors, a urinary-
15 tract tumor, and a mesothelioma of the peritoneal cavity.

Table 4-11. Summary of neoplastic lesions observed in experimental animals exposed to aristolochic acids

System or organ	Tumor type	NMRI mice	Sprague-Dawley rats	Wistar rats		BD-6 rats	Rabbits
		F	F	M	F	M	F
forestomach	papilloma	+		+	+	+	
	squamous-cell carcinoma	+		+	+	+	
stomach	adenocarcinoma	+ (1)					
kidney	adenoma	+		+	+		+ (1)
	adenocarcinoma, carcinoma or unspecified malignant tumor			+	+		+ (1)
	mesenchymal or oncocytoma		+				
	carcinoma of the renal pelvis			+			
urinary bladder or ureter	papilloma			+	+	+	
	carcinoma			+	+ (1)	+ (1)	+ (1)
lung	carcinoma	+		+ (1) ^a			
small intestine	sarcomas or adenocarcinoma			+	+		
thymus	thymoma					+	
ear duct	squamous-cell carcinoma			+			
mammary gland	carcinoma		+ (1)		+ (1)		
pancreas	undetermined morphology			+			
	squamous-cell carcinoma			+ (1) ^a			
heart	fibrosarcoma			+ (1)			
uterus	hemangioma	+					
pituitary gland	adenoma				+ (1)		
hematopoietic system	malignant lymphoma	+		+ (1)			
peritoneum	mesothelioma						+ (1)
skin	injection site fibrohistiocytic sarcoma			+			

+ = Observed in 2 or more exposed animals within a single study or observed across multiple studies.

+ (1) = Observed in only 1 treated animal in a single study and not observed in controls.

^aMetastatic tumor

- 1 4.5.2 Studies using extracts from *Aristolochia* species
- 2 Three studies were reviewed that investigated the carcinogenicity of extracts from
- 3 *Aristolochia* species (one study each of *A. manshuriensis*, *A. clematitis*, or *A. contorta*),
- 4 when administered orally or by injection. Tumors of the forestomach and kidney were the

1 most prevalent findings following oral administration. One study also reported tumors of
 2 the mammary gland, thyroid gland, and skin. Injection site polymorphocellular sarcomas
 3 also were reported in one study. Table 4-12 presents results for studies that used
 4 *Aristolochia* extracts.

Table 4-12. Summary of neoplastic lesions observed in experimental animals exposed to extracts from *Aristolochia* species

System or organ	Tumor type	Sprague-Dawley rats		Albino rats
		M	F	NR
forestomach	papilloma	+	+	
	squamous-cell carcinoma	+	+	
kidney	mesenchymal or oncocytoma		+	
	carcinoma of the renal pelvis	+		
	nephroblastoma		+ (1)	
mammary gland	ductal epithelial		+ ^a	
thyroid gland	follicular epithelium		+ ^a	
skin	appendage epithelial		+ ^a	
injection site	polymorphocellular sarcoma			+

+ = Observed in 2 or more exposed animals within a single study or observed across multiple studies.

+ (1) = Observed in only 1 treated animal in a single study and not observed in controls.

NR = not reported

^aThe number of animals with these tumors were not reported

5 **4.5.3 Studies using botanical products containing aristolochic acids**

6 Forestomach papillomas and squamous-cell carcinomas occurred in male rats given a
 7 weight-loss regimen of herbal ingredients that contained aristolochic acids but not in
 8 controls. One female rate exposed to the herbal mixture developed a forestomach
 9 papilloma; however, this tumor also occurred in two female rats in the control group.

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5 Other Relevant Data

The available epidemiological data for the carcinogenicity of aristolochic acids are reviewed in Section 3, and data from studies in experimental animals are reviewed in Section 4. Other types of data relevant to the evaluation of carcinogenic effects are reviewed below. These include data on absorption, distribution, metabolism, and excretion (Section 5.1), toxicity (Section 5.2), genetic damage and related effects (Section 5.3), and mechanistic studies and considerations (Section 5.4). The data reviewed in this section are summarized in Section 5.5.

5.1 Absorption, distribution, metabolism, and excretion

Aristolochic acids are absorbed following oral exposure, but no estimates or measurements of the relative amounts or absorption rates were located. All known human exposures are from ingestion of various herbal preparations that contained aristolochic acids, or in a clinical trial where volunteers were given aristolochic acids to measure their effect on the phagocytic activity of granulocytes. Other exposures occurred during other clinical trials mentioned in Section 2.1, in which aristolochic acids isolated from the alcoholic extract of *Aristolochia indica* were administered intravenously (i.v.) (Jackson *et al.* 1964). Aristolochic acids were administered orally in most of the experimental animal studies (see Section 4). No data are available on absorption following inhalation or dermal exposure.

DNA adduct data provide evidence of widespread tissue distribution. DNA adducts have been detected in kidney, ureter, bladder, lung, spleen, adrenal gland, liver, stomach, small intestine, and brain of patients exposed to aristolochic acids (Stiborová *et al.* 1999, Arlt *et al.* 2004b) and in the liver, lung, brain, kidney, bladder, forestomach, and stomach of exposed rats (Schmeiser *et al.* 1988).

In vitro metabolism studies suggest that aristolochic acid I is metabolized by oxidative and reductive pathways, while aristolochic acid II is metabolized only by a reductive pathway (Shibutani *et al.* 2007). Schmeiser *et al.* (1986) conducted *in vitro* metabolism studies of aristolochic acids under aerobic and anaerobic conditions. The major metabolites of aristolochic acids I and II incubated with rat liver S9 metabolic activation

1 under anaerobic conditions were the corresponding aristolactams; however, the metabolic
2 rates were different for the two aristolochic acid molecules. After 3 hours, only about
3 10% of aristolochic acid I, compared with about 60% of aristolochic acid II, was
4 metabolized. Under aerobic conditions, aristolochic acid II was not metabolized, and the
5 only metabolite formed from aristolochic acid I was its *O*-demethylated derivative
6 aristolochic acid Ia.

7 The major metabolites of aristolochic acids are produced from nitroreduction, *O*-
8 demethylation, and denitration (Chan *et al.* 2007a). Krumbiegel *et al.* (1987) conducted
9 studies on the metabolism of aristolochic acids I and II in male Wistar rats, female NMRI
10 mice, male guinea-pigs, male rabbits, male beagle dogs, and humans. Test animals
11 (numbers not specified) received a single oral dose of aristolochic acid I or II, and urine
12 and feces samples were collected for up to 72 hours. Doses of aristolochic acids I and II
13 were as follows: 3 mg in rats and guinea-pigs; 10 mg in rabbits, and 10 mg in dogs; mice
14 received aristolochic acid I at 30 mg/kg b.w. or aristolochic acid II at 85 mg/kg b.w. Six
15 healthy human volunteers were given a daily dose of 0.9 mg of a mixture of aristolochic
16 acids I and II for several days, and a 24-hour urine sample was collected on day 3. The
17 same pattern of metabolites was found in the urine of rats and mice, but fewer
18 metabolites were detected in other species, and no information on concentrations of the
19 metabolites was identified (Table 5-1). Most of the metabolites were reduction products
20 (e.g., aristolactams and aristolic acid I). Aristolactam Ia is produced by *O*-demethylation
21 of aristolactam I or by hydroxylation of aristolactam II (Chan *et al.* 2007a). Aristolactams
22 I and II are the only metabolites so far reported in human urine (Krumbiegel *et al.* 1987).
23 Using liquid chromatography/tandem mass spectrometry, Chan *et al.* (2006a) confirmed
24 the presence of aristolactams I, Ia, and II together with the two phenanthrenecarboxylic
25 acids in the urine of rats exposed to a mixture of aristolochic acids I and II by oral
26 administration. Chan *et al.* (2007a) also identified a new Phase I metabolite from the
27 decarboxylation of aristolochic acid I. In addition to these Phase I metabolites, Chan *et*
28 *al.* (2006a, 2007a) identified several Phase II metabolites in the urine of rats. These
29 included the *N*- and *O*-glucuronides of aristolactam Ia and the *N*-glucuronide of
30 aristolactam II (Chan *et al.* 2006a), and the *O*-glucuronide, *O*-acetate, and *O*-sulfate

1 esters of aristolochic acid Ia (Chan *et al.* 2007a). The Phase I metabolism of aristolochic
 2 acids I and II is illustrated in Figure 5-1.

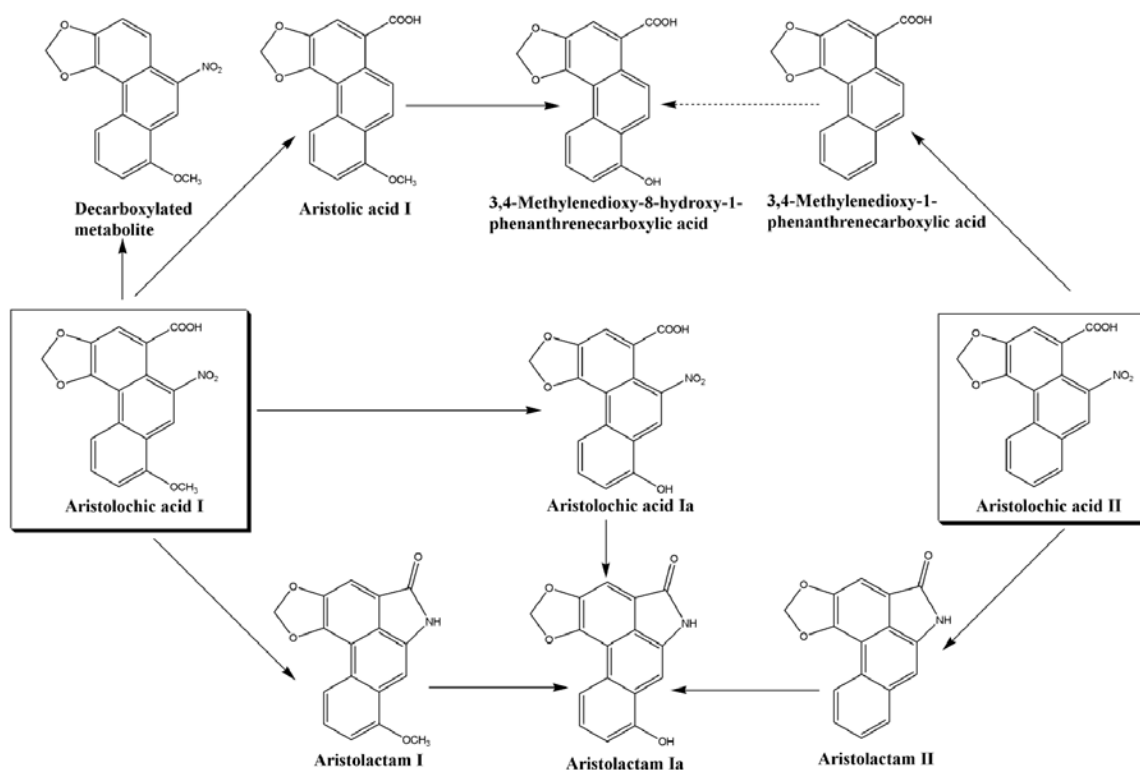


Figure 5-1. Phase I metabolism of aristolochic acids I and II in mammals

Source: Krumbiegel *et al.* 1987.

The dashed arrow indicates that the metabolite was found only after administration of the corresponding precursor.

3 Metabolites of aristolochic acids are excreted in the urine and feces (Krumbiegel *et al.*
 4 1987). The primary metabolite of aristolochic acid I was aristolactam Ia; the average
 5 proportion of the dose in rats was about 46% in the urine (mostly in a conjugated form)
 6 and 37% in feces. Several other minor metabolites of aristolochic acid I (generally
 7 occurring at trace levels to less than 5% of the administered dose) were identified (Table
 8 5-1). Aristolactam II was the primary metabolite of aristolochic acid II, with 4.6%
 9 recovered in the urine and 8.9% in the feces. In rats, metabolites of aristolochic acid I
 10 were excreted within 24 hours, while the metabolites of aristolochic acid II were still
 11 measurable in urine at 48 to 72 hours. Quantitative measurements of the metabolites in
 12 other species were not provided.

Table 5-1. Metabolites of aristolochic acids I and II

Metabolite	Rats & mice	Guinea-pigs	Rabbits	Beagles	Humans
Aristolochic acid I					
Aristolactam Ia	+	+	+	-	-
Aristolactam I	+	+	+	+	+
Aristolochic acid Ia	+	-	-	+	-
MDHPC	+	+	-	-	-
Aristolochic acid I	+	-	-	-	-
Aristolochic acid II					
Aristolactam II	+	+	+	+	+
MDPC	+	+	+	-	-
Aristolactam Ia	+	+	-	-	-

Source: Krumbiegel *et al.* 1987.

MDHPC = 3,4-methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid; MDPC = 3,4-methylenedioxy-1-phenanthrenecarboxylic acid; + = metabolite detected; - = metabolite not detected.

1 Ling *et al.* (2007) administered a single oral dose of 20 mg/kg aristolochic acid I to male
 2 Sprague-Dawley rats and plasma samples were collected at various intervals up to 24
 3 hours to measure concentrations of aristolactam I. Aristolactam I was still detected at 24
 4 hours. The reported half-life of aristolactam I was about 2.5 hours. The maximum
 5 concentration (22.4 µg/L) was reached at 0.5 hour.

6 Chen *et al.* (2007a) conducted a pharmacokinetic and nephrotoxicity (see Section 5.2.2)
 7 study of aristolochic acids in male New Zealand white rabbits. Two studies were
 8 conducted. In the first study, groups of six rabbits each were administered a single i.v.
 9 dose of 0.25, 0.5, 1.0, or 2.0 mg/kg of aristolochic acids (sodium salt) that contained 41%
 10 aristolochic acid I and 56% aristolochic acid II. In the second study, groups of rabbits
 11 were administered increasing i.v. doses (0.5, 1.0, and 2.0 mg/kg) at 7-day intervals.
 12 Plasma samples were collected at 5, 10, 15, 30, 45, 60, and 90 minutes, and 2, 3, 4, 6, 8,
 13 and 10 hours after dosing. Both aristolochic acids I and II were eliminated within 3 hours
 14 at all tested doses. There was a linear relationship between dose and the area under the
 15 plasma concentration curve. In the first study, the half-life for aristolochic acid I was 0.12
 16 hours and that for aristolochic acid II was 0.27 hours. In the second study, clearance rates
 17 for both compounds significantly decreased with escalating dose, and a nonlinear
 18 relationship between dose and the area under the plasma concentration curve was
 19 obtained.

1 **5.2 Toxicity**

2 The kidney is the primary target organ for aristolochic acids toxicity (Mengs and Stotzem
3 1993). As discussed in Section 3, aristolochic acids I and II have been causally linked to a
4 specific kidney disease known as AAN (formerly CHN). Cases of AAN have been
5 reported in a number of countries, including the United States. Two clinical variants of
6 AAN have been described that are characterized by subacute renal failure and adult-onset
7 Fanconi syndrome (Vanherweghem *et al.* 1993, Tanaka *et al.* 2001, Lee *et al.* 2004). This
8 section briefly discusses the toxicity of aristolochic acids in humans (Section 5.2.1) and
9 experimental animals (Section 5.2.2).

10 *5.2.1 Renal toxicity in humans*

11 IARC (2002) reviewed the toxic effects of *Aristolochia* species and aristolochic acids in
12 humans, and reported only effects on the kidney. The clinical spectrum of AAN has also
13 been reviewed by Nortier and Vanherweghem (2007). As noted in Section 2.1,
14 aristolochic acids were tested as an antitumor agent in mice that had been implanted with
15 Adenocarcinoma 755 (Kupchan and Doskotch 1962) and in a Phase I clinical trial
16 involving 20 patients with a variety of malignant tumors (Jackson *et al.* 1964). Although
17 an antitumor effect was reported in mice, aristolochic acids did not have any antitumor
18 effect in the clinical trial. However, it did result in abnormal renal function, with elevated
19 blood urea nitrogen in 8 of 10 patients treated with aristolochic acids at a dose of 1 mg/kg
20 b.w. per day for 3 or more days.

21 A few cases of acute renal failure resulting from an overdose of *A. manshuriensis* also
22 were reported in the Chinese literature between 1964 and 1999 (Li and Wang 2004), but
23 the disease known as AAN was first reported in about 100 patients in Belgium (all but 1
24 of whom were women) who had been treated at a weight-loss clinic and unintentionally
25 exposed to *Aristolochia fangchi* (see Section 3.1.1 and Table 3-1 for more details). Only
26 about 5% of the individuals exposed at the Belgian clinic developed AAN. However, the
27 kidney toxicity was severe in those 5%. AAN has a unique pathological picture marked
28 by anemia, mild tubular proteinuria, extensive hypocellular interstitial fibrosis, tubular
29 atrophy, global sclerosis of glomeruli decreasing from the outer to the inner cortex, and
30 rapid progression to end-stage renal disease (Vanherweghem *et al.* 1993, Cosyns 2003).

1 In one of the Belgian cases, fibrosis extended to the renal pelvis and ureters. Urothelial
2 lesions also were prominent and included urothelial atypia and atypical hyperplasia
3 (Cosyns *et al.* 1994b, Cosyns *et al.* 1999). End-stage renal failure occurred in some
4 patients 3 to 85 months after they stopped taking the pills and was followed by the
5 development of urothelial carcinoma (located primarily in the upper urinary tract) in 40%
6 to 46% of them within a few years after the end of the weight-loss program (Cosyns *et al.*
7 1999, Nortier *et al.* 2000).

8 Another clinical presentation of AAN was later reported in several case reports, mainly
9 from Asian nations, although one case was reported from Germany (see Section 3.1.2 and
10 Table 3-1 for more details). The patients (men and women ranging in age from 19 to 71
11 years) presented with Fanconi syndrome, which is characterized by proximal tubular
12 dysfunction, a generally slower progression to end-stage renal disease, and, in some
13 instances, a reversible clinical course.

14 Another form of endemic nephropathy that may be related to aristolochic acids exposure
15 is BEN (see Section 3.4). BEN is characterized by chronic renal interstitial fibrosis with
16 slow progression to end-stage renal disease and urothelial malignancy (Petronic *et al.*
17 1991, Radovanovic *et al.* 1991, Cosyns 2003, Stefanovic *et al.* 2006, Arlt *et al.* 2007).
18 This disease was first described about 50 years ago and occurs in rural areas of Bulgaria,
19 Bosnia, Croatia, Romania, and Serbia along the Danube river basin. Chronic dietary
20 intoxication from bread made with wheat flour contaminated with seeds of *A. clematitis*
21 has been suggested in the etiology of BEN (Ivic 1970, Hranjec *et al.* 2005, Grollman *et*
22 *al.* 2007). Grollman *et al.* (2007) reported that aristolochic acid adducts were found in the
23 DNA from the renal cortex of Croatian patients with BEN (see Section 5.3.1). Other
24 exposure agents that have been considered as possible etiologic agents in BEN include
25 heavy metals, arsenic, nitrogen species, silica, selenium deficiency, calcium and
26 magnesium deficiency, organic compounds leached from Pliocene lignite deposits,
27 viruses and bacteria, and mycotoxins (Voice *et al.* 2006). Of these, mycotoxins,
28 specifically ochratoxin A, have been the most studied (Kamp *et al.* 2005, Long and Voice
29 2007, Pfohl-Leszkowicz *et al.* 2007).

1 5.2.2 Toxicity in experimental animals

2 The acute and chronic toxicities of aristolochic acids and of herbal preparations
3 containing aristolochic acids have been investigated in a number of *in vivo* studies in rats,
4 mice, and rabbits; these studies demonstrated that the kidneys are the primary site of
5 toxicity, but effects on other organs, including the forestomach, lymphatic system, and
6 liver, have been observed (IARC 2002). The toxic effects of aristolochic acids and
7 botanical products containing aristolochic acids are reviewed below, including general
8 toxicity, non-renal effects, renal toxicity, and metabonomic studies. The reports reviewed
9 by IARC (Mengs 1987 for rats and mice, Mengs and Stotzem 1992, Mengs and Stotzem
10 1993, and Rossiello *et al.* 1993 for rats, and Cosyns *et al.* 2001 for rabbits) are briefly
11 reviewed below. Several of the studies (Mengs *et al.* 1982, Mengs 1983, Hadjiolov *et al.*
12 1993, Cosyns *et al.* 1998, Qiu *et al.* 2000, Debelle *et al.* 2002, and Cui *et al.* 2005 in rats
13 and the study by Mengs 1988 in mice) for which tumor results were reported in Section 4
14 also included information on biochemical or histological evidence of toxicity and are
15 discussed below. Additional reports of toxicity by Liu *et al.* (2003), Debelle *et al.* (2003,
16 2004), Cheng *et al.* (2006), Sun *et al.* (2006), and Pozdzik *et al.* (2007) for rats; by Sato
17 *et al.* (2004), Hu *et al.* (2004), and Shibutani *et al.* (2007) for mice; and by Ivic (1970)
18 and Chen *et al.* (2007a) for rabbits are also reviewed. Most of these studies used pure
19 preparations of aristolochic acids, but herbal preparations (either the plant parts
20 themselves or extracts of the plants) were used in the studies by Ivic (1970), Cosyns *et al.*
21 (1998), Liu *et al.* (2003), Hu *et al.* (2004), Sun *et al.* (2006) and Cheng *et al.* (2006).

22 *General toxicity*

23 Mengs (1987) determined LD₅₀ values for rats and mice exposed to aristolochic acids by
24 either oral or intravenous administration. The LD₅₀ value for aristolochic acids in Wistar
25 rats for oral administration was reported to be 203.4 mg/kg b.w. in males and 183.9
26 mg/kg b.w. in females, while the values for intravenous administration were 82.5 mg/kg
27 b.w. in males and 74.0 mg/kg b.w. in females. The LD₅₀ for aristolochic acids in NMRI
28 mice for oral administration was 55.9 mg/kg b.w. in males and 106.1 mg/kg b.w. in
29 females, while the values for intravenous administration were 38.4 mg/kg b.w. in males
30 and 70.1 mg/kg b.w. in females. Mengs noted that the results suggested that aristolochic
31 acids were slightly more toxic to mice than to rats.

1 Toxicity of aristolochic acids or botanical products containing aristolochic acids in organ
2 systems outside the kidney has also been reported. The toxic effects in the forestomach
3 and other organs are discussed here and renal toxicity is discussed below. Oral exposure
4 to aristolochic acids (usually a mixture of aristolochic acids I and II) caused similar toxic
5 effects in the forestomach of rats (Mengs *et al.* 1982, Mengs 1983, 1987, Hadjiolov *et al.*
6 1993), mice (Mengs 1987, 1988), and rabbits (Cosyns *et al.* 2001), primarily hyperplasia
7 and hyperkeratosis resulting from regeneration of the squamous epithelium. Fibrosis of
8 the gastric mucosa was also reported in the study in rabbits. Within the first 24 hours,
9 reddening of the forestomach mucosa developed in male Wistar rats, followed by
10 papillomatosis and occasional ulceration of the forestomach; histological examination
11 revealed papillomas of the squamous epithelium in addition to the regenerative changes
12 noted above by 14 days after exposure. The studies by Mengs (1983) and Hadjiolov *et al.*
13 (1993) in rats, Mengs (1988) in mice, and Cosyns *et al.* (2001) in rabbits also reported
14 tumor formation in the forestomach after exposure to aristolochic acids (see Section
15 4.2.2).

16 Mengs *et al.* (1982, 1987) also reported atrophy of the lymphatic organs (spleen and
17 thymus) in Wistar rats and NMRI mice. The effects on the lymphatic organs were
18 considered by the authors to be secondary toxic effects caused by the uremia induced by
19 severe renal damage. The adrenal glands were also affected, with some single-cell
20 necrosis; regressive changes were reported for the liver and duodenum; and
21 spermatogenesis was severely curtailed in the testes. In another study, Mengs and
22 Stotham (1992) reported that male Wistar rats exposed to aristolochic acid by gavage at
23 1.0, 5.0 or 25.0 mg/kg b.w. for 4 weeks developed mild testicular degeneration at 5.0
24 mg/kg and severe degeneration at 25.0 mg/kg.

25 The liver has not generally been reported as a target tissue for aristolochic acids toxicity,
26 but as discussed in Section 4.3, Rossiello *et al.* (1993) tested aristolochic acids
27 (unspecified as aristolochic acid I or a mixture) as an initiator together with liver-cell
28 proliferative stimuli (partial hepatectomy and orotic acid). They reported that GST⁺ foci
29 were increased in the two-stage model, but they concluded that aristolochic acids alone
30 were non-necrogenic to the rat liver, although they were capable of acting as initiating

1 agents. Mengs (1987) also noted that intravenous administration of aristolochic acids
2 resulted in severe necrotic lesions of the hepatic parenchyma, particularly in mice.

3 *Renal toxicity*

4 As in humans exposed to aristolochic acids (see Section 5.2.1), renal toxicity is the most
5 pronounced effect in experimental animals or livestock. Dumić (1954, cited in Grollman
6 *et al.* 2007) and Martinčić (1957) reported cases of aristolochic acids nephropathy in
7 horses that were fed hay contaminated with *A. clematidis*. Martinčić (1957) examined
8 kidneys from 26 horses that were poisoned by ingesting contaminated hay, and from 2
9 horses that were experimentally poisoned. Diffuse nephritic alterations of the cortical
10 tubules were reported while the glomeruli were unaffected. Inflammatory processes in the
11 interstitial tissue lead to cirrhosis of both kidneys. The renal toxicity studies in laboratory
12 animals, including details on study design and summaries of renal toxicity, are described
13 in Table 5-2. The major findings from these studies are summarized after the table. (See
14 Section 5.4.1 for mechanistic studies of toxicity.)

Table 5-2. Renal toxicity in experimental animals

Reference	Strain- sex (# animals)	Aristolochic acids preparation [route]	Exposure dose (mg/kg b.w.) [duration]	Renal toxicity- histology	Comments
Rats					
Mengs <i>et al.</i> 1982	Wistar-M (117, 4-18/group)/ F (117, 5-13/group)	aristolochic acids I (77.2%) and II (21.2%) [gavage]	0.1, 1, 10 [3, 6, 12 mo- low dose; 3 mo- mid/high dose]	All dose groups:renal cortex- atypical cells in tubular epithelium renal pelvis and urinary bladder- hyperplasia of transitional epithelium; renal carcinoma in mid/high dose groups \geq 3 mo after treatment.	no toxic effects observed in blood, plasma, or urine forestomach carcinoma was observed in the mid/high dose groups > 3 mo after treatment
Mengs 1987	Wistar-M (20, 10/group)/ F (20, 10/group)	aristolochic acids I (77.2%) and II (21.2%) [gavage]	M: 120–295 F: 150–300 [single dose, 21-d observation]	extensive tubular necrosis in renal cortex	LD ₅₀ (M, p.o.)= 55.9 mg/kg bw LD ₅₀ (F, p.o.)= 106.1 mg/kg bw LD ₅₀ (M, i.v.)= 38.4 mg/kg bw LD ₅₀ (F, i.v.)= 70.1 mg/kg bw Hyperplasia of the forestomach with po administration also was reported
		[i.v.]	M: 62–110 F: 38–86 [single dose, 21-d observation]		
Mengs and Stotzem 1992	Wistar- M (75, 15/group)	aristolochic acids I (77.2%) and II (21.2%) [gavage]	0.2, 1, 5, 25 [4 wk]	hyperplasia in urothelium at 1, 5, 25 doses; necrosis of renal tubular epithelium at high dose	toxic effects increased with dose two rats died in high dose group following renal failure degenerative changes in the testes were noted for the 5 and 25 mg/kg dose groups

Reference	Strain- sex (# animals)	Aristolochic acids preparation [route]	Exposure dose (mg/kg b.w.) [duration]	Renal toxicity- histology	Comments
Mengs and Stotzem 1993	Wistar-F (32, 8/group)	aristolochic acids I (77.2%) and II (21.2%) [gavage]	10, 50, 100 [single dose, 3-d observation]	necrosis of renal tubular epithelium; dose-dependent renal damage	significantly increased serum creatinine and urea at high dose
Cosyns <i>et al.</i> 1998	Wistar-M (12, 6/group)/ F (12, 6/group)	aristolochic acid mixture (% NR) [gavage]	10 [5 d/wk for 3 mo, 3-mo follow-up]	multifocal areas of tubulointerstitial fibrosis- 2/4 M (1/7 control M); not significantly different authors concluded that AA did not induce renal fibrosis	serum creatinine within normal limits
Cosyns <i>et al.</i> 1998	Rats Wistar-M (11, 4-7/group)/ F (9, 4-5/group)	weight-loss regimen with <i>S. tetrandra</i> [gavage (in olive oil)]	0.15 (70 mg <i>S. tetrandra</i> powder) [5 d/wk for 3 mo, 11-mo follow-up]	multifocal areas of tubulointerstitial fibrosis observed in 2/4 treated and 1/7 controls (not significant), no evidence of parenchymal fibrosis	treatment also included other components of weight-loss regimen serum creatinine within normal limits
Qiu <i>et al.</i> 2000	Sprague-Dawley-F (100, 30-40/group)	<i>A. manshuriensis</i> decoction [oral]	A) 50 g/kg/d [7 d] B) 30 g/kg/d [7 d] C) 15 g/kg/d [15 d] all groups followed for 1, 3, and 6 mo	acute tubular necrosis, particularly at corticomedullary junction at end of treatment, with some recovery at 1 and 3 mo and nearly complete at 6 mo	serum creatinine increased significantly at highest dose (group A)

Reference	Strain- sex (# animals)	Aristolochic acids preparation [route]	Exposure dose (mg/kg b.w.) [duration]	Renal toxicity- histology	Comments
Debelle <i>et al.</i> 2002	Wistar-M (66, 6-7/group)	aristolochic acids I (40%) and II (60%) [s.c.]	1, 10 [5 wk]	low dose: slight tubular atrophy on day 10 high dose: tubular necrosis and atrophy with lymphocytic infiltrates on day 10 with severe interstitial fibrosis on day 35	salt depletion induced by furosemide and low-salt, normal protein diet
Liu <i>et al.</i> 2003	Wistar-F (111, 5-10/group)	aristolochic acids I (63%) and II (31%) [oral]	2 mg twice a day [5 d with follow-up for 8, 12, 16 wk]	tubular necrosis in cortex and outer medulla: none at 8 weeks, moderate at 12 weeks, severe at 16 weeks	serum creatinine significantly ($P < 0.05$) increased
		decoction of <i>A. manshuriensis</i> , containing 1 mg aristolochic acid per g of botanical product [oral]	0.2 g, 2 g twice a day [5 d]	low dose: no histological changes high dose: severe tubular necrosis in cortex and outer medulla	serum creatinine significantly ($P < 0.001$) increased at high dose
Cui <i>et al.</i> 2005	Sprague-Dawley-F (44, 3-14/group)	aristolochic acid I (95% purity) extracted from <i>A. manshuriensis</i> [gavage]	50 [3 d, with follow-up for 8 d, 1 mo, 3, mo, or 6 mo]	acute tubular necrosis, focal loss of brush borders, and desquamation of tubular epithelial cells, particularly at corticomedullary junction tubular necrosis was seen at 8 days and at 1 mo but recovered at 3 and 6 mo	plasma creatinine and urea significantly higher at 8 d; returned to normal at 1, 3, and 6 mo

Reference	Strain- sex (# animals)	Aristolochic acids preparation [route]	Exposure dose (mg/kg b.w.) [duration]	Renal toxicity- histology	Comments
Sun <i>et al.</i> 2006	Wistar-F (54, ≥ 8/group)	decoction of <i>A. manshuriensis</i> [gavage, twice a day]	10 mL/kg/d ^a (0.58 mg aristolochic acid I/mL) [8 wk with 8, 12, or 16 wk follow-up]	multifocal tubulointerstitial fibrosis, and tubular atrophy in the medullary rays, deep cortex, and outer medulla interstitial fibrosis increased from no significant fibrosis at 8 weeks to moderate fibrosis at 12 weeks and severe fibrosis at 16 weeks	significant increases in blood urea nitrogen (BUN) and serum creatinine at week 8 ($P < 0.05$), week 12 ($P < 0.01$) and week 16 ($P < 0.01$)
Cheng <i>et al.</i> 2006	Wistar- NS (35, 5-10/group)	aristolochic acids I (58%) and II (36%) [gavage]	10 [5 d/wk for 12 wk, 12 wk follow-up]	no histology reported	chronic renal failure was induced by 5/6 nephrectomy significantly increased serum creatinine
Hwang <i>et al.</i> 2006	Sprague-Dawley- M,F (80, 10 each sex/group)	extract of fruit of <i>A. contorta</i> [gavage]	21.35, 213.5, 2135 [90 d]	Nephrotoxicity (interstitial fibrosis and nephritis, renal tubular necrosis and hyperplasia, hyperplasia and carcinoma in the renal pelvis)	Effects primarily observed in high dose group; however, renal tubular necrosis observed in all treatment groups.
Pozdzik <i>et al.</i> 2007	Wistar- M (60/group)	NR [s.c.]	10 (daily) [1–35 d. rats (6 per group) killed on days 1, 2, 3, 4, 5, 7, 10, 14, 18, & 35]	Acute tubular necrosis, progressive tubular atrophy, and tubulointerstitial fibrosis	Proximal tubular epithelial cells took on a more mesenchymal phenotype and lost some of their epithelial cell phenotype as evidenced by the gain or loss of various cell type markers (E-cadherin, N-cadherin, neutral endopeptidase, vimentin).
Mice					
Mengs 1987	NMRI- M (20, 10/group)/ F (20, 10/group)	aristolochic acids I (77.2%) and II (21.2%) [gavage]	M: 10–70 F: 60–120 [single dose; 21-d follow-up]	kidney- extensive tubular necrosis in cortex	LD ₅₀ (po)= 55.9 (m); 106.1 (f) (mg/kg bw) LD ₅₀ (iv)=38.4 (m); 70.1 (f) (mg/kg bw)

Reference	Strain- sex (# animals)	Aristolochic acids preparation [route]	Exposure dose (mg/kg b.w.) [duration]	Renal toxicity- histology	Comments
	10/group)	[i.v.]	M: 17–102 F: 40–125 [single dose; 21-d follow-up]		bw)
Shibutani <i>et al.</i> 2007	C3H/He mice (M) (10/group)	1) aristolochic acid I [i.p. in saline] 2) aristolochic acid I [gavage] 3) aristolochic acid II [i.p. in saline] 4) aristolochic acid II [gavage]	2.5 [9 d, killed on day 10 or 24]	kidneys of p.o. AAI-treated mice were pale at day 10 with acute tubular necrosis and extensive cortical interstitial fibrosis kidneys of AAI-treated mice had no significant histologic differences compared to controls	AAI appears to be responsible for the nephrotoxicity associated with AAN route of administrations did not significantly affect outcome

Reference	Strain- sex (# animals)	Aristolochic acids preparation [route]	Exposure dose (mg/kg b.w.) [duration]	Renal toxicity- histology	Comments
Sato <i>et al.</i> 2004	BALB/c-M (160, 10-40/group) C3H/He-M (160, 10-40/group) C57BL/6-M (120, 20-40/group)	1) aristolochic acids I (55%) and II (45%) mixture [i.p. in oil] 2) aristolochic acids I (70%) and II (25%) sodium salt mixture [i.p. in saline] 3) aristolochic acids sodium salt mixture [gavage in distilled water] 4) aristolochic acid I [i.p. in oil] 5) aristolochic acid II [i.p. in oil] ^b	2.5 [5 d/wk for 2 wk; follow-up for 1 d or 14 d; aristolochic acid-injected mice also sacrificed one day after 1, 3, 6, or 9 injections]	BALB/c: acute tubular necrosis C3H/He: acute tubular necrosis with interstitial fibrosis C57BL/6: mild and focal tubulointerstitial changes	more severe tubulointerstitial changes were induced by i.p. injection serum creatinine and BUN increased significantly ($P < 0.05$) in BALB/C and C3H/He but not in C57BL/6 mice with aristolochic acid treatment serum creatinine and BUN increased significantly ($P < 0.05$) in BALB/C and C3H/He mice injected with aristolochic acids sodium salt compared to aristolochic acid aristolochic acid I strongly nephrotoxic in BALB/C and C3H/He mice, while aristolochic acid II induced focal mild interstitial change aristolochic acid IVa and aristolactam I were not nephrotoxic
Hu <i>et al.</i> 2004	NIH- M (64, 8/group)/ F (64, 8/group)	<i>A. manshuriensis</i> from 3 Chinese counties or provinces aristolochic acid contents of <i>A. manshuriensis</i> : ranged from 0.45% to 1.06% [oral]	HZ: 1, 2, 4 g/kg/d JL: 1 g/kg/d YQ: 1 g/kg/d [8 wk]	renal tubular hydropic changes observed in treatment groups and in controls	authors suggested that renal changes in both treated and controls groups could be due to technical problem during tissue processing renal function not affected by herbal extracts LD ₅₀ values calculated for extracts, but no correlation found with aristolochic acid contents
Rabbits					

Reference	Strain- sex (# animals)	Aristolochic acids preparation [route]	Exposure dose (mg/kg b.w.) [duration]	Renal toxicity- histology	Comments
Chen <i>et al.</i> 2007a	New Zealand white-M (6/group)	aristolochic acids I (44%) and II (56%) [i.v.]	0.25, 0.5, 1.0, or 2.0 [single injection, i.v.; killed on d 1 and 7.]	moderate to severe proximal tubular atrophy, hyaline cylinders in the distal tubules, interstitial fibrosis, necrosis	Renal tubular damage was progressive and dose-dependent
Cosyns <i>et al.</i> 2001	New Zealand white-F (22, 5-6/group)	aristolochic acids I (44%) and II (56%) [i.p.]	0.1 [5 d/wk for 17 -21 mo]	All treated animals had hypocellular interstitial fibrosis and urothelial atypia.	3/10 AA-treated animals developed tumors in urinary tract
Ivic 1970	Rabbits NS	<i>Aristolochia</i> seeds in wheat flour [oral] 20 mg/kg of mixture	NR [8-14 mo.]	Proteinuria noted at 1 mo exposure; renal interstitial fibrosis	no urothelial atypia or carcinoma noted by the authors

HZ = Hanzhong, Shanxi Province; JL = Changbai County, Jilin Province; YQ = Yuanqu County, Shanxi Province; NR = not reported; NS = not specified; po = *per os* (by mouth).

^aExperimental protocol reports dose as 10 mL/kg/day (twice a day). [It is not clear if the total dose was 10 mL or 20 mL per kg per day.]

^bAristolochic acid IVa and aristolactam I were also tested in this study, and were reported to have no nephrotoxic effect (data not shown).

1 The primary histological finding in the kidneys is severe renal tubular necrosis, which is
2 generally most pronounced at the cortico-medullary junction (Mengs *et al.* 1982, Mengs
3 1987, Qiu *et al.* 2000, Cui *et al.* 2005). Atypical cells in the tubular epithelium of the
4 renal cortex and hyperplasia of the transitional epithelium of the renal pelvis and urinary
5 bladder have also been reported. Mengs and Stotzem (1993) reported that renal lesions
6 developed within 3 days in female rats after oral exposure to aristolochic acids, and the
7 toxicity increased in severity with increasing dose.

8 Pozdzik *et al.* (2007) conducted a detailed study of the time course of kidney damage in
9 male Wistar rats exposed to aristolochic acids (10 mg/kg b.w.) by daily s.c. injections for
10 up to 35 days. This study arbitrarily distinguished two phases of aristolochic acids
11 toxicity: an acute phase marked by transient necrosis of the proximal tubular epithelial
12 cells from day 1 to day 5, and a chronic phase marked by progressive tubular atrophy,
13 leukocyte infiltration, and tubulointerstitial fibrosis from day 7 to day 35. On day 35, foci
14 of collagen I/III deposition indicative of tubulointerstitial fibrosis were found near the
15 external portion of the medullary rays. The renal interstitium was also found to be highly
16 infiltrated by leukocytes consisting of monocytes/macrophages and CD8+ lymphocytes
17 that accumulated around damaged proximal tubular epithelial cells.

18 Biochemical tests have confirmed the renal toxicity of aristolochic acids in many, but not
19 all, studies in rats. As noted in Table 5-2, increased serum or plasma creatinine was
20 reported in studies by Mengs and Stotzem (1993), Qiu *et al.* (2000), Debelle *et al.* (2002),
21 Liu *et al.* (2003), Cui *et al.* (2005), and Cheng *et al.* (2006). These studies used either
22 aristolochic acid I (Cui *et al.*), a mixture of aristolochic acids I and II (Debelle *et al.*, Liu
23 *et al.*), a decoction of *A. manshuriensis* (Liu *et al.*, Qiu *et al.*), or aqueous extracts of the
24 fruit of *A. contorta* (Hwang *et al.* 2006). In a time-course study in Sprague-Dawley rats
25 exposed to aristolochic acid I, Cui *et al.* (2005) reported that plasma urea and creatinine,
26 urine volume, and urinary glucose, protein, and *N*-acetyl- β -glucosaminidase were
27 significantly higher in exposed rats than in controls at day 8; however, all these
28 parameters returned to their normal levels at 1, 3, and 6 months. Some studies, however,
29 have not found the same biochemical changes after aristolochic acids exposure. Mengs *et*
30 *al.* (1982) reported that no biochemical evidence of aristolochic acids toxicity was seen in

1 blood, plasma, or urine of male and female Wistar rats after 3 months of exposure to
2 mixture of aristolochic acids I and II. In the study by Cosyns *et al.* (1998), neither a
3 mixture of aristolochic acids I and II nor the weight-loss regimen of herbal ingredients
4 containing aristolochic acids that was used in the Belgian clinic altered serum creatinine
5 levels.

6 The rapidly progressive interstitial fibrosis of the kidney observed in the individuals that
7 developed herbal medicine nephropathy in the Belgian clinic and other reports has been
8 seen in several studies with rats (Debelle *et al.* 2002, Debelle *et al.* 2003, 2004, Liu *et al.*
9 2003, Sun *et al.* 2006), but not in others (Cosyns *et al.* 1998, Qiu *et al.* 2000, Cui *et al.*
10 2005). Debelle *et al.* (2002) reported that Wistar rats injected s.c. with a mixture of
11 aristolochic acids I and II (10 mg/kg/b.w.) together with furosemide and a low-salt,
12 normal protein diet to produce salt depletion, developed nephropathy, including tubular
13 atrophy and interstitial fibrosis. In the study by Liu *et al.*, no interstitial fibrosis was
14 observed in the rats at 8 weeks after treatment, but at 12 weeks moderate interstitial
15 fibrosis was found ($P < 0.01$ vs. control), and at 16 weeks the fibrosis was severe ($P <$
16 0.01 vs. control). However, Cosyns *et al.* (1998) reported that no fibrosis of the renal
17 interstitium (or any type of renal toxicity) was induced in Wistar rats by exposure for 3
18 months to either a mixture of aristolochic acids I and II or the weight-loss regimen of
19 herbal ingredients containing aristolochic acids that was used in the Belgian clinic. Cui *et*
20 *al.* (2005) reported that oral administration of aristolochic acid I did not cause interstitial
21 fibrosis in Sprague-Dawley rats; however, it did cause renal toxicity, including tubular
22 necrosis, focal loss of brush borders, and desquamation of tubular epithelial cells,
23 predominantly at the corticomedullary junction. [The differences in induction of
24 interstitial fibrosis in studies in rats may be related to the differences in route of
25 administration in the studies, i.e., oral, s.c., or i.p.].

26 Pozdzik *et al.* (2007) investigated the cellular mechanisms responsible for the
27 pathophysiology associated with aristolochic acids toxicity and hypothesized that
28 sustained intoxication by aristolochic acids results in altered regeneration of proximal
29 tubular epithelial cells and apoptosis leading to subsequent irreversible proximal tubular
30 atrophy. They reported that the toxicity of aristolochic acids to kidney tubules resulted in

1 defective activation of antioxidant enzymes (based on a decline in antioxidant catalase
2 and Cu/Zn-SOD activities) and mitochondrial damage (based on increased cytoplasmic
3 staining for cytochrome c released from the internal mitochondrial membrane). They
4 concluded that the accumulation of cells in the interstitial areas that were positive for
5 vimentin and α -SMA (both proteins are mesenchymal phenotype markers) and expressed
6 transforming growth factor- β (a cytokine capable of stimulating fibroblast proliferation,
7 collagen deposition, and epithelial to mesenchymal transition) suggested that resident
8 peritubular fibroblasts were increased in number and were activated into myofibroblasts.
9 Proximal tubular epithelial cell proliferation increased based on Ki-67- or PCNA-positive
10 staining (markers for DNA damage repair and cell proliferation), and these cells showed
11 signs of dedifferentiation toward a mesenchymal phenotype as evidenced by decreased
12 staining for the epithelial phenotype markers, E-cadherin, N-cadherin, and neutral
13 endopeptidase, as well as an increased staining for vimentin. They considered the
14 resulting activated resident fibroblasts to be the main source of collagen deposition in the
15 development of interstitial fibrosis during experimental AAN. They further concluded
16 that epithelial-to-mesenchymal transition, which has been proposed as an important event
17 in native and transplant kidney injury, could be restricted to the thickening of the
18 basement membrane in AAN.

19 Renal fibrosis has also been reported in both rabbits (Ivic 1970, Cosyns *et al.* 2001, Chen
20 *et al.* 2007a) and mice (Sato *et al.* 2004). Cosyns *et al.* (2001) reported that New Zealand
21 White female rabbits exposed to a mixture of aristolochic acids I and II by i.p. injection
22 developed renal hypocellular interstitial fibrosis decreasing from the outer to the inner
23 cortex and urothelial atypia. The authors noted that acute nephrotoxicity from aristolochic
24 acids exposure appeared similar in humans and rabbits but was less in rats and mice. In
25 this study, tumors of the urinary tract and peritoneal cavity were observed (see Section 4).
26 Chen *et al.* (2007a) reported that progressive and dose-dependent tubular damage
27 occurred in male New Zealand White rabbits exposed to aristolochic acids administered
28 as single i.v. doses of 0.25 to 2 mg/kg. In another study in rabbits, feeding of *Aristolochia*
29 seeds for 11 months caused renal interstitial fibrosis similar to that seen in Balkan

1 endemic nephropathy (BEN) (see Section 3.4) [presumably due to the toxicity of
2 aristolochic acids], but no urothelial atypia or carcinoma was reported (Ivic 1970).

3 Sato *et al.* (2004) showed distinct strain differences in the nephrotoxicity of aristolochic
4 acids. A rapidly progressive and severe tubular necrosis occurred in BALB/c and
5 C3H/He mice, while only mild and focal tubulointerstitial changes were reported in
6 C57BL/6 mice. Interstitial fibrosis with mononuclear cell infiltration was most severe in
7 C3H/He mice; however, all three strains showed tubulointerstitial damage without
8 glomerular injury. The authors suggested that differences in metabolism or detoxification
9 may explain the toxicity differences among the strains.

10 It is of interest that the dose levels of aristolochic acids required to induce acute tubular
11 necrosis in rats and mice (20 and 30 mg/kg, respectively) (Mengs 1987) are higher than
12 the dose levels needed in rabbits or humans (around 1 mg/kg), indicating interspecies
13 differences in sensitivity (Mehes *et al.* 1958, Jackson *et al.* 1964, as cited in Cosyns
14 2003). In addition, dogs, cats, frogs, and porpoises seem to be resistant to the acute
15 toxicity of aristolochic acids (Mehes *et al.* 1958, as cited in Cosyns *et al.* 2003).

16 Several studies compared renal toxicity induced by different aristolochic acids, or by
17 aristolochic acids versus the herbal product or component of the herbal products. Sato *et al.*
18 *et al.* (2004) reported that aristolochic acid I was shown to have a much stronger
19 nephrotoxic effect than aristolochic acid II in mice. Shibutani *et al.* (2007) reported that
20 aristolochic acid I but not II caused acute tubular necrosis and extensive cortical
21 interstitial fibrosis in C3H/He mice exposed by i.v. or oral administration. Hu *et al.*
22 (2004) compared the toxicity of *A. manshuriensis* collected from three different areas in
23 China, but renal tubular toxicity was seen in treated groups and controls, possibly due to
24 technical problems with tissue processing. In order to determine the contribution of
25 aristolochic acids to the nephrotoxicity of *A. manshuriensis*, Liu *et al.* (2003) compared
26 the nephrotoxicity of a mixture of aristolochic acids I and II and decoctions of *A.*
27 *manshuriensis* and *Akebia quinata* (which has a chemical composition similar to that of
28 *A. manshuriensis* but does not contain aristolochic acids) in female Wistar rats. Rats
29 exposed to *A. manshuriensis* at the high dose or to aristolochic acids developed

1 progressive tubular damage, decreased renal function, and increased urinary protein
2 excretion. The concentrations of aristolochic acids detected in the serum, urine, and
3 kidney were comparable in these two groups. The authors concluded that the renal
4 toxicity of *A. manshuriensis* was attributable to its aristolochic acids content because no
5 renal toxicity was observed with *A. quinata*. Finally, DeBelle *et al.* (2002) demonstrated
6 that dexfenfluramine, another component of the weight-loss regimen used in the Belgian
7 clinic, did not enhance the nephrotoxic effects of aristolochic acids in their salt-depletion
8 model (see above).

9 *Metabonomic studies*

10 Metabonomic studies, which produce a total profile or “fingerprint” of multiple
11 metabolites present in biological samples such as urine or blood (see also definition in
12 Glossary), show that the renal proximal tubule is the primary target of aristolochic acids
13 in rats (Chen *et al.* 2006a, Zhang *et al.* 2006a, Ni *et al.* 2007). Elevated serum urea and
14 creatinine levels and urinary protein and glucose indicated nephrotoxicity in male Wistar
15 rats exposed to 10 mg/kg b.w. aristolochic acids [not specified, but likely a mixture of I
16 and II] for 5 days (Zhang *et al.* 2006a). Furthermore, increased activity of gamma
17 glutamyl transferase (γ -GT) and *N*-acetyl- β -D-glucosaminidase (NAG) occurred in rats
18 exposed to aristolochic acids, which the authors interpreted as resulting from a lesion of
19 the renal duct epithelial cells. Chen *et al.* (2006a) observed consistent differences among
20 the urinary metabolite profiles of male Wistar rats treated with aristolochic acids (a single
21 oral dose of 50 mg/kg b.w. of material described as an authentic standard obtained from
22 the National Institute for the Control of Pharmaceutical and Biological Products in
23 Beijing, China) or with a water extract of dried and pulverized *A. manshuriensis* (extract
24 of 30 g/kg b.w. per day; equivalent to 96 mg/kg b.w. per day of aristolochic acids)
25 compared with controls. The changes in metabolic patterns with either aristolochic acids
26 or the plant extract were associated with rapidly progressive renal failure.

27 Ni *et al.* (2007) expanded the work of Chen *et al.* (2006a) by combining GC-MS and LC-
28 MS to monitor urinary metabolites in male Wistar rats exposed to aristolochic acids and
29 suggested that metabolic profiling could help unravel the pathological outcomes of
30 aristolochic acids–induced nephrotoxicity. Compared with controls, rats exposed to

1 aristolochic acids had reduced urinary excretion levels of crucial substances of the
2 tricarboxylic acid cycle (citrate, aconitate, isocitrate, and succinate), fatty acids (caprylic
3 acid, valeric acid, and arachidonic acid), *m*-hydroxyphenylpropionate, and methionine.
4 Elevated levels of some amino acids (serine, cystine, cysteine, and homocysteine) and
5 phenyl-containing compounds (*p*-cresol and *p*-hydroxyphenylacetate) were detected in
6 the treatment group. The authors concluded that aristolochic acids–induced acute renal
7 toxicity may be characterized by systemic alterations of metabolic networks involving
8 free fatty acids, energy and amino acid metabolism, and alteration in the structure of gut
9 microbiota.

10 5.2.3 Toxicity to kidney or urinary tract cells *in vitro*

11 Balachandran *et al.* (2005) examined the structure-activity relationships of aristolochic
12 acid analogues based on cytotoxicity as assessed by the neutral red assay *in vitro*. This
13 study tested both cultured proximal tubular cells from pig kidney (LLC-PK₁) and a
14 human epithelial breast cell line (BT-549). More than 20 compounds were tested,
15 including aristolochic acids I, Ia, 7-OH I, II, III, IVa, VIIa, C (IIIa), and D (V), aristolic
16 acid, and seven aristolactam derivatives. Aristolochic acid I was by far the most toxic to
17 LLC-PK₁ cells, followed by aristolochic acids VIIa, II, and Ia. None of the other
18 compounds were toxic to LLC-PK₁ cells. Aristolochic acids were not toxic to BT-549
19 cells, which the authors interpreted as indicating that the cytotoxic action is specific to
20 the kidney. They also concluded that the ring structures, side chains, and location of the
21 side chains are critical determinants of toxicity and that the nitro group (–NO₃) and the
22 methoxy group (–OCH₃) in the locations that they occupy in the aristolochic acid I
23 molecule are associated with maximum toxicity. The authors concluded that any
24 additions, deletions, substitutions, or replacement of the positions of the side chains
25 drastically reduced toxicity.

26 Two other studies reported the cytotoxicity of a series of aristolochic acids and
27 aristolactam derivatives isolated from *Aristolochia contorta*, based on lactate
28 dehydrogenase leakage in the human proximal tubular epithelial cell line HK-2 (Zhang *et al.*
29 *et al.* 2005b, Wen *et al.* 2006). Both Zhang *et al.* and Wen *et al.* tested aristolochic acids I,
30 II, IVa, Va, and 9-OH I and aristolactams I, II, IVa, 7-methoxy IV, and 9-OH I; Wen *et*

1 *al.* also tested 7-OH aristolochic acid III methyl and 5-methoxyl aristolactone I.
2 Aristolochic acid I was cytotoxic to HK-2 cells, but the strongest cytotoxic response in
3 both studies was with 7-methoxy-aristolactam IV. In addition, Wen *et al.* reported
4 significant cytotoxicity of aristolactam I and aristolactam IVa, and Zhang *et al.* noted that
5 aristolochic acid I, aristolactam I, and aristolactam IVa showed moderate cytotoxicity,
6 but they did not report any statistical analyses. Wen *et al.* also carried out MTT assays for
7 metabolic capability and morphological assessments, which suggested that cell injury
8 likely involved interactions with cell membranes and intracellular structures such as
9 lysosomes and mitochondria.

10 The cytotoxicity results reported by Wen *et al.* and Zhang *et al.* differed from those of
11 Balachandran *et al.*, in which aristolochic acid I was the most toxic substance tested;
12 however, the investigators used different cytotoxicity assays, and the specific molecules
13 tested differed considerably between the Balachandran *et al.* study and the studies by
14 Zhang *et al.* and Wen *et al.* Balachandran *et al.* did not test 7-methoxy aristolactam IV,
15 which was the most toxic molecule in the Wen *et al.* and Zhang *et al.* studies, and Wen *et*
16 *al.* and Zhang *et al.* did not test aristolochic acid Ia, which was one of four molecules
17 reported by Balachandran *et al.* to be toxic. However, all three studies included
18 aristolactams I and IVa, and Balachandran *et al.* did not find them to be cytotoxic,
19 whereas the other two studies did. The Zhang *et al.* and Wen *et al.* studies used only a
20 renal cell line and thus did not compare cytotoxicity between different cell types, as did
21 Balachandran *et al.*

22 The cytotoxic effects of aristolochic acids on renal tubular cells may be linked to its
23 effects on intracellular calcium concentrations (Hsin *et al.* 2006). This study
24 demonstrated that aristolochic acids caused a rapid rise in intracellular calcium levels of
25 cultured renal tubular cells. The increased calcium levels caused stress to the
26 endoplasmic reticulum and mitochondria resulting in activation of caspases and
27 apoptosis. Aristolochic acids–induced apoptosis can be suppressed by calcium
28 antagonists, thus supporting a critical role of intracellular calcium levels in aristolochic
29 acids cytotoxicity.

1 Zhang *et al.* (2007) investigated the feasibility of predicting liver and kidney target-organ
2 toxicity by testing the *in vitro* cytotoxicity of selected chemicals (known hepatotoxicants
3 and nephrotoxicants) in human hepatoma (Bel-7402) cells and human renal tubular
4 epithelial (HK-2) cells. Aristolochic acids were among the selected nephrotoxicants. All
5 selected chemicals disrupted mitochondrial permeability transition (MPT) in a dose-
6 dependent manner. In most cases the *in vitro* cytotoxicity was higher in liver cells for
7 hepatotoxicants and higher in kidney cells for nephrotoxicants. However, aristolochic
8 acids showed higher cytotoxicity to liver cells than kidney cells. The authors attributed
9 this discrepancy to the absence of toxicokinetic processes of the whole organism in the
10 cell culture system.

11 Qi *et al.* (2007) reported that the MPT is involved in aristolochic acids–induced renal
12 injury. MPT plays an important role in drug-induced necrosis and apoptosis. Rat kidney
13 mitochondria were isolated and exposed to aristolochic acid I (10 to 50 μM) for up to 20
14 minutes. Mitochondrial swelling, leakage of Ca^{2+} , membrane depolarization, and release
15 of cytochrome c occurred in isolated kidney mitochondria exposed to aristolochic acid I
16 in the presence of Ca^{2+} . Qi *et al.* also exposed human renal tubular epithelial cells (HK-2)
17 to aristolochic acid I at 10 or 25 μM for 24 hours. There was a decrease in cellular ATP,
18 mitochondrial membrane depolarization, cytochrome c release, and an increase in caspase
19 3 activity. These effects were attenuated by MPT inhibitors.

20 Chang *et al.* (2007b) investigated the impact of aristolochic acids on human urinary tract
21 epithelial cells (SV-HUC-1). Cultured cells were exposed to 0.0125 to 0.2 mM
22 aristolochic acids (a mixture of 41% aristolochic acid I and 56% aristolochic acid II) for
23 1, 3, or 5 days. There was a concentration-dependent growth inhibition with an
24 accumulation of cells in the G_0/G_1 phase. Cell-cycle control proteins (p53, p21, and p27)
25 increased in a dose-dependent manner. The authors concluded that aristolochic acids
26 induce cell cycle arrest in SV-HUC-1 cells.

27 Aristolochic acids are specific inhibitors of phospholipase A_2 , blocking the enzymatic
28 activity of purified snake venom (*Vipera russelli*) *in vitro* with a K_i of 9.9×10^{-4} M
29 (Vishwanath and Gowda 1987). Aristolochic acids also are dose-dependent inhibitors

1 (half-maximal inhibitory concentration [IC₅₀] = 40 μM) of phospholipase-dependent
2 release of arachidonate from phosphatidyl choline or phosphatidyl inositol in human
3 neutrophils *in vitro* (Rosenthal *et al.* 1989). Studies of the ability of aristolochic acids to
4 block the arachidonic acid response to inflammation led to the observation that the
5 compound is more acutely toxic to macrophages (IC₅₀ = 2.5 μM) than to neutrophils
6 (IC₅₀ = 100 μM) (Glaser *et al.* 1995). Aristolochic acids also have been shown to inhibit
7 phospholipase A₂-mediated effects of snake venom on local edema *in vivo* (Vishwanath
8 and Gowda 1987) and on neutrophil motility *in vitro* (Sundell *et al.* 2003), effects that
9 might be related to the use of *Aristolochia* species in traditional medical treatments for
10 snakebite.

11 [Thus, aristolochic acid and its derivatives appear to have biochemical targets. Toxicity
12 clearly varies significantly with the cell type and with the structure of the derivative in
13 ways that are not yet well understood. Although it is clear that aristolochic acid I and
14 mixtures of aristolochic acids I and II both are cytotoxic, and that they are indices of
15 *Aristolochia* exposure, they are not necessarily the only (or most potent) cytotoxins
16 present in the botanical extracts. Contributions by aristolactams and other derivatives
17 may be significant.]

18 **5.3 Genetic damage and related effects**

19 The genetic damage and related effects of aristolochic acids were recently reviewed by
20 IARC (2002). Aristolochic acids have been tested for genotoxicity in a number of *in vitro*
21 and *in vivo* test systems. This section reviews formation and detection of AA-DNA
22 adducts in humans and animals and also reviews other genetic damage and related effects
23 of aristolochic acids in prokaryotic, eukaryotic, and mammalian systems.

24 **5.3.1 DNA adduct formation**

25 Aristolochic acids must be activated to form DNA adducts (Figure 5-2). The major
26 activation pathway involves nitroreduction to form an intermediate cyclic *N*-
27 acylnitrenium ion (aristolactam-nitriumion) that has a delocalized positive charge and has
28 been proposed to be the ultimate carcinogen (Chan *et al.* 2007a, IARC 2002). According
29 to Stiborová *et al.* (2007), the primary enzymes involved in activating aristolochic acids
30 in humans include hepatic and renal cytosolic NAD(P)H:quinone oxidoreductase

1 (NQO1), hepatic microsomal CYP1A2, renal microsomal NADPH:CYP reductase, and
2 COX. As noted in Section 5.4.2, additional enzymes have been identified that are
3 involved in the activation of aristolochic acids, including CYP1A1, prostaglandin H
4 synthase, DT-diaphorase, and xanthine oxidase (Stiborová *et al.* 1999, Stiborová *et al.*
5 2001a,b,c, Stiborová *et al.* 2002, Stiborová *et al.* 2003, Stiborová *et al.* 2005a). The
6 available data indicate that the exocyclic amino groups of purines are the preferred
7 binding sites. Numerous *in vitro* studies have demonstrated that aristolochic acids I and
8 II, after metabolic activation, can form adducts with DNA (from calf thymus, MCF-7
9 cells, and plasmids), with polydeoxyribonucleotides and oligodeoxyribonucleotides, and
10 with a variety of individual nucleotides and nucleotide monophosphates (IARC 2002).
11 Several *in vitro* systems are capable of activating aristolochic acids to reactive species
12 including S9 mix from Aroclor 1254- or β -naphthoflavone-pretreated rats, xanthine
13 oxidase, peroxidases (horseradish peroxidases, lactoperoxidase, prostaglandin H
14 synthase), zinc at pH 5.8, and microsomal preparations from various species. Adducts
15 formed by aristolochic acids I and II with adenine and guanine include 7-(deoxyadenosin-
16 N^6 -yl)-aristolactam I (dA-AAI), 7-(deoxyadenosin- N^6 -yl)-aristolactam II (dA-AAII), 7-
17 (deoxyguanosin- N^2 -yl)-aristolactam I (dG-AAI), and 7-(deoxyguanosin- N^2 -yl)-
18 aristolactam II (dG-AAII) (Schmeiser *et al.* 1997). Adducts with cytosine (dC-AAI and
19 dC-AAII) have been reported only *in vitro*, and the structure was not determined (Arlt *et*
20 *al.* 2000, 2001a). Studies have also demonstrated that aristolactams activated with hepatic
21 microsomes or horseradish peroxidase form adducts with calf thymus DNA. Adduct
22 patterns from *in vitro* and *in vivo* studies are similar; thus, the descriptions below are
23 limited to the *in vitro* studies that used intact cells rather than isolated DNA or
24 nucleotides and the *in vivo* studies.

25 Although almost all of the *in vitro* studies used individual nucleotides, oligonucleotides,
26 or calf thymus DNA for the reactions as noted above (see IARC 2002, Table 6, for a
27 detailed description of *in vitro* studies), a few studies have reported formation of DNA
28 adducts in cell lines *in vitro*. Lebeau *et al.* (2001) reported relative adduct labeling (RAL)
29 values for dA-AAI, dG-AAI, and DA-AAII after exposure of opossum kidney (OK) cells
30 to either 10 μ M or 20 μ M aristolochic acids (a mixture of aristolochic acid I and

1 aristolochic acid II with aristolochic acid I predominating) for 15 min to 24 h. RAL
2 values increased with time of exposure and ranged from 0.11 to 58.6 per 10^7 nucleotides
3 for dA-AAI, 0.31 to 25.5 per 10^7 nucleotides for dG-AAI, and from not detectable to 5.6
4 per 10^7 nucleotides for dA-AAII. RAL values that resulted from exposure to the 10 μ M
5 concentration after 24 hours (the only time interval tested for that dose) were 28.1 for dA-
6 AAI, 16.0 for dG-AAI, and 3.0 for dA-AAII, or approximately half those observed with
7 the higher concentration. After a one-day recovery period, no decrease was observed in
8 RAL, but after a six-day recovery period RAL had fallen to 7.7 for dA-AAI, 6.9 for dG-
9 AAI, and 0.74 for dA-AAII. The authors considered the adduct levels after the recovery
10 period of six days to still be significant and they considered this to demonstrate a
11 permanent alteration of DNA by aristolochic acids. In another study, Pfohl-Leszkowicz *et*
12 *al.* exposed human kidney cells (HK2) to 0.1 to 5.0 μ M aristolochic acid I, aristolochic
13 acid II, or a mixture of aristolochic acid I (38%) and aristolochic acid II (62%). The
14 highest level of adducts was approximately 6 adducts per 10^9 nucleotide. The authors of
15 this study reported that after 2 days all adducts had disappeared and concluded the
16 aristolochic acid adducts did not persist. This is not consistent with the *in vitro* data (also
17 in kidney cells) reported by Lebeau *et al.* (2001), where DNA adducts were still present
18 after 6 days. Aristolactam-DNA adducts may persist for many years *in vivo* (Fernando *et*
19 *al.* 1993, Bieler *et al.* 1997, Nortier *et al.* 2000, Arlt *et al.* 2001b, Lord *et al.* 2004).

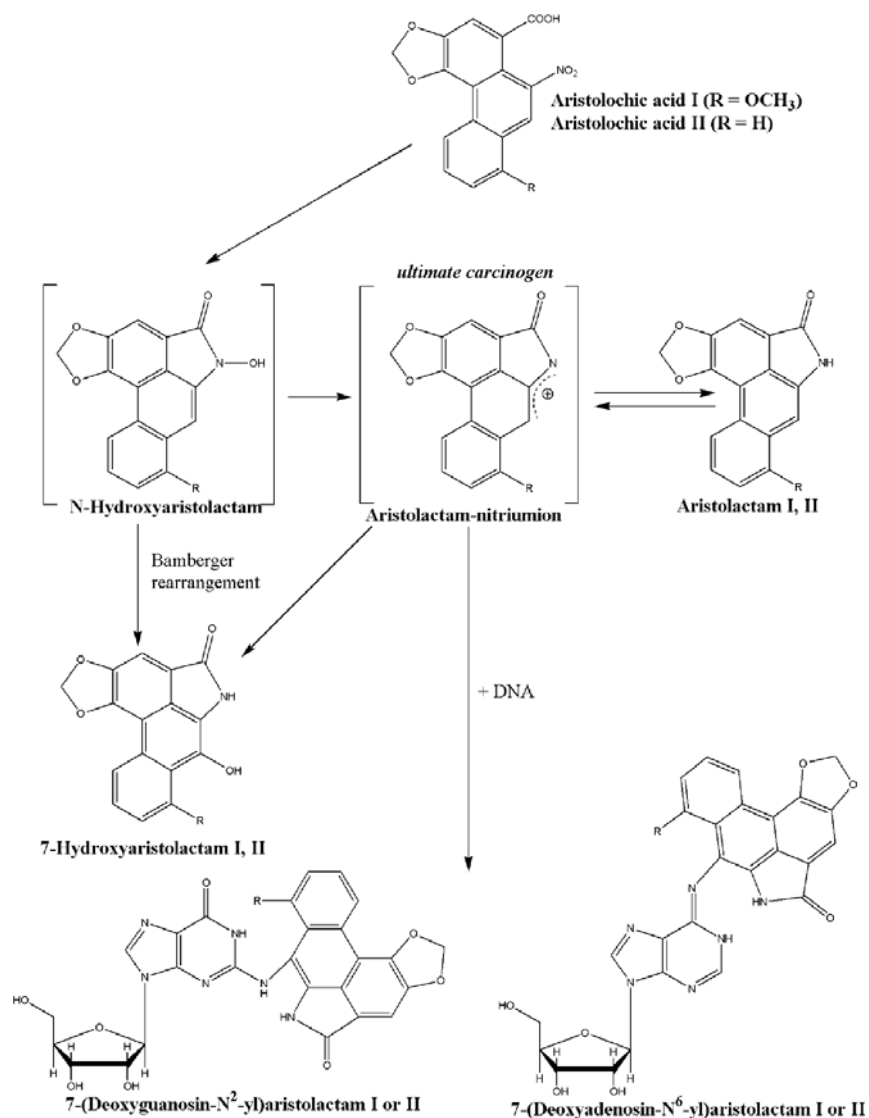


Figure 5-2. Metabolic activation of aristolochic acids and adduct formation

Source: Stiborova *et al.* 2007, Stiborová *et al.* 2003

1 DNA adducts have been detected by ³²P-postlabeling in human tissues from about 50
 2 patients with AAN and in rats and mice exposed to aristolochic acids. All the studies
 3 show that dA-AAI is the major and most persistent adduct formed. This adduct was
 4 detected in all urothelial tissues analyzed from AAN patients in studies reviewed by
 5 IARC (2002) and in reports published subsequent to the IARC review (Arlt *et al.* 2004b,
 6 Lord *et al.* 2004) with the exception of one bladder sample in which the dA-AAI adduct
 7 was not detectable (Nortier *et al.* 2003) (see Table 5-3). Two other adducts, dG-AAI and
 8 dA-AAII, have consistently been reported *in vivo* in humans and in experimental animals,

1 and dG-AAII was observed in rat forestomach after oral administration of aristolochic
2 acids. The data for humans are presented first and summarized in Table 5-3, followed by
3 the data for experimental animals, summarized in Table 5-4.

4 *Studies in humans with AAN or BEN*

5 Several of the studies in humans overlap because the investigators were reporting results
6 from the Belgian cohort. These include the studies of Schmeiser *et al.* (1996), Bieler *et*
7 *al.* (1997), Nortier *et al.* (2000, 2003), and Arlt *et al.* (2001b). Schmeiser *et al.* (1996) and
8 Bieler *et al.* (1997) were the first to report AA-DNA adducts in humans. Tissue samples
9 from the kidneys of 6 female patients from the Belgian cohort were examined with the
10 nuclease P1-enhanced variation of the ³²P-postlabeling assay. In addition, tissue samples
11 taken from the right ureter of 1 patient were analyzed for adducts. These patients had
12 taken the herbal weight-loss pills for 13 to 23 months and had undergone a kidney
13 transplant within 9 to 44 months after the weight-loss regimen. The major adduct was
14 dA-AAI, which occurred in all samples from the 6 AAN patients but was not found in the
15 samples from 6 controls. Minor adducts included dG-AAI and dA-AAII. Nortier *et al.*
16 (2000) reported the same adduct pattern, but somewhat lower levels in kidneys from 38
17 patients and ureters from 11 patients, all from the Belgian cohort. The average period of
18 exposure to the weight-loss pills was 13.3 months, and the interval between discontinuing
19 the weight-loss regimen and prophylactic surgery to remove their kidneys and ureters was
20 56 to 89 months.

21 Several investigators reported AA-DNA adducts in patients outside the Belgian cohort
22 (Gillerot *et al.* 2001, Arlt *et al.* 2004b, Lord *et al.* 2004, Lo *et al.* 2005, Grollman *et al.*
23 2007) (see Section 3 for details on these patients' clinical symptoms). These studies
24 found the major adduct, dA-AAI, in kidney, ureter, and other tissues (see Table 5-3) and
25 confirmed exposure to aristolochic acids from various herbal preparations; however, the
26 levels varied between studies and no tissue was consistently found to contain the highest
27 level of adducts. In three studies in which multiple tissues from the same patient were
28 examined, one study reported the highest level of adducts in kidney (Nortier *et al.* 2003),
29 one in ureter (Lord *et al.* 2004), and one in lung (Arlt *et al.* 2004b); samples from the
30 kidney only were examined from a second patient in the Arlt *et al.* study, and those

1 samples contained the highest individual levels reported in that paper. In contrast to Arlt
2 *et al.* (2004b), Pfohl-Leszkowicz *et al.* (2007) did not detect AA-DNA adducts in French
3 and Belgian patients who had been exposed to slimming regimens that contained
4 aristolochic acids (see sections 3.2 for AAN-related studies and Section 3.4 for findings
5 related to BEN). Pfohl-Leszkowicz *et al.* did not offer an explanation for the discrepancy
6 between their findings and those of others who clearly demonstrated aristolochic acid
7 adducts in tissues of AAN patients. Pfohl-Leszkowicz *et al.* and Arlt *et al.* used slightly
8 different chromatographic conditions (mainly differences in molarity and pH of some of
9 the developing solutions) in their analyses of DNA adducts. Arlt *et al.* (2001b) [Pfohl-
10 Leszkowicz was a coauthor for this paper] (see below) reported that analysis of OTA
11 adducts required different chromatographic conditions than routinely used for detecting
12 aristolochic acid adducts; therefore, these authors used the conditions suitable for OTA-
13 related adducts and demonstrated that aristolochic adducts could be detected with this
14 method. Pfohl-Leszkowicz *et al.* (2007) did not report any results for simultaneous
15 determination of AA-DNA and OTA-related adducts on the same chromatographic plate.
16 Pfohl-Leszkowicz *et al.* did detect aristolochic acid adducts *in vitro* [albeit at lower
17 levels, and for shorter duration than another *in vitro* study (although there were
18 differences in the study conditions) (see Section 5.3.1 above).]

Table 5-3. AA-DNA adducts detected in AAN patients

Tissue examined (no. of patients)	Botanical product, (dose), [mo of exposure]	DNA binding		Reference (Country)
		Adduct(s)	No. per 10 ⁸ nucleotides	
Kidney (6)	weight-loss preparation containing <i>A. fangchi</i> (2 mg/g AA I; 0.2 mg/g AA II) ^a [13–23]	dA-AAI dG-AAI dA-AAII	7–53 0.2–1.2 0.6–2.4	Schmeiser <i>et al.</i> 1996 (Bieler <i>et al.</i> 1997 (Belgium)
Ureter (1)	weight-loss preparation containing <i>A. fangchi</i> (2 mg/g AA I; 0.2 mg/g AA II) [19]	dA-AAI dG-AAI dA-AAII	7.1 0.7 2.0	Bieler <i>et al.</i> 1997 Arlt <i>et al.</i> 2001b (Belgium)
Kidney (38)	weight-loss preparation containing <i>A. fangchi</i> (226 g of herb in patients with carcinoma; 167 g of herb in patients without carcinoma) [mean = 13.3] ^b	dA-AAI dG-AAI dA-AAII	0.12–16.5 0.04–0.82 0.06–0.68	Nortier <i>et al.</i> 2000 (Belgium)
Ureter (11)		dA-AAI dG-AAI dA-AAII	0.22–3.4 NR NR	
Kidney (2) ^c	weight-loss preparation containing <i>A. fangchi</i> (2 mg/g AA I; 0.2 mg/g AA II) ^a [13–24]	dA-AAI dG-AAI dA-AAII	2.9, 5.0 ND 0.3, 0.9	Arlt <i>et al.</i> 2001b (Belgium)
Kidney (1)	various roots and leaves (108 mg AA) [6]	dA-AAI	1.8	Gillerot <i>et al.</i> 2001 (China)
Kidney (1) Liver (1) Pancreas (1) Lymph nodes (1) Stomach (1) Lung (1) Bladder (1)	weight-loss preparation containing <i>A. fangchi</i> (189 g of herb) [14]	dA-AAI	8.1 0.87 0.8 0.5 1.9 0.16 ND	Nortier <i>et al.</i> 2003 (Belgium)
Kidney (1) Ureter (1) Bladder (1) Breast tumor (1) Liver tumor ^d (1) Normal liver (1)	Aristolochic acids- containing herbal preparation for eczema (<i>A. manshuriensis</i>) (NR) [24]	dA-AAI	3.8 40 20 1.0 1.0 16	Lord <i>et al.</i> 2004 (UK)

Tissue examined (no. of patients)	Botanical product, (dose), [mo of exposure]	DNA binding		Reference (Country)
		Adduct(s)	No. per 10 ⁸ nucleotides	
Kidney (2) Ureter (1) Bladder (1) Liver (1) Lung (1) Stomach (1) Small intestine (1) Spleen (1) Adrenal (1) Brain (1) Heart (1)	“Preparation Number 28” and “Preparation Number 23” containing aristolochic acids (NR) [5.5–12]	dA-AAI	0.1–5.4 1.65 0.27 1.75 2.19 1.03 1.0 2.12 1.95 0.19 ND	Arlt <i>et al.</i> 2004b ^e (France)
Kidney (1)	<i>A. mollissima</i> (800 g of herb) [6]	dA-AAI	NR	Lo <i>et al.</i> 2005 (Hong Kong)
Kidney (1)	Herbal remedy containing <i>Aristolochia</i> (NR) [NR]	dA-AAI + -AAII dG-AAI	11–34 0.2–1	Grollman <i>et al.</i> 2007 (U.S.)

dA-AAI = 7-(deoxyadenosin-N⁶-yl)-aristolactam I; dA-AAII = 7-(deoxyadenosin-N⁶-yl)-aristolactam II; dG-AAI = 7-(deoxyguanosin-N²-yl)-aristolactam I; ND = not detected; NR = not reported.

^a Measurements reported for analyses of 2 of 3 samples of herb powders delivered in Belgium under the name of *S. tetrandra* (Bieler *et al.* 1997).

^b Mean for 39 patients examined in study.

^c Three of the patients also were included in Bieler *et al.* 1997 and Schmeiser *et al.* 1996; the adduct levels for the two new cases only are reported above.

^d Metastasis from breast.

^e Pfohl-Leszkowicz *et al.* (2007) did not detect aristolochic acid adducts in these patients.

1 The role of aristolochic acids in BEN is debated. A few studies have reported aristolochic
2 acid adducts in BEN patients. Arlt *et al.* (2002a) analyzed kidney tissues from three
3 female patients with end-stage renal failure (two of these patients also had an upper
4 urinary tract malignancy). Although clinical and renal morphological data were
5 insufficient to clearly identify these individuals as BEN patients, they all lived in villages
6 in Croatia where BEN was endemic. DNA adducts were detected using ³²P-postlabeling.
7 Two of the three patients had one major adduct spot that was indistinguishable from the
8 dA-AAI adduct, which is the most common adduct found in AAN patients. Adduct levels
9 were 5.6 and 17.1 adducts per 10⁹ nucleotides. The authors noted that since the renal
10 tissue samples were collected between 1987 and 1990, the results confirmed that the dA-
11 AAI adduct is a suitable biomarker for exposure to aristolochic acids years later.
12 However, it was not known whether or not these patients had taken herbal medications

1 that might have contained aristolochic acids. Further analysis also showed that the two
2 patients who had aristolochic acid adducts also had OTA-related adducts (3.1 to 4.7
3 adducts per 10^9 nucleotides).

4 Grollman *et al.* (2007) examined renal tissues from four BEN patients for aristolochic
5 acid adducts. Clinical diagnosis of BEN was established using criteria developed by the
6 World Health Organization. AA-DNA adducts were detected in all four patients. Levels
7 of dA-AA and dG-AA adducts ranged from 0.8 to 5.9 and 0.2 to 6.2 adducts per 10^7
8 nucleotides, respectively. These adducts were not detected in five patients with upper
9 urinary tract transitional-cell cancers who resided outside the endemic region of Croatia,
10 or in five patients with common forms of chronic renal disease. In addition, urothelial and
11 renal cortical tissues were obtained from long-term residents of endemic villages who had
12 upper urinary tract malignancies. Three tumor tissues were analyzed for adducts. There
13 were 0.7 to 1.6 dA-AA adducts and 0.3 to 0.5 dG-AA adducts per 10^8 nucleotides.

14 Pfohl-Leszkowicz *et al.* (2007) analyzed OTA-related and AA adducts in 60 formalin-
15 fixed, paraffin-embedded renal tissue samples taken from patients reported to have
16 nephropathy and urothelial cancer from endemic areas of Serbia, Croatia, and Bulgaria
17 and nonendemic areas of Croatia and France. No aristolochic acid adducts were detected
18 in any of the patients; however, OTA-related adducts were reported in 30% of the
19 samples, and in all 7 patients from a rural endemic area. Adduct levels were reported only
20 for the French patients (16 of 18 had OTA-related adducts) and ranged from 1 to 115 per
21 10^9 nucleotides. The C-C8 dGMP-OTA adduct was observed in all samples that exhibited
22 OTA-related adducts. Some of the Croatian and Serbian patients also had the quinone-
23 form of the OTA adduct. [Supporting clinical or pathology data were not provided for
24 these patients.] Interpretation of the data on OTA-related DNA adduct formation is
25 controversial (see Section 5.3.5, “Mutational spectra in tumors from animals and
26 humans”) (Gautier *et al.* 2001, Mally *et al.* 2004, Turesky 2005, Cavin *et al.* 2007, Palma
27 *et al.* 2007). The C-C8 dGMP-OTA adduct used as a standard was synthesized by photo-
28 irradiation. The Panel on Contaminants in the Food Chain of the European Food Safety
29 Authority (EFSA) (2006) stated that advanced chemical analytical procedures had failed
30 to demonstrate the existence of specific OTA-DNA adducts. They considered the data on

1 OTA-DNA adduct formation to be controversial since chemical analyses, even with
2 advanced methods such as ¹⁴C-accelerated mass spectrometry, failed to detect DNA adducts
3 containing OTA or parts of that molecule. Thus, they suggested that the possibility that these
4 adducts represent non-specific oxidative DNA adducts cannot be excluded. (See Section
5 5.3.5, “Mutational spectra in tumors from animals or humans,” for further discussion of
6 the possible role of cellular oxidative damage in the genotoxic effects of OTA.)

7 *Studies in experimental animals*

8 Adduct patterns in animal studies were determined with the nuclease P1-enhanced ³²P-
9 postlabeling assay and are consistent with the adduct patterns reported in AAN patients.
10 Schmeiser *et al.* (1988) was one of the first studies to report that aristolochic acids
11 formed DNA adducts. Aristolochic acids I and II formed one or more adducts in kidney,
12 forestomach, stomach, liver, and lung of male Wistar rats. In addition, aristolochic acid II
13 formed adducts in bladder and brain.

14 Studies reported in Table 5-4 are reviewed briefly here. Pfau *et al.* (1990b) reported that
15 both aristolochic acids I and II formed adducts in various tissues of male Wistar rats, but
16 the specific adducts were not identified. Routledge *et al.* (1990) detected aristolochic acid
17 adducts [the authors did not identify the specific aristolochic acid compound(s) used] in
18 the forestomach of male Wistar rats. Administration of butylated hydroxyanisole before,
19 together with, or after administration of aristolochic acids increased the levels of adducts
20 (data not shown).

21 Fernando *et al.* (1992) exposed male Wistar rats to aristolochic acid I and detected dA-
22 AAI and dG-AAI adducts in exfoliated cells (in the urine), urothelium, and urinary
23 bladder 36 weeks after exposure. Formation and persistence of DNA adducts were
24 investigated by Fernando *et al.* (1993) in male Wistar rats given a single dose of
25 aristolochic acid I; tissues were examined up to 36 weeks after exposure. Both dA-AAI
26 and dG-AAI adducts were found in all organs examined up to 36 weeks, but their
27 removal rates and persistence differed. Both adducts declined rapidly in forestomach
28 during the first 2 weeks, but thereafter, levels of dA-AAI remained constant, while levels
29 of dG-AAI adducts continued to decline. The major adduct in all tissues was dA-AAI, but
30 its removal rate differed among tissues. Based on cancer studies in rats, the target tissue

1 was considered to be forestomach. Adduct levels were lower and removal rates were
2 generally faster in non-target tissues (glandular stomach, liver, lung, and urinary bladder)
3 than in forestomach. [The authors did not provide tabulated adduct data; therefore,
4 estimated adduct levels in Table 5-4 are shown only for forestomach as reported by IARC
5 (2002)].

6 Hadjiolov *et al.* (1993) administered aristolochic acids [the authors did not identify the
7 specific compound(s)] to male BD-6 rats twice a week for 12 weeks. Two major DNA
8 adducts (dA-AAI and dG-AAI) were observed in forestomach of rats sacrificed on day
9 60; four minor adducts also were observed but not identified. Stiborová *et al.* (1994)
10 exposed male Sprague-Dawley rats to either aristolochic acid I, aristolochic acid II, or a
11 mixture for 2 weeks and examined forestomach tissues for DNA adducts. In rats exposed
12 to aristolochic acid I, dA-AAI and dG-AAI were present at the highest levels, with
13 smaller amounts of dA-AAII (the authors noted that the adduct spot was
14 chromatographically indistinguishable from the dA-AII adduct, which could indicate a
15 possible demethoxylation reaction of aristolochic acid I). dA-AAII was the most
16 prevalent adduct in rats exposed to aristolochic acid II, with smaller amounts of dG-AAII
17 and a very small quantity of an unidentified adduct. Smaller amounts of adducts were
18 seen with the mixture of aristolochic acids I and II than with aristolochic acid I or II
19 alone, but dA-AAI, dG-AAI, dA-AAII, and dG-AAII were all detected in the
20 forestomach.

21 Bieler *et al.* (1997) examined the long-term persistence of dA-AAI and dG-AAI adducts
22 in rat kidney in a study with a design and results essentially the same as reported by
23 Fernando *et al.* (1993). Both dA-AAI and dG-AAI adducts were found in rat kidney up to
24 36 weeks post-exposure. Adduct levels declined during the first 2 weeks, after which
25 dA-AAI levels stabilized, but dG-AAI levels continued to decline. The authors concluded
26 that both greater initial DNA binding and greater persistence contributed to the higher
27 levels of dA-AAI adducts.

28 Arlt *et al.* (2001b) investigated DNA adduct formation in the kidneys of male and female
29 Wistar rats exposed to the weight-loss regimen used by the Belgian cohort (Cosyns *et al.*

1 1998). The rats were exposed to aristolochic acids at 0.15 mg/kg b.w. per day for 5 days
2 per week for 3 months and sacrificed 11 months later (see Section 4.2.2 for additional
3 details of the treatment). The three major adducts identified in both male and female rats
4 were dA-AAI, dG-AAI, and dA-AAII; four additional adducts were observed but not
5 identified. Female rats had significantly higher levels of dG-AAI adducts than did males.

6 Mei *et al.* (2006) investigated DNA adduct formation in rat kidney and liver. Groups of
7 six male Big Blue rats were administered oral doses of aristolochic acids (mixture, 40%
8 aristolochic acid I, 56% aristolochic acid II) at 0, 0.1, 1.0, and 10 mg/kg b.w. 5 days/week
9 for 3 months. Rats were sacrificed the day after the final treatment. Three major adducts
10 were identified (dA-AAI, dA-AAII, and dG-AAI), and there was a strong linear dose
11 response. Although DNA adducts were detected in both the kidneys and livers of rats
12 exposed to aristolochic acids, the kidneys ($4,598 \pm 148 \times 10^{-8}$ nucleotides) had about
13 twice the level of DNA adducts observed in the liver ($1,967 \pm 468 \times 10^{-8}$ nucleotides) at
14 the 10 mg/kg b.w. dose of aristolochic acids.

Table 5-4. Aristolochic acid–DNA adduct formation in rodents

Strain (sex)	Compound & dose	Tissues	DNA binding		Reference
			adduct(s)	no. per 10 ⁸ nucleotides	
Wistar rats (M)	AA I 10 mg/kg b.w. × 5	forestomach stomach liver kidney urinary bladder	NI	330 180 56 42 17	Pfau <i>et al.</i> 1990b
	AA II 10 mg/kg b.w. × 5	forestomach stomach liver kidney urinary bladder	NI	25 25 53 80 24	
Wistar rats (M)	aristolochic acids 1 mg/kg b.w. × 5	forestomach liver	NI NI	7.7 6.3	Routledge <i>et al.</i> 1990
Wistar rats (M)	AA I 10 mg/kg b.w., 5 d/wk for 3 mo	exfoliated cells (urine)	dA-AAI dG-AAI	0.27, 2.31 ^a 0.31, 1.46 ^a	Fernando <i>et al.</i> 1992
		urothelium	dA-AAI dG-AAI	9.61, 28.2 ^a 2.97, 3.5 ^a	
		urinary bladder	dA-AAI dG-AAI	2.32, NR ^a 1.41, NR ^a	
Wistar rats (M)	AA I 5 mg/kg b.w. × 1	forestomach	dA-AAI dG-AAI	30/2 ^b 21/0.4 ^b	Fernando <i>et al.</i> 1993
BD-6 rats (M)	aristolochic acids 10 mg/kg b.w., 2 d/wk for 12 wk	forestomach	dA-AAI dG-AAI spots 3–6	49 19 0.85–11	Hadjiolov <i>et al.</i> 1993
Sprague- Dawley rats (M)	AA I 10 mg/kg b.w., 2 d/wk for 2 wk	forestomach	dA-AAI dG-AAI dA-AAII	385 207 31.6	Stiborová <i>et al.</i> 1994
	AA II 10 mg/kg b.w., 2 d/wk for 2 wk		dA-AAII dG-AAII unknown	20 4.6 0.8	
	AA I and II (mixture) 10 mg/kg b.w., 2 d/wk for 2 wk		dA-AAI dG-AAI dA-AAII dG-AAII	15.8 10.0 5.1 1.2	
Wistar rats (M)	AA I 5 mg/kg b.w. × 1	kidney	dA-AAI dG-AAI	6.5/1.6 ^b 3.8/0.5 ^b	Bieler <i>et al.</i> 1997
Wistar rats (M/F)	Weight-loss (<i>S.</i> <i>tetrandra</i>) 0.15 mg/kg b.w., 5d/wk for 3 mo	kidney	dA-AAI dG-AAI dA-AAII	2.2/2.0 ^c 2.1/4.6 ^c 0.8/1.7 ^c	Arlt <i>et al.</i> 2001b

Strain (sex)	Compound & dose	Tissues	DNA binding		Reference
			adduct(s)	no. per 10 ⁸ nucleotides	
Big Blue rats (M)	AA I and II (mixture) 10 mg/kg b.w. 5d/wk for 3 mo	kidney	dA-AAI	911.4	Mei <i>et al.</i> 2006
			dG-AAI	1,676.6	
			dA-AAII	2,010.3	
		liver	dA-AAI	684.1	
			dG-AAI	720.9	
			dA-AAII	561.8	

AA I = aristolochic acid I; AA II = aristolochic acid II; dA-AAI = 7-(deoxyadenosin-N⁶-yl)-aristolactam I; dA-AAII = 7-(deoxyadenosin-N⁶-yl)-aristolactam II; dG-AAI = 7-(deoxyguanosin-N²-yl)-aristolactam I; dG-AAII = 7-(deoxyguanosin-N²-yl)-aristolactam II; NI = specific molecular forms of adducts were not identified; total adduct levels are given.

^aThe first value is for nuclease P1 extraction and the second for *n*-butanol extraction.

^bInitial adduct level/level at 36 weeks as reported by IARC 2002.

^cLevel in males/level in females.

- 1 Dong *et al.* (2006) exposed 3 male Wistar rats to aristolochic acid I or II or aristolactam I
- 2 at 5 mg/kg b.w. per day for 7 days by gavage. Nine different tissues were collected. The
- 3 highest adduct levels were detected in intestine, kidney, and liver of rats exposed to
- 4 aristolochic acid I and in kidney, bladder, and intestine of rats exposed to aristolochic
- 5 acid II (Table 5-5). Rats exposed to aristolochic acid II had the highest adduct levels;
- 6 however, other studies found higher adduct levels in rats exposed to aristolochic acid I.
- 7 Levels of adducts in rats exposed to aristolactam I were much lower, ranging from 2 to
- 8 24 adducts per 10⁸ nucleotides.

Table 5-5. Formation of DNA adducts by aristolochic acids I and II and aristolactam I in various tissues of male Wistar rats

DNA source	No. of adducts per 10 ⁸ nucleotides ± SD					
	aristolochic acid I		aristolochic acid II		aristolactam I	
	dA-AA I	dG-AA I	dA-AA II	dG-AA II	dA-AA I	dG-AA I
Kidney (pelvis)	401	44	1,410	294	24	8
Kidney (cortex)	485	54	1,970	506	1	1
Bladder	120	15	1,380	185	6	3
Forestomach	276	44	484	72	9	4
Glandular stomach	250	39	239	33	5	2
Intestine	686	115	811	106	22	4
Liver	411	43	333	127	3	1
Spleen	47	7	102	15	5	3
Lung	203	27	237	44	4	2

Source: Dong *et al.* 2006.

dA-AA I = 7-(deoxyadenosin-N⁶-yl)-aristolactam I; dA-AA II = 7-(deoxyadenosin-N⁶-yl)-aristolactam II; dG-AAI = 7-(deoxyguanosin-N²-yl)-aristolactam I; dG-AAII = 7-(deoxyguanosin-N²-yl)-aristolactam II.

1 Shibutani *et al.* (2007) measured DNA adducts in groups of 10 male C3H/He mice
2 exposed to 2.5 mg/kg/day of aristolochic acid I or aristolochic acid II (see Section 5.2.2).
3 The route of administration did not significantly affect the outcome. Similar levels of
4 DNA adducts were found in target tissues (kidney and bladder) in mice treated with
5 aristolochic acid I or aristolochic acid II; however, adduct levels in nontarget tissues
6 (liver, stomach, intestine, and lung [although lung tumors were observed in NMRI mice
7 exposed to aristolochic acids, see Table 4-1), were significantly higher in mice treated
8 with aristolochic acid I (Table 5-6). All adduct data were collected from mice killed on
9 day 10. The authors concluded that aristolochic acid I and aristolochic acid II have
10 similar genotoxic and carcinogenic potential.

Table 5-6. DNA adducts in male C3H/He mice exposed to aristolochic acids I and II

Treatment/organs	Adducts per 10 ⁶ nucleotides ± S.D. ^a			
	AAI		AAII	
	dA-AA I	dG-AA I	dA-AA II	dG-AA II
i.p				
Kidney (cortex)	12.3 ± 0.90	1.10 ± 0.23	14.1 ± 6.38	2.47 ± 0.91
Kidney (medulla)	12.9 ± 2.88	1.63 ± 0.15	12.5 ± 4.95	2.30 ± 0.61
Bladder	6.49 ± 1.68	0.71 ± 0.05	6.73 ± 5.51	0.88 ± 0.45
Stomach	2.02 ± 0.86	0.79 ± 0.24	0.87 ± 0.11	0.31 ± 0.13
Intestine	1.73 ± 0.61	0.46 ± 0.16	0.43 ± 0.30	0.09 ± 0.08
Liver	6.52 ± 3.20	1.15 ± 0.38	0.66 ± 0.53	0.46 ± 0.36
Spleen	0.13 ± 0.10	0.07 ± 0.11	0.13 ± 0.09	0.05 ± 0.03
Lung	3.32 ± 1.42	0.50 ± 0.13	0.60 ± 0.46	0.14 ± 0.09
oral				
Kidney (cortex)	17.2 ± 6.40	2.58 ± 0.79	22.1 ± 4.10	5.20 ± 1.57

Source: Shibutani *et al.* 2007.^aMeans based on analyses from three mice.

1 *In vitro studies in cell-free systems*

2 The affinity of aristolochic acids I and II to form adducts at the first adenine of codon 61

3 (CAA) in the *H-ras* gene was assessed in *in vitro* studies using a polymerase arrest assay

4 in a plasmid (pNPR) containing exon 2 of the mouse *H-ras* gene (Arlt *et al.* 2000).

5 Aristolochic acids I and II modified by chemical reduction with zinc were incubated with

6 the pNPR plasmid, and the sites of polymerase arrest 3' to the bulky aristolochic acid

7 adducts were determined. Both aristolochic acids showed a preference for adduct

8 formation and arrest sites at purine bases; however, the polymerase arrest spectra differed

9 for the two molecules. Aristolochic acid I preferentially formed adducts at guanine

10 residues, but polymerase arrest sites were primarily at adenine residues. Conversely,

11 aristolochic acid II reacted preferentially to form adducts with adenine residues, but

12 polymerase arrest occurred relatively equally at guanine, adenine, and cytosine residues.

13 Neighboring bases affected adduct formation for both aristolochic acids I and II, with

14 flanking pyrimidine residues favoring binding. The differences in adduct formation sites

15 and polymerase arrest sites were suggested to result from structural characteristics of the

16 DNA adducts formed by the two aristolochic acid molecules. The authors also suggested

17 that the mutation “hot spot” at the first adenine of codon 61 of *H-ras* did not result from

18 initial adduct formation but could be due to non-random action of DNA repair processes,

19 because analysis of adducts by ³²P-postlabeling showed formation of adducts at both

20 adenines in codon 61.

1 In a study using human DNA, Arlt *et al.* (2001a) mapped the distribution of DNA
2 adducts formed by aristolochic acids I and II using an adduct-specific polymerase arrest
3 assay together with terminal transferase-dependent PCR. Human mammary carcinoma
4 (MCF-7) DNA was incubated with aristolochic acids I and II activated by zinc dust, and
5 an adduct pattern was obtained that consisted of dA-AAI, dG-AAI, dA-AAII, dG-AAII,
6 and dC-AAII. The polymerase arrest assay indicated that most arrests occurred at purine
7 residues; however, the authors noted that the method must be considered semiquantitative
8 because of variability of one or two nucleotides in identification of the termination site.
9 The pattern of adduct formation in p53 DNA did not predict AA-specific hotspots in
10 urothelial tumors of the p53 database, which the authors suggested could be due to the
11 small number of mutations for urothelial carcinomas recorded in the database. However,
12 they also suggested that aristolochic acids are not likely to be the cause of non-CHN
13 related urothelial tumors, which is consistent with the predominance of A:T → T:A
14 mutations in urothelial cancers from patients exposed to aristolochic acids compared with
15 less than 5% of TCC containing this mutation in the p53 database (Debelle *et al.* 2008).

16 5.3.2 Prokaryotic systems

17 The genetic effects of mixtures of aristolochic acids, of aristolochic acids I and II, and of
18 metabolites of aristolochic acids (aristolactams I and II and aristolic acid) have been
19 investigated in *Salmonella typhimurium* and *Escherichia coli*, and the results are
20 reviewed below. In addition, one study of the mutagenic effects of aristolochic acid IV in
21 *S. typhimurium* is reviewed. Results are summarized in Table 5-7.

22 *Salmonella typhimurium*

23 Robisch *et al.* (1982) tested aristolochic acids (reported by IARC [2002] as an
24 aristolochic acid mixture) in *S. typhimurium* strains TA100, TA1537, TA1535, TA1538,
25 and TA98. The mixture induced reverse mutation in TA100 and TA1537 either with or
26 without metabolic activation; however, negative results were reported for TA1535,
27 TA1538, and TA98 with or without metabolic activation.

28 Aristolochic acid I induced reverse mutation in *S. typhimurium* strains TA98, TA100,
29 TA102, TA1535, TA1537, YG1020, YG1021, YG1024, YG1025, YG1026, and YG1029
30 (Schmeiser *et al.* 1984, Chakrabarty *et al.* 1987, Pezzuto *et al.* 1988, Götzl and Schimmer

1 1993, Zhang *et al.* 2004). The YG strains contain multiple copies of plasmids for
2 bacterial nitroreductase or *O*-acetyltransferase; the first three YG strains are derived from
3 TA98 (sensitive to frameshift mutagens) and the latter three from TA100 (sensitive to
4 base-pair-substitution mutagens). Negative results were reported for strains TA98NR and
5 TA100NR (nitroreductase-deficient strains of TA98 and TA100) (Pezzuto *et al.* 1988,
6 Schmeiser *et al.* 1984) and for TA1978 and strains containing the *hisG46* or *hisD3052*
7 allele (Chakrabarty *et al.* 1987). Aristolochic acid I induced forward mutation to 8-
8 azaguanine resistance in *S. typhimurium* strain TM677 (Pezzuto *et al.* 1988).

9 Aristolochic acid II induced reverse mutations in many of the same strains as aristolochic
10 acid I (TA98, TA100, YG1020, YG1021, YG1024, YG1025, YG1026, YG1029)
11 (Pezzuto *et al.* 1988, Götzl and Schimmer 1993). All studies that were reviewed reported
12 positive results for aristolochic acid II.

13 Aristolochic acid IV was extracted from *Aristolochia rigida* and tested for mutagenic
14 activity in *S. typhimurium* TA100 with and without metabolic activation (Pistelli *et al.*
15 1993). Aristolochic acid IV induced a dose-related increase in the number of revertants in
16 the absence of metabolic activation, but no significant dose-related effect with metabolic
17 activation. The authors concluded that aristolochic acid IV had weak direct mutagenic
18 activity.

19 Aristolochic acid metabolites also were tested for mutagenic activity in *S. typhimurium*.
20 Aristolactams I and II were mutagenic with or without metabolic activation in one study
21 (Schmeiser *et al.* 1986); however, in a second study (Chakrabarty *et al.* 1987),
22 aristolactam I gave negative results in a number of strains. Another metabolite, aristolic
23 acid, gave consistently negative results both without metabolic activation (Chakrabarty *et*
24 *al.* 1987, Götzl and Schimmer 1993) and with metabolic activation (Chakrabarty *et al.*
25 1987).

26 *Escherichia coli*

27 Kevekordes *et al.* (1999) tested an aristolochic acids plant extract and aristolochic acids I
28 and II in the SOS chromotest in *E. coli* PQ37. Both the aristolochic acids plant extract
29 and aristolochic acid I were genotoxic with or without metabolic activation, but the

- 1 response was much greater without activation. Aristolochic acid II also was considered to
- 2 be genotoxic without metabolic activation and marginally genotoxic with activation.

Table 5-7. Genetic effects of aristolochic acids, aristolactam, and aristolic acid in prokaryotes

Test system	End point	LED or HID (µg/plate)	Without S-9	With S-9	Reference
Aristolochic acids mixture or plant extract					
<i>S. typhimurium</i> TA100, TA1537	reverse mutation	50 (mixture)	+	+	Robisch <i>et al.</i> 1982
<i>S. typhimurium</i> TA1535, TA1538, TA98	reverse mutation	200 (mixture)	–	–	Robisch <i>et al.</i> 1982
<i>E. coli</i> PQ37	DNA damage (SOS chromotest)	0.38 µg/assay (plant extract)	+ ^a	+	Kevekordes <i>et al.</i> 1999
Aristolochic acid I					
<i>S. typhimurium</i> TA100, TA1537	reverse mutation	100	+	+	Schmeiser <i>et al.</i> 1984
<i>S. typhimurium</i> TA100NR ^b	reverse mutation	200	–	–	Schmeiser <i>et al.</i> 1984
<i>S. typhimurium</i> TA100, TA98, TA1535	reverse mutation	50	+	+	Chakrabarty <i>et al.</i> 1987
<i>S. typhimurium</i> TA1978, <i>hisG46</i> , <i>hisD3052</i>	reverse mutation	1,000	–	–	Chakrabarty <i>et al.</i> 1987
<i>S. typhimurium</i> TA100, TA102, TA1537	reverse mutation	100	+	NT	Pezzuto <i>et al.</i> 1988
<i>S. typhimurium</i> TA98NR ^b , TA100NR ^b	reverse mutation	200	–	NT	Pezzuto <i>et al.</i> 1988
<i>S. typhimurium</i> TM677	forward mutation (<i>hprt</i> locus)	8.5 µg/mL	+	NT	Pezzuto <i>et al.</i> 1988
<i>S. typhimurium</i> TA98, YG1020, YG1021	reverse mutation	170	(+)	NT	Götzl and Schimmer 1993
<i>S. typhimurium</i> TA1537	reverse mutation	85	+	NT	Götzl and Schimmer 1993
<i>S. typhimurium</i> YG1024, TA100, YG1025, YG1026, YG1029	reverse mutation	34	+	NT	Götzl and Schimmer 1993
<i>S. typhimurium</i> TA98, TA100	reverse mutation	100	+	+	Zhang <i>et al.</i> 2004
<i>E. coli</i> PQ37	DNA damage (SOS chromotest)	0.17 µg/assay	+ ^a	+	Kevekordes <i>et al.</i> 1999
Aristolochic acid II					
<i>S. typhimurium</i> TA98, YG1020, YG1021	reverse mutation	78	(+)	NT	Götzl and Schimmer 1993
<i>S. typhimurium</i> YG1021, YG1024, TA100, YG1025, YG1026, YG1029	reverse mutation	[31] ^c	+	NT	Götzl and Schimmer 1993
<i>S. typhimurium</i> TA1537	reverse mutation	78	+	NT	Götzl and Schimmer 1993

Test system	End point	LED or HID (µg/plate)	Without S-9	With S-9	Reference
<i>E. coli</i> PQ37	DNA damage (SOS chromotest)	0.16 µg/assay	+	(+)	Kevekordes <i>et al.</i> 1999
Aristolochic acid IV					
<i>S. typhimurium</i> TA100	reverse mutation	100	(+)	–	Pistelli <i>et al.</i> 1993
Aristolactams					
<i>S. typhimurium</i> TA100, TA1537	reverse mutation	50 (AL I, II)	–	+	Schmeiser <i>et al.</i> 1984
<i>S. typhimurium</i> TA100, TA98, TA1535, TA1978, and strains carrying <i>hisG46</i> or <i>hisD3052</i>	reverse mutation	1,000 (AL I)	–	–	Chakrabarty <i>et al.</i> 1987
Aristolochic acid					
<i>S. typhimurium</i> TA100, TA98, TA1535, TA1978, and strains carrying <i>hisG46</i> or <i>hisD3052</i>	reverse mutation	1,000	–	–	Chakrabarty <i>et al.</i> 1987
<i>S. typhimurium</i> TA98, TA100, YG1021, YG1026	reverse mutation	276	–	NT	Götzl and Schimmer 1993
<i>S. typhimurium</i> TA1537	reverse mutation	207	–	NT	Götzl and Schimmer 1993

AL = aristolactam; HID = highest ineffective dose; LED = lowest effective dose; NT = not tested; + = positive results in all listed strains; (+) = weakly positive results; – = negative results.

^aThe response was much greater without metabolic activation.

^bNitroreductase-deficient strains.

^c[IARC reported 34 µg/plate for TA100, YG1025, YG1026, YG1029 in Table 8; however, the correct value is 31, based on the molecular weight of aristolochic acid II.]

- 1 **5.3.3 Lower eukaryotes**
- 2 Exposure of *Drosophila melanogaster* to aristolochic acids (composition not specified)
- 3 caused sex-linked recessive lethal mutation, chromosome damage in the sex-chromosome
- 4 loss test, and recombinogenic damage in the somatic mutation and recombination test
- 5 (Frei *et al.* 1985), demonstrating strong genotoxic activity *in vitro* (Table 5-8).

Table 5-8. Genetic effects of aristolochic acids^a in *Drosophila melanogaster*, without metabolic activation

End point	Dose range (mM)	Result
Sex-linked recessive lethal mutation	0.05–0.1	+
Sex-chromosome loss	0.5–1.0	+
Somatic recombination	0.005–0.15	+

Source: Frei *et al.* 1985.

+ = positive results.

^aThe test agent was identified as aristolochic acid (CAS #313-67-7; i.e., aristolochic acid I), but the authors noted that the relative amounts of different aristolochic acids were not determined, [which would imply that a mixture was used].

1 5.3.4 *In vitro studies in mammalian cells*

2 The genetic end points examined *in vitro* in mammalian systems include DNA strand
3 breaks, mutation, sister chromatid exchange (SCE), micronucleus induction, and
4 chromosomal aberrations. The studies reviewed previously by IARC (2002) reported
5 mostly positive results and are included in Table 5-9 but are not reviewed in detail here.
6 Briefly, in the studies reviewed by IARC, aristolochic acids I and II induced *hprt* gene
7 mutations in Chinese hamster ovary (CHO) cells and rat fibroblast cells (aristolochic acid
8 I only) but did not cause DNA strand breaks in rat hepatocytes. Aristolochic acid
9 mixtures caused SCE and chromosomal aberrations in human lymphocytes and
10 micronucleus formation in human lymphocytes and hepatoma cells.

11 Li *et al.* (2006a) exposed porcine proximal tubular epithelial cell lines (LLC-PK1 cells)
12 to aristolochic acid I at concentrations of 0.08, 0.32, and 1.28 $\mu\text{g}/\text{mL}$ for 24 hours and
13 evaluated DNA damage with the comet assay. Aristolochic acids caused DNA damage in
14 LLC-PK1 cells in a dose-dependent manner. No DNA damage was detected in the
15 control group or low-dose group; however, DNA damage was significantly increased at
16 the two higher doses ($P < 0.01$) compared with controls. Wu *et al.* (2007b) exposed
17 human hepatoma HepG2 cells to aristolochic acids and identified genotoxic effects with
18 the comet assay and micronucleus test (see below). Aristolochic acids induced a dose-
19 dependent increase in DNA migration in the comet assay at concentrations of 25 to 200
20 μM . These investigators also noted that aristolochic acids caused a significant increase in
21 the levels of nitric oxide formation and 8-hydroxydeoxyguanosine (8-OHdG) at
22 concentrations $\geq 50 \mu\text{M}$. The authors concluded that aristolochic acids may exert
23 genotoxic effects through nitric oxide and its derivative peroxynitrite (ONOO^-).

24 Zhang *et al.* (2004) used several *in vitro* screening assays to test for genotoxic effects of
25 aristolochic acid I. These included reverse mutation in *S. typhimurium* (see
26 Section 5.3.2), forward mutation in mouse lymphoma L5178Y cells, and chromosomal
27 aberrations and micronuclei in CHO cells (see below). Mouse lymphoma L5178Y cells
28 (with or without S9 metabolic activation) were exposed to aristolochic acid I (1.57 to 100
29 $\mu\text{g}/\text{mL}$) for 4 hours and then incubated for 2 days. Aristolochic acid I increased mutations

1 at the tk locus in a concentration-dependent manner (at concentrations ≥ 25 $\mu\text{g/mL}$) with
2 or without metabolic activation.

3 Liu *et al.* (2004) used embryonic fibroblast cells from a human *p53* knock-in (Hupki)
4 mouse strain to generate human *p53* DNA-binding domain mutations. Fibroblasts were
5 harvested from 13.5-day-old embryos homozygous for the humanized *p53* allele.

6 Twenty-four cultures of the primary Hupki cells were exposed to 100 μM aristolochic
7 acid I for 48 hours and then passaged for 8 to 10 weeks. Ten of the 24 cultures were
8 established (defined as having acquired a uniform morphology and a population-doubling
9 time of 72 hours or less) within this timeframe and were analyzed for *p53* mutations. Six
10 base substitutions were identified in five of the established cultures. Four of the
11 substitutions were A:T \rightarrow T:A transversions on the nontranscribed strand, and two were
12 C:G \rightarrow G:C transversions. The authors noted that A:T \rightarrow T:A transversions are relatively
13 rare in spontaneous or UV-induced mutations, but are a hallmark of mutations induced by
14 aristolochic acid I. Feldmeyer *et al.* (2006) reported similar results in a study with the
15 same cell line exposed to 50 μM aristolochic acid I. Eighteen immortalized cultures were
16 examined for *p53* mutations, and six cell lines were found with base changes, five of
17 which were A:T \rightarrow T:A transversions. Feldmeyer *et al.* also found that one of the
18 mutations in their cell lines was at the same location (codon 139) that was reported in an
19 aristolochic acid-exposed patient (Nortier *et al.* 2000).

20 Chromosomal aberrations and micronuclei also were evaluated in CHO cells (Zhang *et*
21 *al.* 2004). For the chromosomal aberration test, CHO cells were exposed to aristolochic
22 acid I (6.25 to 50 $\mu\text{g/mL}$) with or without S9 metabolic activation for 3 hours and
23 incubated for 17 hours. For the micronucleus test, cells were exposed to aristolochic
24 acid I (0.79 to 100 $\mu\text{g/mL}$) with or without S9 for 4 hours and incubated for 20 hours; in
25 addition, separate cell cultures were exposed to aristolochic acid I for 23 hours without
26 S9. Significant increases in chromosomal aberrations and micronuclei occurred at
27 25 $\mu\text{g/mL}$ with activation and at 50 $\mu\text{g/mL}$ without activation. However, micronuclei
28 were not increased following continuous 23-hour exposure without activation. The
29 authors did not provide an explanation for the different responses in the micronucleus test

- 1 following 4 hours or 23 hours of exposure to the test agent. Wu *et al.* (2007b) (see above)
- 2 also found that aristolochic acids (12.5 to 50 μM .) increased the frequency of micronuclei
- 3 in human hepatoma HepG2 cells.

Table 5-9. Genetic effects of aristolochic acids in mammalian *in vitro* systems

Test system	Exposure	LED or HID (µg/mL)	End point	Without S-9	With S-9	Reference
Rat hepatocytes	AA I, AA II	not reported	DNA strand breaks	–	NT	Pool <i>et al.</i> 1986 ^a
Porcine proximal tubular epithelial cells	AA I	0.32	DNA damage	+	NT	Li <i>et al.</i> 2006a
Human hepatoma (HepG2) cells	AA mixture	25 µM	DNA damage	+	NT	Wu <i>et al.</i> 2007b
Rat fibroblast-like cells	AA I, AA II	20	mutation at <i>hprt</i> locus	+	NT	Maier <i>et al.</i> 1987 ^a
CHO cells	AA I	18.2	mutation at <i>hprt</i> locus	+	NT	Pezzuto <i>et al.</i> 1988 ^a
Mouse lymphoma cells	AA I	25	forward mutation	+	+	Zhang <i>et al.</i> 2004
Hupki mouse fibroblasts (human <i>p53</i> knock-in strain)	AA I	100 µM	<i>p53</i> DNA-binding domain mutation	+	NT	Liu <i>et al.</i> 2004
Hupki mouse fibroblasts (human <i>p53</i> knock-in strain)	AA I	50 µM	<i>p53</i> DNA-binding domain mutation	+	NT	Feldmeyer <i>et al.</i> 2006
Human lymphocytes	AA mixture	1	chromosomal aberrations	+	NT	Abel and Schimmer 1983 ^a
CHO cells	AA I	25–50	chromosomal aberrations	+	+	Zhang <i>et al.</i> 2004
Human lymphocytes and hepatoma cells	AA mixture	17	micronucleus induction	+	+	Kevekordes <i>et al.</i> 2001 ^a
CHO cells	AA I	25–50	micronucleus induction	+	+	Zhang <i>et al.</i> 2004
Human hepatoma (HepG2) cells	AA mixture	12.5 µM	micronucleus induction	+	NT	Wu <i>et al.</i> 2007b
Human lymphocytes	AA mixture	1	sister chromatid exchange	+	NT	Abel and Schimmer 1983 ^a

HID = highest ineffective dose; LED = lowest effective dose; NT = not tested; + = positive results; – = negative results.

^aCited in IARC 2002.

1 5.3.5 *In vivo studies*

2 Relatively few *in vivo* studies of genotoxic effects of aristolochic acids in mammals were
3 found. The genetic end points examined include mutation, mutational spectra in tumors
4 from animals or humans exposed to aristolochic acids, DNA damage, unscheduled DNA
5 synthesis in rats, and micronucleus induction in mice.

6 *Mutation in rodents*

7 Maier *et al.* (1985, 1987) investigated the mutagenicity of aristolochic acids in
8 subcutaneous tissue in male Sprague-Dawley rats. Aristolochic acids were injected in 1-
9 mL volumes into an air pouch formed by the injection of germ-free air into the loose
10 connective tissue between the shoulder blades of the rats. Two days after exposure, the
11 granulation tissue was dissected and dissociated enzymatically into single cells; it was
12 then cultured *in vitro* for 6 days, harvested, and exposed to 15 μ M 6-thioguanine culture
13 medium for 7 days, and the mutation frequency (frequency of 6-thioguanine-resistant
14 cells) was measured. In the first study, three groups of rats received aristolochic acids by
15 s.c. injection at a dose of 40, 160, or 320 μ g; another group received aristolochic acids by
16 gavage at 45 or 90 mg/kg b.w.; and a control group received a s.c. injection of air only.
17 [The proportions of aristolochic acids I and II in the mixture were not specified.] Dose-
18 related increases in the mutation frequency were observed following both s.c. and gavage
19 administration. In the second study, aristolochic acids I and II were studied separately.
20 Rats received an s.c. injection of aristolochic acid I at 80 μ g or aristolochic acid II at
21 320 μ g. The second study also investigated the effects of oxygen tension on mutation by
22 using different oxygen tensions (5% or 19%) in the cultures. At equimolar exposure
23 levels, aristolochic acid I induced 16 times as many mutations as aristolochic acid II at
24 19% oxygen tension and 19 times as many at 5% oxygen tension. The authors concluded
25 that the genotoxic activity of aristolochic acids in mammals is caused primarily by
26 aristolochic acid I, and that exposure of cells to aristolochic acids *in vitro* at low oxygen
27 tension corresponded most closely to the metabolic situation *in vivo*.

28 Aristolochic acids (a mixture of 50% aristolochic acid I and 40% aristolochic acid II)
29 were injected intragastrically into groups of 4 male *lambda/lacZ* transgenic mice (Muta
30 mice) at 15 mg/kg b.w., once a week for 4 weeks (Kohara *et al.* 2002). Total genomic

1 DNA was isolated from liver, bone marrow, urinary bladder, kidney, colon, lung,
2 forestomach, glandular stomach, spleen, and testis. The mutation frequencies for *lacZ* and
3 *cII* were significantly higher in exposed than in control mice in the target organs
4 (forestomach, kidney, and bladder) and the colon, but only slightly increased in the other
5 non-target organs (liver, bone marrow, lung, glandular stomach, spleen, and testis).
6 Sequencing showed primarily A:T → T:A transversions, which would be consistent with
7 mutagenesis induced by aristolochic acid I.

8 Chen *et al.* (2006b) and Mei *et al.* (2006) also investigated the mutagenicity of
9 aristolochic acids (mixture 40% aristolochic acid I and 56% aristolochic acid II) in male
10 Big Blue rats (in addition to the study of adduct formation discussed in Section 5.3.1).
11 Rats were exposed to oral doses of aristolochic acids at 0, 0.1, 1.0, and 10 mg/kg b.w. 5
12 days per week for 3 months and were sacrificed one day after the final treatment. Mei *et*
13 *al.* reported results for both kidney and liver tissue while Chen *et al.* reported results only
14 for the kidney. There was a strong linear dose-response relationship in mutant
15 frequencies for both kidney and liver, with the kidneys having at least two-fold more
16 mutations than the livers. The authors also reported that the relationship between total
17 AA-DNA adducts and mutant frequency was linear over the dose range studies for both
18 liver and kidney [no significance level or correlation coefficient was reported]. Sequence
19 analysis indicated that there was a statistically significant ($P < 0.001$) difference between
20 the mutation spectra observed in exposed rats and controls but not between liver and
21 kidney. A:T → T:A transversion was the predominant mutation type observed in exposed
22 rats, while G:C → A:T transition was the predominant type in the control group.

23 The results of *in vivo* mutagenicity studies in rodents are summarized in Table 5-10.

Table 5-10. Mutation frequencies in rodents exposed to aristolochic acids *in vivo*

Species (sex) End point	Compound Route	Tissues	Dose ^a	Mutation frequency × 10 ⁶	Reference
Sprague-Dawley rats (M) 6-TG resistance	AA mixture s.c. injection	s.c. granulation tissue ^b	control	3.7	Maier <i>et al.</i> 1985
			40 µg	10.7*	
			160 µg	172.5*	
			320 µg	305.3*	
Sprague-Dawley rats (M) 6-TG resistance	AA mixture gavage	s.c. granulation tissue ^b	45 mg/kg b.w.	18.1*	Maier <i>et al.</i> 1985
			90 mg/kg b.w.	54.5*	
Sprague-Dawley rats (M) 6-TG resistance	AA I s.c. injection	s.c. granulation tissue ^b	control (5%)	3.4	Maier <i>et al.</i> 1987
			control (19%)	4.0	
			80 µg (5%)	68.5*	
			80 µg (19%)	59.5*	
Sprague-Dawley rats (M) 6-TG resistance	AA II s.c. injection	s.c. granulation tissue ^b	control (5%)	3.4	Maier <i>et al.</i> 1987
			control (19%)	4.0	
			320 µg (5%)	17.3*	
			320 µg (19%)	17.5*	
Muta mice (M) <i>lacZ</i> mutation	AA mixture (56% I, 40% II) 4 intragastric injections	forestomach	control 15 mg/kg b.w.	33 1,129* ^c	Kohara <i>et al.</i> 2002
		kidney	control 15 mg/kg b.w.	81 851* ^c	
		bladder	control 15 mg/kg b.w.	60 1,026* ^c	
		colon	control 15 mg/kg b.w.	70 616* ^c	
Big Blue rats (M) <i>cII</i> gene	AA mixture (40% AAI, 56% AAII) gavage	kidney	control	29	Chen <i>et al.</i> 2006b, Mei <i>et al.</i> 2006
			0.1 mg/kg b.w.	78***	
			1.0 mg/kg b.w.	242***	
			10 mg/kg b.w.	1,319***	
Big Blue rats (M) <i>cII</i> gene	AA mixture (40% AAI, 56% AAII) gavage	liver	control	28	Mei <i>et al.</i> 2006
			0.1 mg/kg b.w.	37	
			1.0 mg/kg b.w.	113***	
			10 mg/kg b.w.	666***	

*Significantly different from the control group at $P < 0.05$.

***Significantly different from the control group at $P < 0.001$.

AA = aristolochic acids; TG = thioguanine.

^aThe value in parentheses is the oxygen tension of the cell cultures.

^bRats were exposed *in vivo*, but cells were harvested and cultured *in vitro*.

^cThe P -value was not reported by the authors.

- 1 *Mutational spectra in tumors from animals or humans*
- 2 Schmeiser *et al.* (1990) examined *ras* gene activation in various tumors from 18 rats
- 3 exposed to aristolochic acid I (Table 5-11). These included 14 squamous-cell carcinomas

1 of the forestomach, 7 squamous-cell carcinomas of the ear duct, 8 tumors of the small
2 intestine, 3 tumors of the pancreas, 1 adenocarcinoma of the kidney, 1 lymphoma, and 1
3 metastatic tumor each in the lung and the pancreas. A:T → T:A transversions were found
4 at the second position of codon 61 of the c-Ha-*ras* gene in 13 of 14 of the forestomach
5 squamous-cell carcinomas, all 7 squamous-cell carcinomas of the ear duct, and the lung
6 metastatic tumor. Additional analysis of the one forestomach tumor that initially failed to
7 show a *ras* point mutation revealed that the primary transfectant of this tumor contained a
8 c-Ha-*ras* mutation identical to that in the other forestomach tumors. In addition, c-Ki-*ras*
9 mutations at codon 61 were observed in 1 ear-duct tumor and 1 small-intestine tumor,
10 and c-N-*ras* mutations were observed in transformants of 2 pancreatic tumors and in the
11 lymphoma.

12 In a subsequent study, Schmeiser *et al.* (1991) analyzed tissue sections of tumors induced
13 by aristolochic acids in male Wistar rats and female NMRI mice for mutations at codon
14 61 of the Ha-*ras* gene (Table 5-11). The investigators examined 2 forestomach tumors
15 and 1 pancreatic tumor in rats and 1 forestomach tumor and 3 lung tumors in mice. The
16 same A:T → T:A transversions were observed in rat forestomach tumors and in mouse
17 forestomach and lung tumors, but not in the adjacent normal tissue. Cheng *et al.* (2006)
18 also identified the A:T → T:A transversion at codon 61 of the H-*ras* proto-oncogene in
19 DNA isolated from stomach tissue of rats with induced chronic renal failure exposed to
20 aristolochic acids for 12 weeks. No mutations were found in other tissues of these rats, in
21 control rats exposed to aristolochic acids, or in rats with chronic renal failure not exposed
22 to aristolochic acids.

23 Lord *et al.* (2004) looked for *p53* mutations in a patient with AAN (Table 5-11). This
24 patient had a kidney transplant three years after she had stopped taking an herbal
25 preparation containing aristolochic acids to treat eczema. Three years after the kidney
26 transplant, she had a bilateral nephroureterectomy which showed microinvasive TCC of
27 the ureter. One year later, the patient presented with a palpable tumor in the right breast
28 with metastases to the liver. Tissues from the breast tumor, normal breast tissue,
29 metastatic liver tumors, normal liver, bladder, transplanted kidney, and the original
30 urothelial tumor were analyzed for DNA adducts (see Section 5.3.1) and mutations. An

1 identical missense mutation in codon 245 of exon 7 of *p53* (GGC → GAC) was detected
2 in the breast and liver tumors. In contrast, the urothelial tumor contained an AAG →
3 TAG mutation in codon 139 of exon 5. The authors noted that the A → T transversion
4 observed in the urothelial tumor is the typical mutation observed in the *H-ras* gene of
5 rodent tumors induced by aristolochic acids and corresponds to DNA adducts at
6 adenosine residues. Cosyns *et al.* (1999) also reported overexpression of p53 in urinary-
7 tract tumors collected from patients with AAN. The authors noted that overexpression of
8 p53 strongly suggests that the p53 gene is mutated in AAN-associated tumors.
9 Sequencing analysis of a papillary TCC from the bladder in one AAN patient showed an
10 A → C transversion and a G → A mutation in exon 7 of *p53* (Cosyns 2003) (Table 5-11).

11 Grollman *et al.* (2007) examined urothelial and renal cortical tissue from 11 patients (7
12 women and 4 men) who had resided for at least 15 years in villages of Croatia where
13 BEN was endemic. All patients had upper urinary tract malignancies, and 8 patients
14 exhibited changes in their renal cortex that were diagnostic or highly suggestive of BEN.
15 (Two tissues had insufficient tissue analysis for histology.) DNA was isolated from fresh
16 tumor tissues from 6 patients and from formalin-fixed, paraffin-embedded tissues from 5
17 patients and examined for *p53* mutations. Mutational analysis was performed only on
18 tumors that were positive for *p53* mutations by immunohistochemistry (> 10% of tumor
19 cells staining positive with a highly specific p53 monoclonal antibody). Nineteen base
20 substitutions were identified in exons 2 to 11. Mutations at A:T base pairs accounted for
21 89% of all mutations, and 78% of these were A → T transversions. The authors noted
22 that these data are consistent with the mutational spectra of aristolochic acids, but differ
23 from the mutational spectra for sporadic TCC reported in the October 2006 edition of the
24 IARC p53 mutational database. They reported the mutation frequency of the A:T → T:A
25 transversion from that database as 4.8% of TCC in the bladder, 5.0% in the ureter, and
26 0% in the renal pelvis. The frequency and predominance of A:T → T:A transversions are
27 suggestive of a mutational signature for human exposure to aristolochic acids (Debelle *et*
28 *al.* 2008)

29 Arlt *et al.* (2007) reviewed the use of mutational spectra as a means for studying the
30 etiology of BEN-associated cancer. They discussed the mechanism of aristolochic acids–

1 induced carcinogenesis and the available data evaluating OTA and cancer. They noted
2 that, although unequivocal proof of OTA-specific DNA binding is lacking, or has been
3 disputed, two DNA adduct standards have been obtained by photooxidation, which
4 indicates that OTA can react with dG to yield C-C8-dG OTA and O-C8-dG OTA
5 adducts. The C-C8-dG OTA adduct has been detected in rodents treated with OTA and in
6 human bladder and kidney tumors exposed to OTA. Neither the mutagenic potential nor
7 specificity of this adduct is currently known; however, related C8-aryl adducts and C8-
8 phenyl-dG adducts have generated G:C → T:A and G:C → C:G transversions. It may be
9 difficult to distinguish between mutations induced directly by OTA or caused indirectly
10 by oxidative DNA damage. Regardless, the mutation pattern induced by OTA would be
11 different from that induced by aristolochic acids.

12 Evidence for OTA-DNA adducts remains controversial despite the various reports of
13 OTA adducts detected by ³²P-postlabeling techniques under different conditions (EFSA
14 2006). Advanced chemical analytical procedures have failed to verify the existence of
15 specific OTA-DNA adducts and it cannot be excluded that the reported adducts represent
16 non-specific oxidative DNA adducts (Gautier *et al.* 2001, Mally *et al.* 2004, Turesky
17 2005, EFSA 2006, Cavin *et al.* 2007, Palma *et al.* 2007,).

18 Kamp *et al.* (2005) noted that reactive metabolites of OTA and DNA adducts have not
19 been unambiguously identified but that oxidative damage has been observed *in vitro*.
20 These authors investigated whether or not OTA induces oxidative damage *in vivo*. Male
21 F344 rats were dosed with 0, 0.03, 0.1, and 0.3 mg/kg OTA daily for 4 wk by gavage.
22 OTA-mediated oxidative DNA damage was detected in liver and kidney DNA of all
23 dosed groups.

24 Several publications have concluded that OTA does not play a role in BEN or its
25 associated upper urothelial cancer (Grollman and Jelakovic 2007, Grollman *et al.* 2007, de
26 Jonge and Vanrenterghem 2008) based on the findings of the A:T → T:A transversions in
27 urinary tumors from patients with probable BEN. However, some recent reviews still
28 consider OTA to be a potential risk factor for BEN (Peraica *et al.* 2008, Stefanovic and
29 Radovanovic 2008).

Table 5-11. Tumor mutations in rodents and humans exposed to aristolochic acids

Species (sex)	Tumor location	Mutation		Reference
		Type	Incidence	
Wistar rats (M)	forestomach	Ha-ras 61 CAA→CTA	14/14	Schmeiser <i>et al.</i> 1990
	ear duct	Ha-ras 61 CAA→CTA	7/7	
	ear duct	Ki-ras 61 CAA→CAT	1/7	
	small intestine	Ki-ras 61 CAA→CTA	1/8	
	pancreas	N-ras 61 CAA→CTA	2/4 ^a	
	lymphatics	N-ras 61 CAA→CTA	1/1	
	kidney	ND	0/1	
	lung	Ha-ras 61 CAA→CTA	1/1 ^a	
Wistar rats (M)	forestomach	Ha-ras 61 CAA→CTA	2/2	Schmeiser <i>et al.</i> 1991
	pancreas	ND	0/1	
NMRI mice (F)	forestomach	Ha-ras 61 CAA→CTA	1/1	
	lung	Ha-ras 61 CAA→CTA	1/3	
Wistar rats with induced chronic renal failure (not specified)	stomach	H-ras 61 CAA→CTA	NR	Cheng <i>et al.</i> 2006
	kidney	ND	NR	
	ureter	ND	NR	
	bladder	ND	NR	
	liver	ND	NR	
Human AAN patient (F)	bladder	<i>p53</i> 230 ACC→CCC	1/1	Cosyns 2003
	bladder	<i>p53</i> 248 CGG→CAG	1/1	
Human AAN patient (F)	ureter	<i>p53</i> 139 AAG→TAG	1/1	Lord <i>et al.</i> 2004
	breast	<i>p53</i> 245 GGC→GAC	1/1	
	liver	<i>p53</i> 245 GGC→GAC	1/1	
Human BEN patients (N = 11) with urothelial cancer ^b	renal pelvis and/or ureter or bladder	<i>P53</i> (19 base substitution mutations) at A:T pairs	89%	Grollman <i>et al.</i> 2007
		A:T → T:A transversions	15/19	

ND = not detected; NR = not reported.

^aIncludes 1 metastatic tumor.

^bExposure to aristolochic acids was presumed by the authors but not documented.

- 1 *Unscheduled DNA synthesis, DNA damage, and micronucleus induction in rodents*
- 2 A single intragastric administration of aristolochic acids [it was not clear from the
- 3 publication whether it was aristolochic acid I or a mixture of aristolochic acids] did not
- 4 induce unscheduled DNA synthesis in the pyloric mucosa of male PVG rats at dose of 30
- 5 to 300 mg/kg b.w. (Burlinson 1989) or of male F344/Du Crj rats at a dose of 400 mg/kg
- 6 b.w. (Furihata *et al.* 1984).

1 Nesslany *et al.* (2007) investigated the ability of the alkaline *in vivo* Comet assay to
2 distinguish between genotoxic carcinogens from epigenetic carcinogens in freshly
3 isolated kidney cells from male Sprague-Dawley rats. Aristolochic acids (a mixture
4 containing 27% aristolochic acid I and 65% aristolochic acid II) were administered once
5 by gavage at 20 or 40 mg/kg to groups of 4 animals. Controls were given saline. Kidneys
6 were removed 3 to 6 hours after treatment or at 22 to 26 hours after treatment.
7 Aristolochic acids treatment significantly increased DNA fragmentation at both dose
8 levels in the 22- to 26-hour expression period.

9 Mengs and Klein (1988) administered single i.v. injections of aristolochic acids (77.2%
10 aristolochic acid I and 21.2% aristolochic acid II) at 6, 20, or 60 mg/kg b.w. to male and
11 female NMRI mice. A negative control group was given distilled water, and a positive
12 control group was given cyclophosphamide at 100 mg/kg b.w. Groups of 5 male and 5
13 female mice were killed at 24, 48, and 72 hours, and the bone marrow from both femurs
14 was examined for micronuclei in polychromatic erythrocytes. The high-dose groups
15 showed evidence of cytotoxicity. The numbers of micronuclei were significantly
16 increased in males in all dose groups at 24 hours and in the two highest dose groups at 48
17 hours and in females in the two highest dose groups at 24 and 48 hours. However, at 72
18 hours, the numbers of micronuclei in males or females did not differ significantly from
19 control levels. The authors did not offer an explanation for the negative results at 72
20 hours.

21 Kohara *et al.* (2002) also examined micronucleus induction in peripheral blood in male
22 Muta mice. Aristolochic acids (56% aristolochic acid I; 40% aristolochic acid II) was
23 administered by gavage at a dose of 15 mg/kg b.w. to groups of 4 mice once a week for 4
24 weeks. The control group received olive oil. Peripheral blood samples were collected
25 from the tail vein and examined for micronuclei 48 hours after the first exposure. The
26 mean frequency of micronucleated reticulocytes in the exposed group was 0.18%, which
27 did not differ significantly from that in the control group (0.13%). The authors noted that
28 different doses and routes of administration might explain the differences between their
29 results and those of Mengs and Klein (1988).

1 **5.4 Mechanistic studies and considerations**

2 Since the first AAN cases were reported in the early 1990s, many studies have
3 investigated the toxicity of aristolochic acids. Arlt *et al.* (2002b) and Cosyns (2003)
4 reviewed the toxicity data for aristolochic acids and evaluated the evidence for an
5 association between aristolochic acids exposure and AAN or AAN-associated urothelial
6 cancer in humans. [Although the precise mechanism has not been determined, the
7 available evidence suggests that DNA damage is responsible for the potential
8 carcinogenic effects of aristolochic acids and that the destructive fibrotic effects in the
9 kidney result from damage to the proximal tubular cell. Whether a mutation induces renal
10 interstitial fibrosis remains to be demonstrated.] This section discusses mechanistic
11 studies related to (1) renal toxicity (Section 5.4.1), (2) carcinogenesis in animals (Section
12 5.4.2), and (3) carcinogenesis in humans (Section 5.4.3). It is based primarily on reviews
13 by Arlt *et al.* (2002b) and Cosyns (2003), but also includes studies published after these
14 reviews.

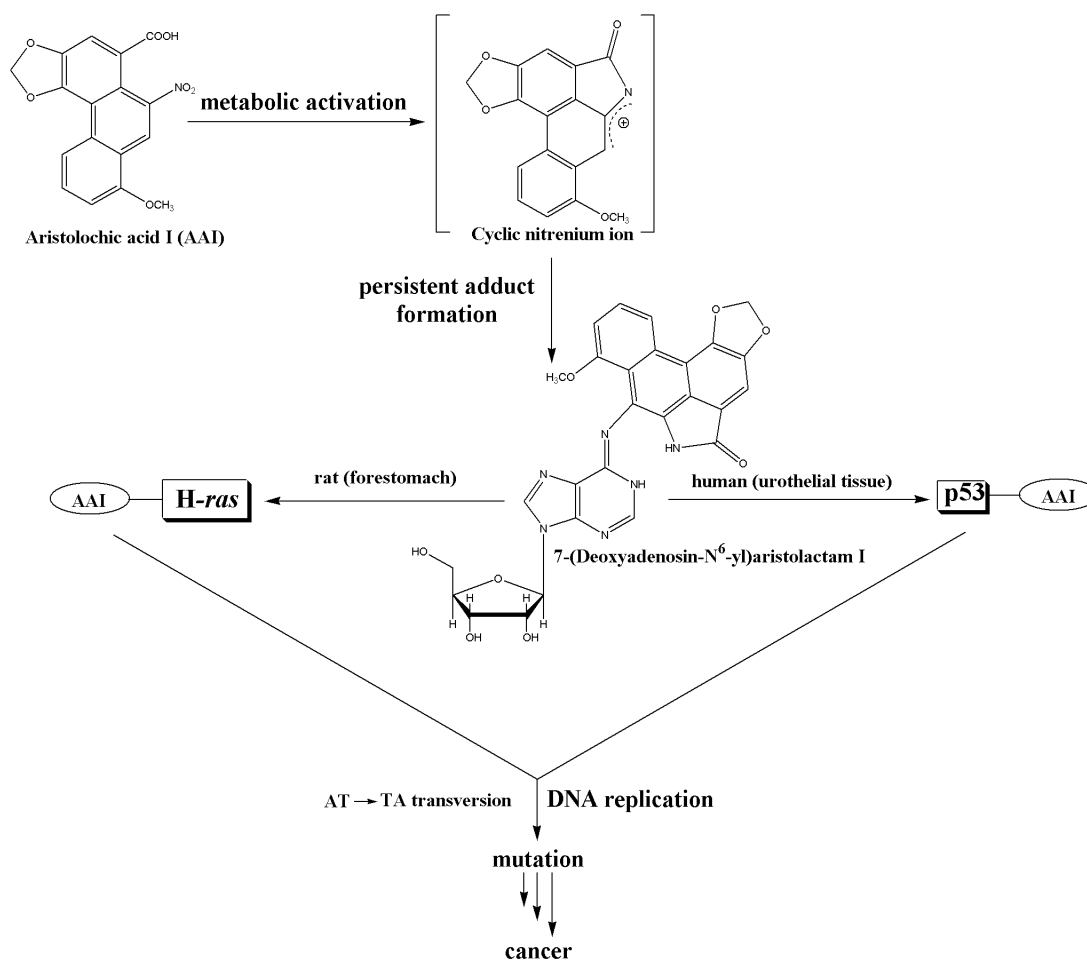


Figure 5-3. Proposed mechanism for aristolochic acids-induced carcinogenesis

Source: adapted from Arlt *et al.* 2002b.

- 1 5.4.1 Renal toxicity
- 2 Studies in experimental animals have shown that aristolochic acids exposure causes acute
- 3 tubular necrosis and renal failure in rodents that are reminiscent of AAN in humans.
- 4 Proteinuria is one of the earliest signs of AAN; thus, impairment of proximal tubular
- 5 function is thought to be one of the first manifestations of aristolochic acids toxicity.
- 6 Rodents exposed to high doses of aristolochic acids and renal biopsies from AAN
- 7 patients show selective proximal tubule lesions (Mengs 1987, Cosyns *et al.* 1994a,
- 8 Depierreux *et al.* 1994). Sun *et al.* (2006) (see Section 5.2.2 for details of the treatment)
- 9 reported that ischemia and hypoxia (measured by upregulation of HIF-1 α) were the most
- 10 important causes of renal interstitial fibrosis in female Wistar rats administered oral doses

1 of an *A. manshuriensis* decoction for 8 weeks. Although the exact mechanism is
2 unknown, Arlt *et al.* (2002b) noted the suggestion that AA-DNA adducts may trigger the
3 progressive fibrotic process in the kidneys. Lebeau *et al.* (2001, 2005) investigated the
4 effects of aristolochic acids on the proximal tubules *in vivo* in Wistar rats and *in vitro* in
5 opossum kidney cells. The proximal tubules reabsorb low-molecular-weight plasma
6 proteins (e.g., albumin and β_2 -microglobulin) through receptor-mediated endocytosis.
7 Exposure to aristolochic acids significantly decreased expression of megalin (one of the
8 receptor proteins) and resulted in formation of the same DNA adducts found in AAN
9 patients. The authors concluded that their data supported the role of aristolochic acids in
10 the early proximal tubule dysfunction observed in AAN patients and suggested a causal
11 relationship between DNA adduct formation, decreased megalin expression, and
12 inhibition of receptor-mediated reabsorption of low-molecular-weight proteins.

13 Yang *et al.* (2007) compared renal biopsy tissues from 8 patients with aristolochic acids-
14 induced acute tubular necrosis (AA-ATN) and 9 cases of antibiotic-induced ATN (a-
15 ATN). All patients diagnosed with AA-ATN had taken unspecified amounts of
16 medications containing guan mu tong (*A. manshuriensis*), and both the AA-ATN and the
17 a-ATN patients had significantly ($P < 0.01$) elevated serum creatinine at the time of renal
18 biopsy. Although neither group of patients had histologically confirmed interstitial
19 fibrosis by light microscopy, the AA-ATN renal tissue showed changes consistent with a
20 tendency toward fibrosis, which the authors proposed could be due to diminished renal
21 tubular epithelial cell repair, impaired anti-fibrosis mechanisms, and loss of peritubular
22 capillaries. The authors suggested that the combination of elevated α -smooth muscle
23 actin expression and limited expression of proliferating cell nuclear antigen in AA-ATN
24 tissue were consistent with transdifferentiation of renal tubular epithelial cells to
25 myofibroblasts, which would participate in interstitial fibrosis rather than in cell repair.
26 The lack of cellular regeneration also could be due in part to the observed suppression of
27 epidermal growth factor expression in the AA-ATN kidney tissue. Impairment of anti-
28 fibrosis mechanisms was suggested by the expression of components of extracellular
29 matrix, i.e., fibronectin and collagens III and IV, in the tissue from the AA-ATN patients
30 only, even though both groups of patients had increased expression of transforming

1 growth factor- β_1 and connecting tissue growth factor, both of which regulate tissue repair
2 in different diseases. Finally, there was a severe loss of peritubular capillaries in the AA-
3 ATN patients, which could result in hypoxia and decreased blood flow in the
4 tubulointerstitium, contributing to the tubulointerstitial damage; similar findings of
5 hypoxia were also reported in rats by Sun *et al.* (2006) (see above).

6 5.4.2 Carcinogenesis in animals

7 As described in Section 4, exposure to aristolochic acids increased incidences of tumors
8 in forestomach, kidney, lung, and lymphoid tissues in mice exposed for 3 weeks and in
9 forestomach, kidney, ear duct, small intestine, and other organs in rats exposed for 3 days
10 to 12 months. These studies also showed that aristolochic acids exposure causes acute
11 tubular necrosis and renal failure in rodents that are reminiscent of AAN in humans.

12 *In vitro* and *in vivo* studies with experimental animal systems show that the critical step
13 in metabolic activation of aristolochic acids is nitroreduction by CYP1A1 and CYP1A2
14 and, to a lesser extent, NADPH:CYP reductase (see Section 5.4.2 for additional enzymes
15 involved in other activation steps). [The ultimate carcinogenic species is believed to be a
16 cyclic *N*-acylnitrenium ion that binds to exocyclic nitrogen groups of purine nucleotides
17 (see Figure 5-2).] However, adducts were detected in both target (forestomach and
18 kidney) and non-target tissues (stomach, liver, and lung) of rats (see Tables 5-4 and 5-5).
19 [One Wistar rat was reported to have a metastatic tumor in the lung (Table 4-6), but no
20 primary lung tumors were reported in this species.] While adduct levels generally were
21 somewhat higher in forestomach and kidney, the presence of similar levels of adducts in
22 non-target tissues suggests that adduct formation alone may not be sufficient to explain
23 tumor formation.

24 The overall binding activity of aristolochic acid I was reported to be about 10 times that
25 of aristolochic acid II (Pfau *et al.* 1990b). Although both target and non-target tissues
26 showed the same relative amounts of the individual aristolochic acid I adducts in their
27 study, overall DNA binding by aristolochic acid I was highest in forestomach and lowest
28 in kidney and urinary bladder. Adduct levels were lower for aristolochic acid II than for
29 aristolochic acid I, and the highest levels were detected in kidney, with lower levels in
30 liver, stomach, and urinary bladder epithelia. Later studies reported different results for

1 tissue distribution and the relative numbers of adducts for aristolochic acids I and II.
2 Dong *et al.* (2006) reported higher adducts levels for aristolochic acid II than for
3 aristolochic acid I, and adduct levels were higher in kidney than in forestomach for both
4 aristolochic acids in Wistar rats (see Table 5-5). Mei *et al.* (2006) also reported higher
5 adduct levels in kidney than in liver of Big Blue rats (see Table 5-4).

6 The predominant and most persistent adduct, dA-AAI, is consistent with possible direct
7 mutagenicity of aristolochic acid adducts, as a high frequency of A:T → T:A
8 transversions of the first adenine of codon 61 (CAA) of the H-*ras* oncogene was reported
9 in aristolochic acids–induced tumors in rats and mice (Schmeiser *et al.* 1990, Schmeiser
10 *et al.* 1991). Chen *et al.* (2006b) (see Section 5.3.5) also demonstrated that aristolochic
11 acids–induced mutations in the cII gene in the kidneys of Big Blue transgenic rats were
12 likely the result of AA–DNA adducts because the dA-AAI adducts were persistent and
13 frequently resulted in A:T → T:A transversions due to incorporation of dAMP opposite
14 the adenine adducts. The authors noted that the AA–DNA adducts induced the same type
15 of mutation that was shown to result in activation of H-*ras* and initiation of tumors.
16 Furthermore, DNA binding studies using the DNA polymerase arrest assay confirm that
17 aristolochic acids bind to adenines of codon 61 in the mouse H-*ras* gene (Arlt *et al.* 2000)
18 and to purines in the human *p53* gene (Arlt *et al.* 2001a, Lord *et al.* 2004) (see “In vitro
19 *studies in cell-free systems*” in Section 5.3.1). [Thus, the formation of persistent dA-AAI
20 adducts in target tissues is consistent with the mutation spectra in those tissues. These
21 data suggest that dA-AAI adducts occupy genomic sites that are resistant to repair, and
22 are subsequently converted into mutations in cellular oncogenes.]

23 The mutagenic activity of AA–DNA adducts was investigated by Broschard *et al.* (1994,
24 1995). Synthetic oligonucleotides containing either a single deoxyadenosine or
25 deoxyguanosine residue were treated with aristolochic acid I or II. The adducted
26 oligonucleotides were then used as templates in primer extension reactions catalyzed by
27 modified bacteriophage T7 DNA polymerase or human DNA polymerase α . The authors
28 found that dAMP and dTMP were incorporated equally well across from the
29 deoxyadenosine adducts, but that deoxyguanosine adducts allowed preferential
30 incorporation of dCMP. Thus, the guanine adducts have a lower mutagenic potential than

1 adenine adducts. These data demonstrate that the A:T → T:A transversions are caused by
2 the adenosine adducts and provide a plausible explanation for the mutations found at
3 adenine residues in codon 61 of the *H-ras* gene in rodent tumors.

4 Although the urothelial cancer reported in humans exposed to aristolochic acids (see
5 Section 3.2) has been proposed to be linked to AA–DNA adducts, the cellular
6 mechanisms, such as the effects of aristolochic acids exposure on expression of specific
7 genes, by which aristolochic acids induce cancer is not known. In order to examine the
8 tissue-specific toxicity and tumorigenicity of aristolochic acids, Chen *et al.* (2006c)
9 defined differences in gene expression profiles in kidney and liver of rats treated with
10 aristolochic acids using the Rat Genome Survey Microarray. Aristolochic acids
11 significantly altered the gene expression profiles in both organs; however, there were
12 significantly more ($P < 0.01$) altered genes involved in cancer-related pathways in kidney
13 than in liver. Furthermore, genes associated with defense responses (i.e., apoptosis and
14 immune response) were significantly altered in the kidney but not in the liver. [Thus,
15 differences in the gene expression profiles may be responsible for the tissue-specific toxic
16 and carcinogenic effects of aristolochic acids.]

17 Chang *et al.* (2006) investigated the possible role of activation of cell-cycle progression
18 via cyclin D₁/cdk4 and cyclin E/cdk2 in the induction of the urothelial proliferation in
19 male Wistar rats exposed to an aristolochic acids mixture (41% aristolochic acid I; 56%
20 aristolochic acid II) at either 5 or 10 mg/kg b.w. per day. The authors reported that dose-
21 dependent urothelial proliferation was detected histologically, and at doses of 5 and 10
22 mg/kg, respectively, induction of cyclin D₁/cdk4 increased 1.57- and 1.95-fold, and
23 induction of cyclin E/cdk2 increased 1.46- and 1.62-fold. Phosphorylation of the
24 retinoblastoma tumor suppressor protein (Rb) also increased 1.75-fold at the low dose
25 and 2.07-fold at the high dose, while Rb/E2F complexes were reduced to 0.65 of the
26 control level at the low dose and 0.24 of the control level at the high dose. The authors
27 suggested that induction of cyclin-cdk complexes could result in phosphorylation of Rb
28 and release of E2F from Rb, resulting in promotion by E2F of cell-cycle transition from
29 the G1 to the S phase, which could cause urothelial proliferation as a pro-carcinogenic
30 phenomenon in tumorigenesis.

1 Stemmer *et al.* (2007) investigated gene expression profiles in male wild-type and Eker
2 rats exposed to aristolochic acids or ochratoxin A (OTA). Eker rats are heterozygous for
3 a dominant germline mutation in the *tuberous sclerosis 2 (Tsc2)* tumor suppressor gene.
4 Rats were gavaged daily with 10 mg/kg aristolochic acids or 0.21 mg/kg OTA for 1, 3, 7,
5 or 14 days. Renal histopathology, tubular cell proliferation, and gene expression profiles
6 from the renal cortex/outer medulla were analyzed at the end of each exposure period.
7 Aristolochic acids–treated Eker and wild-type rats were qualitatively comparable in all
8 variables assessed, suggesting that *Tsc2* was not involved in the mechanism of action. In
9 contrast to the effects of aristolochic acids, OTA induced distinctly different gene
10 expression profiles when in OTA-treated Eker and wild-type rats. The authors concluded
11 that the gene expression changes, which were more prominent in the *Tsc2* mutant Eker
12 rat, suggested involvement of *Tsc2* in OTA-mediated toxicity and carcinogenicity.
13 Aristolochic acids caused a slightly greater inflammatory response than in controls but
14 did not induce pronounced nonneoplastic renal pathology in either strain. Aristolochic
15 acids were not cytotoxic or mitogenic under the conditions of this study but did result in
16 significant deregulation of gene expression that increased with duration of exposure.
17 There was a prominent up-regulation of genes encoding Phase I or Phase II
18 biotransformation enzymes and of several *p53* pathway genes. In addition, antiapoptotic
19 genes and genes involved in DNA replication and cell-cycle progression were down-
20 regulated while proapoptotic genes were upregulated.

21 *5.4.3 Metabolic activation and toxic effects in humans*

22 As discussed in Section 3.1.1, an estimated 1,500 to 2,000 people were exposed to the
23 herbal weight-loss regimen in Belgium, yet only about 100 people developed AAN.
24 Differences in dose, duration of exposure, and metabolic activation may account for the
25 differences in susceptibility. However, no mechanistic explanation for the unusual
26 rapidity of the onset of urinary-tract carcinoma in humans following *Aristolochia*
27 consumption has been found.

28 Although there are some differences between the aristolochic acids metabolites detected
29 so far in humans and experimental animals, the metabolic activation pathways and DNA
30 adducts are the same. As in experimental animals, a number of cytosolic and microsomal

1 enzymes are involved in aristolochic acids activation in humans. These include
2 cytochrome P450 enzymes (CYP1A1, CYP1A2, and NADPH-CYP reductase),
3 peroxidases (prostaglandin H synthase), cytosolic nitroreductases (DT-diaphorase and
4 xanthine oxidase), COX, and NAD(P)H:quinone oxidoreductase (Sato *et al.* 2004,
5 Stiborová *et al.* 1999, Stiborová *et al.* 2001a,b,c, Stiborová *et al.* 2002, Stiborová *et al.*
6 2003, Stiborová *et al.* 2005a, Stiborova *et al.* 2007). These enzymes are affected by
7 several factors, including nutrition, smoking, drugs or environmental chemicals, and
8 genetic polymorphisms. Because prostaglandin H synthase is the most abundant
9 peroxidase found in kidney and ureter, it may be particularly important for the toxic and
10 carcinogenic effects of aristolochic acids.

11 Activation of aristolochic acids to their DNA-reactive and mutagenic metabolites requires
12 reduction of their aryl nitro group (Meinl *et al.* 2006). The biological activity of many
13 nitro- and aminoarenes after Phase I metabolism is enhanced by acetyltransferases or
14 sulphotransferases. Meinl *et al.* demonstrated that expression of human sulfotransferases
15 (SULT1A1 and SULT1B1) in bacterial and mammalian target cells enhanced the
16 mutagenicity of aristolochic acids. The mutagenic effects were reduced by exposure to
17 pentachlorophenol, an inhibitor of SULT1A1. Both SULT1A1 and SULT1B1 are
18 expressed in human kidney, but at lower levels than in liver. SULT1A1 is polymorphic
19 with substantial differences in expression. Potent inhibitors of this enzyme include many
20 phytochemicals, drugs, and food additives. Thus, SULT1A1 may be an important
21 modifier of the nephrotoxic and carcinogenic effects of aristolochic acids in humans.

22 Nortier *et al.* (2000) demonstrated a significant relationship between cumulative dose of
23 *A. fangchi* and the risk of developing urothelial cancer in the Belgian AAN patients (see
24 Section 3.2.2), but the levels of DNA adducts did not correlate with dose. The mean
25 levels of dA-AAI adducts in renal tissue samples did not differ significantly between
26 patients who had developed urothelial carcinoma and those who had not developed
27 cancer. The authors noted that this observation was “not disturbing,” because DNA
28 adduct levels reflect the balance between their formation and loss from repair or
29 apoptosis, and because the aristolochic acids content of the various powders differed as
30 much as 10-fold from batch to batch. Furthermore, all but 2 of the tumor-free patients had

1 urothelial atypia or preneoplastic lesions. AA-DNA adducts also have been identified in
2 urothelial cancer patients who were not part of the Belgian cohort (Gillerot *et al.* 2001,
3 Arlt *et al.* 2004b, Lord *et al.* 2004, Lo *et al.* 2005).

4 Urothelial tissues from AAN patients have been shown to contain relatively high levels
5 of dA-AAI adducts up to 89 months after exposure (Nortier *et al.* 2000). This adduct also
6 was predominant and highly persistent in rat forestomach and kidney, where high
7 incidences of tumors occurred. Urothelial carcinoma and urothelial atypia from AAN
8 patients have been associated with overexpression of p53 protein (Cosyns *et al.* 1999).
9 Arlt *et al.* (2001a) showed that both aristolochic acids I and II formed DNA adducts at
10 purine bases in human *p53 in vitro*, and Lord *et al.* (2004) reported mutations in exon 7
11 of *p53* that included an A → T transversion, which is the typical mutation observed in the
12 *H-ras* gene of rodent tumors induced by aristolochic acids (see “*Mutational spectra in*
13 *tumors from animals or humans*” in Section 5.3.5). It is likely that aristolochic acids–
14 induced mutations in *p53* could lead to tumors in the same way as reported in rats with
15 *H-ras* mutations. Grollman *et al.* (2007) also reported that urothelial cancer tissues
16 obtained from BEN patients contained *p53* mutations. Mutations at A:T base pairs
17 accounted for 89% of all *p53* mutations, and 78% of these were A → T transversions.

18 **5.5 Summary**

19 *5.5.1 Absorption, distribution, metabolism, and excretion*

20 Aristolochic acids are absorbed from the gastrointestinal tract and distributed throughout
21 the body, as evidenced by observation of specific DNA adducts in kidney, urinary tract,
22 liver, lung, brain, stomach, and other tissues of humans and experimental animals. The
23 available data indicate that aristolochic acid I is metabolized by both oxidative and
24 reductive pathways, whereas aristolochic acid II is metabolized only by a reductive
25 pathway. The metabolites of aristolochic acid I in rats and mice include aristolactam I,
26 aristolactam Ia, aristolochic acid Ia, aristolic acid I, 3,4-methylenedioxy-8-hydroxy-1-
27 phenanthrenecarboxylic acid, and a decarboxylated metabolite. The metabolites of
28 aristolochic acid II include aristolactam II, aristolactam Ia, and 3,4-methylenedioxy-1-
29 phenanthrenecarboxylic acid. Only aristolactam I and II have been reported in humans,
30 although full metabolic profiles determined through sensitive techniques have not been

1 reported. Phase II metabolites include the *N*- and *O*-glucuronides of aristolactam Ia, the
2 *N*-glucuronide of aristolactam II, and the *O*-glucuronide, *O*-acetate, and *O*-sulfate esters
3 of aristolochic acid Ia. The metabolites are excreted in the urine and the feces. Reported
4 half-lives in New Zealand White rabbits for aristolochic acids I and II were 0.12 hours
5 and 0.27 hours, respectively. Aristolactam Ia is the major metabolite of aristolochic acid I
6 detected in both urine (46%, primarily in a conjugated form) and feces (37%).
7 Aristolactam II is the primary metabolite of aristolochic acid II, but less than 10% of a
8 dose is recovered as this form in the urine and feces; the other metabolites account for 5%
9 or less of the administered dose. Studies in rats show that the metabolites of aristolochic
10 acid I are excreted within 24 hours, whereas metabolites of aristolochic acid II are still
11 present in the urine at 72 hours.

12 5.5.2 Toxicity

13 The kidney is the primary target organ for aristolochic acids toxicity. A specific kidney
14 disease known as AAN has been described in more than 100 cases (all but 1 in women)
15 exposed at a weight-loss clinic in Belgium and in more than 100 other sporadic cases in
16 Europe, Asia, and the United States (Table 3-1). Two clinical presentations of AAN are
17 described. One is marked by the rapid onset of acute renal failure and the other by adult-
18 onset Fanconi syndrome characterized by a slower and possibly reversible onset of
19 similar symptoms.

20 Only about 5% of the exposed population from a Belgian clinic developed AAN.
21 However, the kidney toxicity was severe in those 5%. The disease was marked by
22 anemia, mild tubular proteinuria, extensive and usually hypocellular interstitial fibrosis
23 decreasing from the outer to the inner cortex, tubular atrophy, global sclerosis of
24 glomeruli, and rapid progression to renal failure. Another clinical presentation (Fanconi
25 syndrome) has been described in a few cases in China, Korea, Japan, and Germany. This
26 form is characterized by proximal tubular dysfunction, and a generally slower
27 progression to end-stage renal disease. Balkan endemic nephropathy (BEN or EN), which
28 is characterized by chronic interstitial fibrosis progressing slowly to end-stage renal
29 disease and urothelial malignancy has been proposed to result from exposure to

1 aristolochic acids in wheat contaminated with seeds of *Aristolochic clematitis* (reviewed
2 by Debelle *et al.* 2008) (see Section 3.4).

3 Rats and mice exposed to high doses of aristolochic acids developed acute renal failure.
4 The primary features included tubular necrosis, elevated plasma creatinine and urea
5 levels, atrophy of the lymphatic organs, superficial ulceration of the forestomach, and
6 hyperplasia and hyperkeratosis of the squamous epithelium. Lower doses fed to rats over
7 several months resulted in chronic renal failure. Hypocellular interstitial fibrosis
8 decreasing from the outer to the inner cortex was observed in a study in rabbits and in
9 some, but not all, studies in rats and mice. Rabbits exposed to aristolochic acids also
10 developed renal fibrosis of the gastric mucosa, and urothelial atypia. Species and strain
11 differences in susceptibility to the toxic effects of aristolochic acids are apparent. Rabbits
12 appear to be more susceptible to renal and extrarenal fibrosis than rats or mice, and
13 BALB/c and C3H/He mice were more susceptible than C57BL/6 mice to the nephrotoxic
14 effects. Most animal studies used purified aristolochic acids rather than the crude extracts
15 or relatively unprocessed botanical material (*e.g.*, ground, dried root) consumed by
16 humans.

17 Metabonomic studies in rats identified changes in serum and urinary metabolites that
18 indicate that the renal proximal tubule is the primary target of aristolochic acids.
19 Aristolochic acids and a plant extract containing aristolochic acids produced similar
20 effects that were associated with rapidly progressive renal toxicity.

21 Aristolochic acids and their aristolactam derivatives are cytotoxic to cells growing in
22 culture, including kidney cells and human epithelial breast cells. The cytotoxic effects of
23 aristolochic acids may be linked to a rapid increase in intracellular calcium that promotes
24 apoptosis. Other studies reported that aristolochic acids disrupted mitochondrial
25 permeability transition in human renal tubular epithelial cells, an effect that may be
26 involved in renal injury, and one study reported cell-cycle arrest in human urinary tract
27 epithelial cells. Aristolochic acids are also specific inhibitors of phospholipase A₂ and
28 may have other specific biochemical targets that explain its renal toxicity and its
29 widespread use in traditional plant-based medical therapies throughout the world.

1 5.5.3 Genetic damage and related effects

2 Aristolochic acids are metabolically activated by reductive pathways to form a reactive
3 intermediate cyclic *N*-acylnitrenium ion that forms adducts at purine bases in DNA.
4 These adducts include dA-AAI, dG-AAI, dA-AAII, and dG-AAII. Of these, dA-AAI is
5 the most persistent and appears to be responsible for most of the mutagenic properties of
6 aristolochic acids. Aristolochic acids I and II are mutagenic in a number of strains of *S.*
7 *typhimurium*, with negative results reported only for several nitroreductase-deficient
8 strains. Aristolochic acids I and II were genotoxic in the SOS chromotest in *E. coli*, and
9 aristolochic acid I was genotoxic in *D. melanogaster*. In mammalian *in vitro* studies,
10 aristolochic acid I or II or mixtures of aristolochic acids increased the frequency of
11 chromosomal aberrations, DNA damage, oxidative DNA damage (as evidenced by
12 increased levels of nitric oxide formation and 8-OHdG adducts), sister chromatid
13 exchange, micronuclei, and mutations. In mammalian *in vivo* studies, aristolochic acids
14 were mutagenic and caused DNA damage.

15 5.5.4 Mechanistic studies and considerations

16 The carcinogenic action of aristolochic acids appears to be mediated through a cyclic *N*-
17 acylnitrenium ion, a reactive intermediate that forms adducts at purine bases in DNA. A
18 number of cytosolic and microsomal enzymes are capable of bioactivating aristolochic
19 acids to the reactive species (see Section 5.4.2). The DNA adducts have been associated
20 with the mutagenic and carcinogenic effects of aristolochic acids. In particular, the
21 persistence of the major dA-AAI adduct (lifelong in rats and at least 89 months in
22 humans) indicates that it is nonrepairable. These DNA adducts have been associated with
23 an A:T → T:A transversion mutation at adenine residues in codon 61 of the *H-ras* gene in
24 rodent tumors and overexpression of p53 in malignant urothelial cells and papillary TCC
25 in humans. Aristolochic acid adducts were found in urothelial and renal cortical tissues
26 from four patients with BEN confirmed by WHO criteria. A group of 11 patients (7
27 women and 4 men) who had resided in endemic villages for a minimum of 15 years and
28 who had upper urinary tract tumors were analyzed for mutations in the p53 gene; 8 of the
29 9 patients with adequate tissue samples for histopathologic analysis had changes in their
30 renal cortex that were diagnostic or suggestive of BEN. A:T → T:A transversion
31 mutations in the p53 gene were identified in tumor tissue from 10 of the 11 patients and

1 this mutation accounted for the majority (78%) of mutations found in the urinary tract
2 tumors (10 localized in the renal pelvis and/or ureter and 1 in the bladder) from these
3 patients. Gene expression profiles of kidney and liver of rats exposed to aristolochic acids
4 identified significant alterations of expression of cancer-related pathways, including
5 apoptotic and immune responses, in kidney but not in liver.

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Glossary of Terms

Adenocarcinoma 755: A transplantable, spontaneous mammary adenocarcinoma in the C57Bl mouse strain that does not metastasize but kills the host by local growth and invasion.

Adulterated: Being made impure by mixing in a foreign or inferior substance.

Antihelminthic: A drug used to treat parasitic infestations caused by protozoa or worms.

Atypia: A general term describing cells that vary in appearance from normal cells because of inflammation or as a cancerous or precancerous condition.

Black foot disease: A disease caused by exposure to arsenic via drinking water in Taiwan; severe damage to the blood vessels of the lower limbs leads to gangrene.

Boiling point: The boiling point of the anhydrous substance at atmospheric pressure (101.3 kPa) unless a different pressure is stated. If the substance decomposes below or at the boiling point, this is noted (dec). The temperature is rounded off to the nearest °C.

Contaminant: A substance inappropriately present in the environment that might cause harmful effects.

Decoction: An extract obtained by boiling.

Density: The density for solids and liquids is expressed in grams per cubic centimeter (g/cm^3) and is generally assumed to refer to temperatures near room temperature unless otherwise stated. Values for gases are generally the calculated ideal gas densities in grams per liter at 25°C and 101.325 kPa.

Emmenagogue: An agent or measure that induces menstruation.

Fanconi syndrome: A complex of proximal renal tubular dysfunctions defined by renal glycosuria, generalized aciduria, phosphaturia, and renal tubular acidosis and often associated with hypokalemia, hypophosphatemia, and osteomalacia. Also called Fanconi's syndrome.

Glutathione-S-transferase 7-7: A synonym for rat glutathione-S-transferase P (GST class-pi).

Henry's Law constant at 25°C: The ratio of the aqueous-phase concentration of a chemical to its equilibrium partial pressure in the gas phase. The larger the Henry's law constant the less soluble it is (greater tendency for vapor phase).

Hydronephrosis: A physical condition of the kidney or kidneys in which the pelvis and calyces (the urine-collection structure of the kidney) become distended because urine is unable to drain from the kidney down the ureter into the bladder.

Log octanol-water partition coefficient (log K_{ow}): The ratio of concentrations of a substance in octanol and in water, when dissolved in a mixture of octanol and water. For convenience, the logarithm of K_{ow} is used. The octanol/water partition coefficient of a substance is useful as a means to predict soil adsorption, biological uptake, lipophilic storage, and bioconcentration.

Megalin: A receptor protein expressed on the luminal surface of the proximal renal tubules that acts as a component of the mechanism by which essential metabolites, including small protein molecules, are retrieved from the ultrafiltrate by endocytosis for degradation or recycling to the blood stream.

Melting point: The melting point of the substance at atmospheric pressure (101.3 kPa). When there is a significant difference between the melting point and the freezing point, a range is given. In case of hydrated substances (i.e., those with crystal water), the apparent melting point is given. If the substance decomposes at or below its melting point, this is noted (dec). The temperature is rounded off to the nearest °C.

Mesotherapy: A general term for a technique developed in France in the 1940s involving a series of injections of medications and other substances into the subcutaneous fat for treatment of a variety of medical conditions, but often for cosmetic purposes and weight loss.

Metabonomics: A method for simultaneous quantitative measurement of the amounts of multiple metabolites, which generates a profile or “fingerprint” for the metabolites present in a biological sample. Uses of metabonomic data include: (1) comparisons of normal physiologic states and pathologic changes or disease states, (2) comparisons between control and treated, including determining the effects of toxic or unknown chemicals, (3) comparisons between different species/strains or sexes, (4) comparisons of changes over time, (5) identification of the source of the differences, e.g., target organs or cells or the chemical exposure causing the differences, and (6) identification of a sample from its fingerprint.

Molecular weight: The molecular weight of a substance is the weight in atomic mass units of all the atoms in a given formula. The value is rounded to the nearest tenth.

MTT assay: A colorimetric assay for measuring cell proliferation. Yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is reduced to purple formazan in the mitochondria of living cells, and the absorbance of the purple formazan is determined with a spectrophotometer.

Mu Tong: Chinese herbal medicine ingredient that may describe *Aristolochia manshuriensis* and certain *Clematis* and *Akebia* species. Alternate spellings include Mutong and Mu-Tong.

Neoplasm: An abnormal group of cells.

Negative log acid dissociation constant (pK_a): A measure of the degree to which an acid dissociates in water (a measurement of acid strength). The pK_a is the negative logarithm (to the base 10) of the acid dissociation constant (K_a); the lower the pK_a, the stronger the acid.

Nephroureterectomy: Excision of a kidney and all or part of its ureter; the term ureteronephrectomy may also be used.

Physical state: Substances may either be gases, liquids, or solids according to their melting and boiling points. Solids may be described variously as amorphous, powders,

pellets, flakes, lumps, or crystalline; and the shape of the crystals is specified if available. Solids also may be described as hygroscopic or deliquescent depending upon their affinity for water.

Pin Yin: A form of Chinese language phonetic notation converting Standard Mandarin to Roman script (*pin* means spell and *yin* means sound).

Pyelonephritis: An infection of the kidney and the ducts (ureters) that carry urine away from the kidney.

Solubility: The ability of a substance to dissolve in another substance and form a solution.

Transgenic: An animal that carries a foreign gene that has been deliberately inserted into its genome.

Tumor: An abnormal mass of tissue.

Ureteronephrectomy: Excision of a kidney and all or part of its ureter; the term nephroureterectomy may also be used.

Urothelial: Pertaining to the urothelium, the lining of the urinary tract, including the renal pelvis, ureters, urinary bladder, and urethra.

Vapor density, relative: A value that indicates how many times a gas (or vapor) is heavier than air at the same temperature. If the substance is a liquid or solid, the value applies only to the vapor formed from the boiling liquid.

Vapor pressure: The pressure of the vapor over a liquid (and some solids) at equilibrium, usually expressed as mm Hg at a specific temperature (°C).

Appendix A: Botanical Products Available on the Internet

11/08/07- Edits to Gold and Slone tables are enclosed in square brackets ([]).

In 2003 Gold and Stone submitted a letter to the FDA in which they noted that they were able to identify 112 botanical products that either contained or had the potential to contain aristolochic acids despite the FDA safety warnings in 2000 and 2001. The botanical species listed by Gold and Slone were included in their tables because the botanicals were either known to contain aristolochic acids, i.e., *Aristolochia* species or *Asarum canadense* (Table A-1 here), because of the possibility for substitution by *Aristolochia* species for other botanicals (i.e., *Akebia* spp., *Asarum* species other than *Asarum canadense*, *Clematis* spp., *Cocculus* spp., *Saussurea lappa*, *Sinomenium acutum*, and *Stephania* spp.) (Table A-2 here), or because they are likely to be an *Asarum* because the name of the product is reported as “wild ginger” (Table A-3 here).

The information presented in the original Gold and Slone (2003) tables is now at least 4 years old and some of that information might not be current in 2007. Therefore the following tables contain updated information available as of September 2007 (searches completed 9/5/07 – 9/14/07). Some of the websites listed in the Gold and Slone tables were found to still be current; however, there were numerous scenarios where some or all of the information has changed. The various scenarios were addressed as detailed below.

- When the website and product were confirmed to still contain the specific botanical as an ingredient, that fact is noted with a dagger (†) after the URL (53 of the original listings were confirmed as still current).
- If any part of the information could not be confirmed, the following steps were taken and the results are enclosed in brackets to indicate updated information:
 - If the website still exists and the product is still listed, but the presence of the botanical could not be confirmed because no ingredients are listed or because the ingredients list does not include the botanical, these outcomes are noted.
 - If the website still exists, but the product is no longer listed, that is noted and the URL has been deleted.
 - If the website no longer exists, a search was conducted to identify a new website for the retailer and any new URL is noted.
 - When neither the product nor the website was found, searches were also conducted for the product name and manufacturer’s name if available.

Any information obtained through these searches has been added to the table.

- Finally, any products containing any of the botanicals listed by Gold and Slone that were not listed in the original tables but were identified on the current version of the websites have been added here. However, no attempt was made to identify additional websites or retailers beyond those originally reported in by Gold and Slone in 2003.

Table A-1. Botanical products for oral use available as of March 4, 2003 on the web that list ingredients known to contain aristolochic acids

Species	Medicinal name	Retailer	Manufacturer	2007 update
<i>Aristolochia clematis</i>	PMS-Ease	InnerLife Wellness Center	Växa	[Product not found on the Innerwellness.com website.]
<i>Aristolochia fangji</i>	Tong Xue Pian Tablets	Merchant America	[NA]	[Retailer no longer found on the Internet.]
<i>Aristolochia manshuriensis</i> [<i>manshuriensis</i>]	Long Dan Xie Gan Wan / Long Dan Xie Gan Pian / Lung Tan Xie Gan	[Morningstar Health]	[Min Shan brand]	[http://www.morningstarhealth.com/store/Min-Shan-Brand-Long-Dan-Xie-Gan-Wan.html] [Long Dan Xie Gan Wan confirmed, but ingredients are not listed.]
		[Vita Springs]	[NA]	http://www.vitasprings.com/londanxiegan1.html †
		Wing Hop Fung	[NA]	[Product not found on the Winghopfung.com website.]
		Ginseng 4 Less	[NA]	[http://www.ginseng4less.com/chinese2.html] [Long Dan Xie Gan Wan confirmed, but ingredients are not listed.] [Note: <i>Akebia</i> stem (mu tong) is also sold in bulk on this website- http://www.ginseng4less.com/herbs.html ; see entry in Table 2, below]
		Angel Herb: Herbs for Health	[NA]	[Retailer no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	2007 update
		MaxNature	[Guang Ci Tang (Chinese Patent Medicine Series) (Shanghai TongHanChun Herbs Factory)- http://www.guangcitan.com/]	[http://maxnature.stores.yahoo.net/lodanxieganw.html] [Long Dan Xie Gan Wan with <i>Aristolochia manshuriensis</i> still available for sale.] [Three other products containing <i>Akebia</i> as an ingredient are listed in Table A-2.]
		TCM Healing Center for Men's Diseases [associated with Eastern Chinese Medicine Export Company, see below]	[NA]	[No Long Dan Xie Gan Wan or similar products found on website; however, other products containing <i>Aristolochia</i> plant parts were identified, and are listed below as <i>Aristolochia sp.</i>]
		Oriental Chinese Medicine Wholesale Retail Company- [now called Eastern Chinese Medicine Export Company]	[NA]	[See entry for TCM Healing Center, above.]
		[Chinese Wonder Herbs]	[NA]	http://www.chineseherb.com/Merchant2/merchant.mv?Screen=PROD&Store_Code=CWH&Product_Code=CWH42 [Lung Tan Xie Gan Wan confirmed, but ingredients are not listed.]
		[Hierbas Chinas (Spanish version of Chinese Wonder Herbs)]	[NA]	[See entry above.]
		Chinese Patent Medicines	[NA]	[Retailer no longer found on the Internet.]
		China guide [now listed as CGC Mall.com]	[NA]	[http://www.cgcmall.com/ProductDetails.asp?ProductCode=hr00ld1] [Long Dan Xie Gan Wan confirmed, but <i>Aristolochia</i> is not listed as an ingredient.]

Species	Medicinal name	Retailer	Manufacturer	2007 update
		[Herbswest LLC]	[NA]	http://www.herbswest.net/items/BL2080.shtml [Product ingredients now include <i>Akebia</i> root rather than <i>Aristolochia manshuriensis</i> - see new listing below in Table 2.]
[<i>Aristolochia</i> sp.]	[Ma dou ling] [Qing mu xiang]	[Eastern Chinese Medicine Export Company] [Eastern Chinese Medicine Export Company]	[Eastern Chinese Medicine Export Company] [Eastern Chinese Medicine Export Company]	[http://www.tcm-treatment.com/images/wholesale/herb-price/6.htm] and [http://www.tcm-treatment.com/herbs/0-madouling.htm] [<i>Aristolochia</i> fruit: <i>Aristolochiae fructus</i>] [http://www.tcm-treatment.com/images/wholesale/herb-price/7.htm] [<i>Aristolochia</i> root: <i>Aristolochiae radix</i>]
[<i>Aristolochiae Mollissimae</i>]	[Xun gu feng]	[Eastern Chinese Medicine Export Company]	[Eastern Chinese Medicine Export Company]	[http://www.tcm-treatment.com/images/wholesale/herb-price/9.htm] [<i>Mollissima: Aristolochiae mollissimae</i>]
<i>Aristolochia manshuriensis</i> [<i>manshuriensis</i>]	Q13: Five Types Stranguria Pill (Wu Lin Wan)	TCM Healing Center for Men's Diseases (Eastern Chinese Medicine Export Company) [TCM Healing Center for Men's Diseases formerly called Oriental Wholesale & Retail Company]	[Guangdong Guoyitang Pharmaceutical Co., Ltd.]	[http://www.mentcm.com/images/drugstore/product-17-q02.htm]
[<i>Aristolochia manshuriensis</i> (<i>manshuriensis</i>)]	[Q19: Strangury Clearing Soluble Granule (qing ling chong ji)]	[TCM Healing Center for Men's Diseases (Eastern Chinese Medicine Export Company (TCM Healing Center for Men's Diseases formerly called Oriental Wholesale & Retail Company)]	[Haerbing TCM Sixth Factory Co., LTD]	[http://www.mentcm.com/images/drugstore/product-17-q02.htm] [Product ingredients list includes Manshurian <i>aristolochia</i> stem.]

Species	Medicinal name	Retailer	Manufacturer	2007 update
[<i>Aristolochia manshuriensis</i> (<i>manshuriensis</i>)]	[Q20: Stone-Expelling Granule (pai shi ke li)]	[TCM Healing Center for Men's Diseases (Eastern Chinese Medicine Export Company (TCM Healing Center for Men's Diseases formerly called Oriental Wholesale & Retail Company)]	[Jiangxi Nanxchang Jisheng Manufacturing Co., LTD]	[http://www.mentcm.com/images/drugstore/product-17-q02.htm] [Product ingredients list includes Manshurian <i>aristolochia</i> stem.]
<i>Aristolochia</i> sp.	Chi Kuan Yen Wan	Angel Herb: Herbs for Health Opane.com	[NA] [NA]	[Retailer no longer found on the Internet.] http://www.opane.com/coughchikuany.html † Health Canada reports this to contain aristolochic acid: http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/2001/2001_100_e.html .
<i>Aristolochia</i> sp.	Guan Xin Su He / Circulatory Cardioflex	Angel Herbs: Herbs for health Opane.com	[NA] [NA]	[Retailer no longer found on the Internet.] [Product not found on the Opane.com website.]
<i>Aristolochia</i> sp.	Gui Pi Wan	Doc4Pain.com	[NA]	[Retailer no longer found on the Internet.]
[<i>Aristolochia</i> sp.]	[Virginia Snake]	[Taylor's Organic Gardens]	[NA]	[http://www.taylorgarden.com/Products/BulkHerbList.asp] [Product is listed in the bulk herbs list as Virginia Snake (<i>Aristolochia serpentaria</i>).]
[<i>Aristolochia</i> sp.]	[Ma Dou Ling Aristolochia fruit (<i>Aristolochiae Fructus</i>)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/6.htm] [Product available in wholesale price list of Chinese herbs.]
[<i>Aristolochia</i> sp.]	[Qing Mu Xiang Aristolochia root (<i>Aristolochiae Radix</i>); Vladimiria root (<i>Vladimiriae Radix</i>)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/7.htm] [Product available in wholesale price list of Chinese herbs.]

Species	Medicinal name	Retailer	Manufacturer	2007 update
<i>Aristolochia</i> sp. (+ Coltsfoot)	Chuan Ke Wan	[Herbs West, LLC]	[Herbal Times brand]	http://herbswest.net/items/BL1355.shtml †
<i>Aristolochia</i> sp. + <i>Clematis</i> sp.	Circula	Opone.com PlazaQ.com	[NA] [NA]	[<i>Aristolochia</i> and <i>clematis</i> could not be confirmed on either website. Another product was found with an ingredients list including <i>Aristolochia</i> and <i>clematis</i> (see below) and several products containing <i>clematis</i> sold through these websites are listed in Table 2, below.]
[<i>Aristolochia</i> sp. + <i>Clematis</i> sp.]	[Eucommiae Musculoskeletal Support Pills: Du Zhong Zhuang Gu Wan]	[Opone.com] [PlazaQ.com]	[NA] [NA]	[http://opone.stores.yahoo.net/eucmussup100.html] [The ingredients listed include <i>clematis</i> root and Woolly Dutchmanspipe (i.e., <i>Aristolochia tomentosa</i> - http://plants.usda.gov/java/profile?symbol=ARTO3 - and wild ginger.)] [http://plusq.stores.yahoo.net/eucmussup100.html] [Same ingredients list as on Opone.com website.]
<i>Asarum canadense</i>	Wild ginger capsules	Taylor's Organic Gardens	Taylor's Organic Gardens [Does not appear to be a manufacturer]	http://www.taylorgarden.com/Products/Bulk_New.asp?Common_Name=Wild%20Ginger [Wild ginger and wild ginger capsules are still listed on the website, but the website identifies the product as <i>Zingiber officinale</i> , which is the botanical name for ginger. The listing below is for a product identified on the website as <i>Asarum canadense</i> (wild ginger).]
[<i>Asarum canadense</i>]	[Canada snake]	[Taylor's Organic Gardens]	NA	[http://www.taylorgarden.com/Products/BulkHerbList.asp] [Listing is for Canada snake (<i>Asarum canadense</i>).]
<i>Asarum (canadense)</i>	Old Indian Herbal Syrup	iHerb, Inc. (Herbal Advisor)	Planetary Formulas	[Old Indian Syrup no longer found in search of website. Another product (Joint 4-Way Support System) lists <i>Asarum</i> herb as an ingredient (see listing in Table 2, below).]

Species	Medicinal name	Retailer	Manufacturer	2007 update
<i>Asarum canadense</i>	Cold Away [Now called Winter Coat]	Sunrise Herbal Remedies	[Sunrise Herbal Remedies]	http://www.sunriseherbfarm.com/coldaway.html †
<i>Asarum canadense</i>	Cramp Relief	Sunrise Herbal Remedies	Sunrise Herbal Remedies	http://www.sunriseherbfarm.com/cramprelie.html †
<i>Asarum canadense</i>	Formula 208	Web Vitamins	Heritage Products	[Product no longer available from retailer.]
<i>Asarum canadense</i>	Mother Earth's Cough Syrup / Mother Earth's Respiratory System Tonic	InterNatural Kalyx DiscountBlvd.com NutritionBlvd.com	Heritage Products [Store] Heritage Products [Store] [NA]	http://www.international-alternative-health.com/ingr/ingr179190.cfm † http://www.kalyx.com/store/proddetail.cfm/ItemID/569659.0/CategoryID/6000.0/SubCatID/985.0/file.htm † [Retailers DiscountBlvd.com and NutritionBlvd.com were not found on the web.]
<i>Asarum canadense</i>	Viral Resolve [called "Viral Vanish" in 2007]	[Sunrise Herbal Remedies]	[Sunrise Herbal Remedies]	http://www.sunriseherbfarm.com/viralresolve.html †
<i>Asarum canadense</i>	Wild Ginger tincture	Crucible Catalog	Spagyric Tinctures [Not a manufacturer but a potential product preparation method.]	http://www.crucible.org/spagyricsS-Z.htm †

Species	Medicinal name	Retailer	Manufacturer	2007 update
<i>Asarum canadense</i>	Wild Ginger tincture [Teeter Creek Herbs Asthmaid Tincture]	Spring Valley Herbs and Natural Foods [Spring Valley Herbs and Natural Foods]	Teeter Creek [Teeter Creek]	http://www.springvalleyherbs.com/catalog.php?itemID=2025 [Wild Ginger tincture containing <i>Asarum canadense</i> is listed as sold out on the Spring Valley Herbs and Natural Foods website; the product was not found in a search of www.teetercreekherbs.com .] [Teeter Creek Herbs Asthmaid Tincture containing wild ginger is available at http://www.springvalleyherbs.com/catalog.php?itemID=2045 .]
<i>Asarum canadense</i> + <i>Akebia trifoliata</i>	Aller Relief	Spanda	Neo Concept	[<i>Asarum</i> is no longer listed as an ingredient in Aller Relief- http://www.spanda.com/catalog/product_info.php?cPath=1_31&products_id=51 .] [Gold and Slone (2003) noted that the manufacturer had recalled this product and reformulated it to remove <i>Asarum</i> , which was confirmed from the product information on the Neo Concept website- (http://www.neoconcept.com/1_welcome.html).]

Source: Gold and Sloan 2003a.

Table A-2. Botanical products for oral use, available as of March 4, 2003 on the web, that list ingredients that may be adulterated with aristolochic acids

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Akebia</i> sp.	Akebia	Botanicum	[NA]	[Retailer no longer found on the Internet.]
<i>Akebia</i> sp.	Alive Energy: Mental and Emotional Strength Women's Courage 60's	InterNatural	[NA]	[Product not found on the Internatural.com website.]
<i>Akebia</i> sp.	Circulation: Specific Rubrella Care [Feng Zhen hwan]	Opane.com	[NA]	http://www.opane.com/cirspeclubca.html †
<i>Akebia</i> sp.	Eye Relief Capsules	diabetes-alternativemedicine.com	[NA]	[Retailer no longer found on the Internet.]
<i>Akebia</i> sp.	Genpriv	Mandarin Herbs	[NA]	[Product not found on the Mandarinherbs.com website]
<i>Akebia</i> sp.	K-C	The Herb Nook Virtualherbs.com	Nature's Sunshine	[Retailers no longer found on the Internet]
<i>Akebia</i> sp.	Lung Tan Xie Gan Wan Combination	Wing Hop Fung	[NA]	[Product not found on the Winghopfung.com website.]
<i>Akebia</i> sp.	Shi Chuan Xiu Xue Tang (General Purpose Stop Blood Formula)	Ancient Way Accupuncture & Herbs	[NA]	http://www.ancientway.com/Pages/MartialArtsFormulas.html †
<i>Akebia</i> sp.	Wind-Dispelling Powder (Xiao Feng San)	Nature's Health	[NA]	http://www.nature-s-health.com/products/theproduct1.asp?pid=287 †
[<i>Akebia</i> sp.]	[Yu Zhi Zi Foreknowledge Akebiae Fructus]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/10.htm] [Product available in wholesale price list of Chinese herbs.]
<i>Akebia</i> sp. + <i>Asarum</i> sp.	Nasixx	MyHerbalRx.com	[NA]	http://myherbalrx.net/products/nasixx2.htm †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Akebia</i> sp. + <i>Asarum</i> sp.	Sinus Clear Ephedra Free	Vitanet	Ridge Crest Herbals	http://store.yahoo.com/vitanet/sinclearnoep1.html †
<i>Akebia</i> sp. + <i>Stephania</i> sp.	Chinese Kidney Activator (formerly K-C)	Blessed Nutrition, Inc Herbshop.com	[NA] [NA]	[Product not found on the Blessednutrition.net website] [http://www.herbshop.com/urinary.htm#kc The website notes that the Chinese Kidney Activator product, which lists <i>Akebia</i> stem and <i>Stephania</i> root, is unavailable while it is reformulated to meet new FDA regulations.]
<i>Akebia</i> sp. + <i>Stephania</i> sp.	Chinese Kidney Activator (K-C) [Eliminate Moisture] Qu Shi	Mind, Body & Soul Healer	[NA]	http://www.soulhealer.com/1872-5.htm [Product confirmed, but <i>Akebia</i> and <i>Stephania</i> not present in ingredients list.]
		The Reynolds Office of Health and Nutrition	[NA]	http://www.reynoldsoffice.com/1872-5.htm [Product confirmed, but <i>Akebia</i> and <i>Stephania</i> not present in ingredients list.]
		Go With Herbs [The website opens the same information as The Reynolds Office of Health and Nutrition]	[NA]	http://www.gowithherbs.com/1872-5.htm [Product confirmed, but <i>Akebia</i> and <i>Stephania</i> not present in ingredients list.]
		Plain Herb [The website opens the same information as The Reynolds Office of Health and Nutrition]	[NA]	http://www.plainherb.com/1872-5.htm [Product confirmed, but <i>Akebia</i> and <i>Stephania</i> not present in ingredients list.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Akebia</i> sp. + <i>Stephania</i> sp.	K-C (Eliminate Moisture/Qu Shi) - Kidney Support	Superlative Soundness	[NA]	[Retailer no longer found on the Internet.]
[<i>Akebia trifoliata</i>]	[Ba Yue Zha (Akebia fruit; 5:1 Extract Powder)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/286084.0/CategoryID/1000.0/SubCatID/2565.0/file.htm] [A search of the Kalyx.com website identified the 11 products listed below as containing <i>Akebia trifoliata</i> in the ingredients. An additional product contained both <i>Akebia trifoliata</i> stem and <i>Stephania tetrandra</i> root (see listing below), and 3 products containing <i>Asarum sieboldii</i> (see listings below) also were identified.]
	[Akebia Fruit (Ba Yue Zha) Cut & Sifted]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/286087.0/CategoryID/13000.0/SubCatID/2850.0/file.htm] [Product for sale is <i>Akebia</i> fruit.]
	[Dang Gui Si Ni Teapills (Frigid Extremities- Dang Gui Si Ni Tang Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290675.0/CategoryID/13000.0/SubCatID/12095.0/file.htm] [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
	[Eight Righteous Teapills (Eight Herb Powder for Rectification- Ba Zheng San Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290823.0/CategoryID/8000.0/SubCatID/1045.0/file.htm] [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
	[Great Mender Teapills (Muscle Bone Traumatic Injury - Jin Gu Die Shang Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290695.0/CategoryID/13000.0/SubCatID/12095.0/file.htm] [Product ingredients list includes <i>Akebia trifoliata</i> stem.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
	[Great Windkeeper Teapills (Disperse Wind- Xiao Feng Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290571.0/CategoryID/13000.0/SubCatID/12095.0/file.htm [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
	[Ji Sheng Ju He Wan (Abundant Life Tangerine Seed Pills)]	[Kalyx]	[Min Shan]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290762.0/CategoryID/13000.0/SubCatID/12095.0/file.htm [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
	[Kai Kit Wan (Prostate Gland Pills)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290840.0/CategoryID/8000.0/SubCatID/2220.0/file.htm [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
	[Long Dan Xie Gan Wan (Gentiana Drain the Liver Pills)]	[Kalyx]	[Min Shan]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290843.0/CategoryID/8000.0/SubCatID/1055.0/file.htm [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
	[Magnolia Flower Teapills (Xin Yi Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290586.0/CategoryID/13000.0/SubCatID/12095.0/file.htm [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
	[Red Door Teapills (Guide Out the Red - Dao Chi Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290599.0/CategoryID/13000.0/SubCatID/12095.0/file.htm [Product ingredients list includes <i>Akebia trifoliata</i> stem.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
	[Snake & The Dragon Teapills (Gentiana Drain the Liver - Long Dan Xie Gan Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290861.0/CategoryID/8000.0/SubCatID/1055.0/file.htm] [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
<i>Akebia trifoliata</i>	Bai Ji Li (5:1 herb extract powder)	Kalyx	Plum Flower brand	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290254.0/CategoryID/1000.0/SubCatID/10.0/file.htm] [Bai Ji Li confirmed, but its ingredients include (or it consists of) <i>Tribulus terrestris</i> rather than <i>Asarum</i> . See listing below.] [<i>Akebia</i> fruit was found on the Kalyx.com website (see entry above for Ba Yue Zha).]
<i>Akebia trifoliata</i>	Eight Righteous / Ba Zheng San Wan	Herbswest, LLC Jade Chinese Herbs & Extracts	[NA] [Same ingredients as in Plum Flower brand sold on the Kalyx website.] [NA]	[http://www.herbswest.net/items/13325.shtml] † [Retailer no longer found on the Internet.]
<i>Akebia trifoliata</i>	Hepataplex	2000 + Nutrition Center	[NA]	[Retailer no longer found on the Internet.]
<i>Akebia trifoliata</i>	Kai Kit Wan (Reduce Prostate Swelling Pills)	Herbswest, LLC	[NA] [Same ingredients as in Plum Flower brand sold on the Kalyx website.]	[http://www.herbswest.net/items/13956.shtml] †
<i>Akebia trifoliata</i>	Prostate: Kai Kit Pills	Opane.com	Hanyang pharmaceutical	[http://www.opane.com/proskaikitpi.html] †
<i>Akebia trifoliata</i>	Prostate: Kai Kit Wan	Healing Herbs of China	Plum Flower	[http://store.yahoo.com/healingherbschina/prosenkaikit.html] †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Akebia trifoliata</i>	Prostate: Prostate Gland Care	Opone.com	[NA]	http://www.opone.com/prosproglan.html [<i>Akebia</i> not found in ingredients list.]
[<i>Akebia trifoliata</i>]	[Snake & The Dragon Teapills]	[MaxNature]	[Plum Flower Brand]	[http://maxnature.stores.yahoo.net/sndrteldanxi.html] [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
[<i>Akebia trifoliata</i>]	[Snake & The Dragon Teapills]	[MaxNature]	[Min Shan Brand (Lanzhou Foci herb factory)]	[http://maxnature.stores.yahoo.net/lodanxieganwl.html] [Product ingredients list includes <i>Akebia trifoliata</i> stem.]
[<i>Akebia trifoliata</i>]	[Coptis Purge Fire Formula]	[MaxNature]	[Health Concerns]	[http://maxnature.stores.yahoo.net/copufifoloda.html] [Product ingredients list includes <i>Akebia trifoliata</i> caulis (Mu Tong).]
[<i>Akebia trifoliata</i> + <i>Stephania tetrandra</i>]	[Xuan Bi Teapills (Drain Away Obstruction - Xuan Bi Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290634.0/CategoryID/13000.0/SubCatID/12095.0/file.htm] [Product ingredients list includes <i>Akebia trifoliata</i> stem and <i>Stephania tetrandra</i> root.]
<i>Asarum heterotropoides</i>	Bio-Antihist	Natural Health Consultants	Ameriden	http://www.naturalhealthconsult.com/Monographs/BioAntihist.html †
<i>Asarum heterotropoides</i>	100% Herbal Treatment for Tinnitus	Young Again Nutrients [Supplement Spot Nutrients (2007)]	[NA]	[http://www.supplementspot.com/tinnitus.html]
<i>Asarum heterotropoides</i>	Asarum	Botanicum	[NA]	[Retailer no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[<i>Asarum sieboldii</i>]	[Chui Feng Tou Gu Wan (Dispel Wind Penetrate Bone - Zhui Feng Tou Gu Wan)]	[Kalyx]	[Plum Flower brand]	[http://www.kalyx.com/store/proddetail.cfm/ItemID/290670.0/CategoryID/13000.0/SubCatID/12095.0/file.htm] [Product ingredients list includes <i>Asarum sieboldii</i> herb.]
<i>Asarum</i> sp.	AsthmaClear	LifeHealthEnergy.com	[NA]	[Retailer no longer found on the Internet.]
<i>Asarum</i> sp.	Azarina	Qlife Batory Asset Management	[NA] [NA]	http://www.qlife.com/azarina.html † http://www.merchantamerica.com/qlife/index.php?ba=product_enlarge&category=1843&product_id=6747 †
<i>Asarum</i> sp.	Beijing Tong Ren Tang Qi Guan Yan Ke Sou Tan Chuan Wan	Opane.com	Tong Ren Tang	http://www.opane.com/beijtonrenta24.html †
		[PlazaQ.com]	[NA]	http://store.yahoo.com/plusq/beijtonrenta24.html [Product ingredients list includes <i>Asarum</i> herb.]
<i>Asarum</i> sp.	Breath Easy	NutraCompute	[NA]	[Product not found on Nutracompute.com website.]
<i>Asarum</i> sp.	Chuan Qiong Cha Tiao Pian	Vita Springs	[NA]	[http://www.vitasprings.com/chuan-qiong-cha-tiao-pian-headache.html] †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Asarum</i> sp.	Clear Tinnitus	AlzheimerSupport.com	Clear Products	https://www.alzheimersupport.com/shop/product.cfm?product_code=N0161 †
		ProHealth, Inc.	[NA]	https://www.prohealthnetwork.com/TreatmentCenter/product.cfm?product_code=N0161 †
		ChronicFatigueSyndromSupport. Com [Part of ProHealth, Inc.]	[NA]	http://www.chronicfatiguesyndromsupport.com/shop/product.cfm?product_code=N0161 †
		LifesVigor and many others	[NA]	[http://www.lifesvigor.com/17668.html] †
<i>Asarum</i> sp.	M05: Brain-Conquering Calmness Capsule (Zhen Nao Ning Jiao Nang)	TCM Healing Center for Men's Diseases Oriental Wholesale & Retail Company [These companies share the same website.]	[NA]	[http://www.mentcm.com/images/drugstore/product-13-m.htm] †
<i>Asarum</i> sp.	Migrex	MyHerbalRx.com	[NA]	[http://www.figueroa.net/store/product_info.php?cPath=22&products_id=95&osCsid=7f251fd22c3df99ca2a8d77706c3b4b0] HTML [MigreX confirmed, but <i>Asarum</i> not found in list of ingredients.]
<i>Asarum</i> sp.	Notoptergium Decoction with Nine Herbs (Jiu Wei Qiang Huo Tang)	Nature's Health	[NA]	[http://www.nature-s-health.com/products/theproduct1.asp?pid=289] †
<i>Asarum</i> sp.	Xiao Qing Long Wan (Concentrated Chinese Herb for Common Cold)	MaxNature.com	[NA]	[http://maxnature.stores.yahoo.net/xiqilowan.html] †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[<i>Asarum</i> sp.]	[Pure Essence, Advanced Holistics, Joint 4 Way Support System]	[iHerb.com]	[Pure Essence]	[http://www.iherb.com/ProductDetails.aspx?c=1&pid=3200&at=0] [Product ingredients list includes <i>Asarum</i> herb.]
[<i>Asarum</i> sp.]	[Bei Xi Xin Northern asarum Asiasari Herba cum Radice Septentionalis]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/herb.index.htm] [Product available in wholesale price list of Chinese herbs.]
[<i>Asarum</i> sp.]	[Bei Dou Gen Northern asarum Menispermi Daurici Radix]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/herb.index.htm] [Product available in wholesale price list of Chinese herbs.]
[<i>Asarum</i> sp.]	[Xi Xin Asarum Asiasari Herba cum Radice]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/9.htm] [Product available in wholesale price list of Chinese herbs.]
<i>Clematis chinensis</i>	Diabetics Yu Xiao San 8804	VitaSprings.com	[Dr. Chong Brand]	[http://www.vitasprings.com/diabetics-yu-xiao-san-8804-preventing-diabetes.html]. [Product confirmed, but <i>Clematis</i> not found in list of ingredients.]
		Chong's Health Care	[Dr. Chong Brand]	[http://store.yahoo.com/cljhealth/yuxiaosan88052.html] [This link automatically redirects to this website- http://cljhealth.stores.yahoo.net/yuxiaosan88052.html] [Product confirmed, but <i>Clematis</i> not found in list of ingredients.]
		[MaxNature]	[Dr. Chong Brand]	[The same product is listed at this website- http://www.maxnature.com/yuxbasontrad.html () with Chinese <i>clematis</i> as an ingredient.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Clematis chinensis</i>	Flex N Spring	Health Products Distributors, Inc.	[NA]	[Product not found on the Health Products Distributors, Inc. (Integratedhealth.com) website.]
<i>Clematis chinensis</i>	Joint Health	N101, Inc.	Rainbow Light	[Product not found on the N101.com website.] [A search of the Rainbow Light website (http://www.rainbowlight.com/) also failed to identify a product by this name.]
<i>Clematis chinensis</i>	Kam Wo Herbal Tea	PlazaQ.com	Sing-lin	http://store.yahoo.com/plusq/kamwoherateak.html [Product confirmed, but <i>Clematis</i> not found in list of ingredients.]
[<i>Clematis chinensis</i>]	[Gam Wo Herbal Tea]	[MaxNature Health Products Co.]	[Sing-lin]	[http://maxnature.stores.yahoo.net/gamwoheteagh.html] [Product containing <i>Clematis chinensis</i> was found on this website by searching for Sing-Lin brand.]
<i>Clematis chinensis</i>	Tien Hsien Natural Nutritious Liquid	Cancerth.com	[NA]	[Retailer no longer found on the Internet]
<i>Clematis chinensis</i>	Yu Xiao San 8805	MaxNature Health Products Co	[Chong's Health Care, Inc.]	http://www.maxnature.com/yuxbasontrad.html †
<i>Clematis chinensis</i>	40+ Nutritional System Joint Health 90's	InterNatural	Rainbow Light	[Product not found on the Internatural.com website.]
<i>Clematis chinensis</i>	Clematis extract	Stakich, Inc.	Stakich, Inc.	[http://stakich.com/Merchant2/merchant.mvc?Screen=PROD&Product_Code=2052&Category_Code=] †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[<i>Clematis chinensis</i>]	[Sciatica Pills]	[Opone. Com] [PlazaQ]	[NA] [NA]	[http://opane.stores.yahoo.net/arsciatpil12.html] [Product ingredients list includes <i>Clematis rhinensis</i> [<i>chinensis</i>] Osbeck.] [http://www.plazaq.com/arscpi1zh.html] [The same product containing <i>Clematis rhinensis</i> [<i>chinensis</i>] Osbeck is available at this website.]
<i>Clematis</i> sp.	Eucommia Extract [Eucommia Extract (Du Jhong Waji Hwan)]	Opone.com [PlazaQ.com]	[NA] [NA]	http://store.yahoo.com/opane/eucex20cap.html † [http://plusq.stores.yahoo.net/euex20cadujh.html] [Product ingredients list includes <i>Clematis</i> root.]
<i>Clematis</i> sp.	Eucommiae Musculoskeletal [sic] Support	PlazaQ.com	[NA]	http://store.yahoo.com/plusq/eucmussup100.html † [Product ingredients list includes <i>Clematis</i> and Woolly Dutchmanspipe (<i>Aristolochia tomentosa</i>) and wild ginger.]
<i>Clematis</i> sp.	Head Rescue Extract	[Afterglow of Sedon]	NOW brand	http://www.sedonalive.com/nowforms.html [Product confirmed, but <i>Clematis</i> not found in ingredients list.]
<i>Clematis</i> sp.	Joint Health	NutritionBlvd.com DiscountBlvd.com	[NA]	[Retailers no longer found on the Internet.]
<i>Clematis</i> sp.	Neck and Shoulders Support	iHerb.com	Planetary Formulas	[Product not found on the Iherb.com website.] [Product was found at VitaNet, LLC (see below).]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[<i>Clematis</i> sp.]	[Neck and Shoulders Support]	[VitaNet, LLC]	[Planetary Formulas]	[http://vitanetonline.com/description/PF0416/vitamins/Neck-and-Shoulder-Support/] [Product ingredients list includes Chinese <i>clematis</i> root extract.]
[<i>Clematis</i> sp.]	[Touku Rheumatic Pills]	[Opene. Com]	[NA]	[http://opane.stores.yahoo.net/rheumtoukrhe.html] [Product ingredients list includes <i>Clematis</i> root.]
[<i>Clematis</i> sp.]	[Mobility 2 (Clematis Combination Herbal Supplement)]	[MaxNature]	[Health Concerns]	[http://maxnature.stores.yahoo.net/mo2cohesuta.html] [Product ingredients list includes <i>Clematis</i> root (Wei Ling Xian).]
[<i>Clematis</i> sp.]	[AC-W Tabs (Da Huo Luo Dan Herbal Supplement)]	[MaxNature]	[Health Concerns]	[http://maxnature.stores.yahoo.net/acq.html] [Product ingredients list includes <i>Clematis</i> root (Wei Ling Xian).]
[<i>Clematis</i> sp.]	[Dao Chi San (Rehmannia & Armand's clematis Formula)]	[MaxNature]	[NA]	[http://maxnature.stores.yahoo.net/daochisanrar.html] [<i>Clematis</i> listed as part of product name; other ingredient information provided on website in Chinese only.]
[<i>Clematis</i> sp.]	[Shan Mu Tong Finet's clematis (Clematidis Finetianae Radix, Caulis et Folium)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/7.htm] [Product available in wholesale price list of Chinese herbs.]
[<i>Clematis</i> sp.]	[Wei Ling Xian Clematis root (Clematidis Radix)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/9.htm] [Product available in wholesale price list of Chinese herbs.]
<i>Clematis</i> sp. + <i>Stephania</i> sp.	Clematis & Stephania	TCMM Formulas	[NA]	[http://www.tcmformulas.com/studentliquidself.htm] [<i>Clematis</i> & <i>Stephania</i> confirmed in product list, but no other details found.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[<i>Clematis</i> sp. + <i>Stephania</i> sp.]	Circula (Shu Jing Juo Zue Tang) (Clematis & Stephania Combination)]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/cisjihuoquet.html] [Product identified as <i>Clematis</i> & <i>Stephania</i> combination.]
[<i>Clematis</i> sp. + <i>Stephania</i> sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) capsules]	[MaxNature]	[KPCformulas]	[http://maxnature.stores.yahoo.net/shujihuoquet.html] [<i>Clematis</i> and <i>Stephania</i> confirmed as part of product name.]
[<i>Clematis</i> sp. + <i>Stephania</i> sp.]	[Shu Jing Huo Xue Tang (no Aristolochic Acid) (Clematis and Stephania Combination) tablets]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/shujihuoquet1.html] [<i>Clematis</i> and <i>Stephania</i> confirmed as part of product name; however, it specifies “No Aristolochic Acid.”]
[<i>Clematis</i> sp. + <i>Stephania</i> sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) tablets]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/shujihuoquet2.html] [<i>Clematis</i> and <i>Stephania</i> confirmed as part of product name.]
[<i>Clematis</i> sp. + <i>Stephania</i> sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) herbal powder]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/shujihuoquet3.html] [<i>Clematis</i> and <i>Stephania</i> confirmed as part of product name.]
[<i>Clematis</i> sp. + <i>Stephania</i> sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) tablets]	[MaxNature]	[KPCformulas]	[http://maxnature.stores.yahoo.net/shujihuoquet4.html] [<i>Clematis</i> and <i>Stephania</i> confirmed as part of product name.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Cocculus cordifolia</i>	Guduchi	Herbal Remedies USA, LLC	Vadik Herbs	[http://www.herbalremedies.com/guduchi-capsules.html] † [Product ingredients list includes <i>Tinospora cordifolia</i> , which is a synonym for <i>Cocculus cordifolia</i> (http://www.plantnames.unimelb.edu.au/Sorting/Tinospora.html)]
<i>Cocculus indicus</i>	Neuran	InnerLife Wellness Center	Vāxa	http://www.innerlifewellness.com/products/neuran.html †
<i>Cocculus indicus</i>	PMS	Spring Valley Herbs	Hyland	http://www.springvalleyherbs.com/catalog.php?itemID=923 †
<i>Saussurea lappa</i>	BotaniGest	Vitatest	Metagenics	http://www.vitatest.com/ProductDetail.asp?ProductCode=BOTA&Store=METAGENICS †
<i>Saussurea lappa</i>	Cardio Flow	Emerson Ecologics	PL	http://www.emersonecologics.com/ProductInformation.asp?BrowseBy=CAR18 †
<i>Saussurea lappa</i>	Chinese Mood Elevator (AD-C)	1Dietstore.com	Nature's Sunshine	http://www.onedietstore.com/chinese_mood-elv.htm [The ingredients list still includes <i>Saussurea lappa</i> , but the website says the product is not available.]
<i>Saussurea lappa</i>	Chinese Spleen Activator (Wen Zhong) (K3-C)	country-spice.com	Nature's Sunshine	[Retailer no longer found on the Internet.]
[<i>Saussurea sp.</i>]	[Spleen Activator (Chinese)]	[1001 Herbs]	[NA]	[http://www.1001herbs.com/uc-c/] [Product ingredients list includes <i>Saussurea</i> root.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[<i>Saussurea sp.</i>]	[Spleen Activator (UC-C)]	[Klies Herbal Wellness and Colon Care]	[Nature's Sunshine]	[http://www.kliescolon.com/1880-8.htm] [Product ingredients list includes <i>Saussurea</i> root.]
	[Spleen Activator (formerly UC-C)]	[Dr. Mary's Wholesale Herbs Shop]	[Nature's Sunshine]	[https://www.shop.marysherbs.com/displayProductDocument.hg?categoryId=1&productId=228] [Same product as above; product ingredients list includes <i>Saussurea</i> root.]
	[Chinese Spleen Activator (Wen Zhong)]	[Greatest Herbs on Earth]	[Nature's Sunshine]	[http://www.greatestherbsonearth.com/nsp/chinese_spleen_activator.htm] [Same product as above; product ingredients list includes <i>Saussurea</i> root.] [NB: The Nature's Sunshine website does not list <i>Saussurea</i> in the ingredients for their "Spleen Activator, Chinese" product.] [http://www.naturessunshine.com/us/products/catalog/product/default.aspx?stocknum=1880]
<i>Saussurea lappa</i>	Chinese Stress Relief (STR-C)	Goherbal, Inc. Greatest Herbs on Earth HerbNook.com	Nature's Sunshine Nature's Sunshine Product confirmed, but [NA]	[http://goherbal.stores.yahoo.net/1863-5.html †] [http://www.greatestherbsonearth.com/nsp/chinese_stress_relief.htm] [Product confirmed, but <i>Saussurea lappa</i> not found in ingredients list.] [Retailer no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Saussurea lappa</i>	Complete Antioxidant Support	Betterlife.com, LLC N101, Inc.	Rainbow Light	[Product not found on the Betterlife.com, N101, or Rainbow Light website.]
<i>Saussurea lappa</i>	Gastrogen (formerly TCB 6)	EGeneral Medical, Inc. [Vitatest]	Metabotanica Method [Metagenics]	[Product not found on the Egeneralmedical.com website.] [Gastrogen (formerly TCB 6) was found at Vitatest website- http://www.vitatest.com/ProductDetail.asp?ProductCode=GA005&Store=METAGENICS . <i>Saussurea lappa</i> is listed in the ingredients.]
		[Healthy Store]	[Metagenics]	[The same product containing <i>Saussurea lappa</i> in the ingredients was also found at- http://www.healthstores.com/store/stores/HealthyStore/Browse_Item_Details.asp?Shopper_id=427632635234276&Item_ID=1107]
<i>Saussurea lappa</i>	Liver/Gallbladder Support	Health Designs International	Botanigest	[Link to “Liver/Gallbladder Support” product not found.]
<i>Saussurea lappa</i>	UC-C [Enhance Earth] Wen Zhong	HerbNook.com	Nature’s Sunshine	[Retailer no longer found on the Internet.]
<i>Saussurea lappa</i>	Ultra Energy Plus	NutritionBlvd.com DiscountBlvd.com	Rainbow Light	[Retailers no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Saussurea lappa</i>	Ultra Energy Plus	Internatural.com	Rainbow Light	[http://www.international.com/ingr/ingr845210.cfm] [Product confirmed, but <i>Saussurea lappa</i> not listed in the ingredients.]
		Eng Natural [now called Enk Store]	[NA]	[http://www.enkueros.net/301086.html] [Product confirmed, but <i>Saussurea lappa</i> not listed in the ingredients.]
		Thymely Solutions	[NA]	[http://www.absolutelythepurest.com/realestatesurvivalkit/ultraenergy.html] [Product confirmed, but <i>Saussurea lappa</i> not listed in the ingredients.]
		Life's Vigor	[NA]	[http://www.lifsvigor.com/10087.html] [Product confirmed, but <i>Saussurea lappa</i> not listed in the ingredients.]
		Herbal Advisor and many others	[NA]	[Retailer no longer found on the Internet]
[<i>Saussurea</i> sp.]	[Yun Mu Xiang Yunnan saussurea root (<i>Saussureae Radix Sichuanensis</i>)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/10.htm] [Product available in wholesale price list of Chinese herbs.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[<i>Saussurea</i> sp.]	[Chuan Mu Xiang sichuan saussurea root (Vladiniriae Souliei Radix)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/2.htm http://www.tcm-treatment.com/images/wholesale/herb-price/9.htm] [Product is listed in 2 places in the wholesale price list of Chinese herbs.]
<i>Sinomenium acutum</i>	Vine Essence Pills	Solstice Medicine Company	Vine Essence	http://www.sosusaco.com/product/productDetail.asp?iProductID=227 †
[<i>Sinomenium</i> sp.]	[Qing Feng Teng Orient Vine (<i>Sinomenii</i> seu <i>sabiae</i> Caulis et Rhizoma)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/7.htm] [Product available in wholesale price list of Chinese herbs.]
<i>Stepania</i> sp.	Water Balance Tonic	Elixir	Elixir	[The URL for www.elixir.net redirects to http://www.elixirtonics.com/ , but the product was not found in a search of that website.]
<i>Stephania clematis</i>	OrthoFlex Plus	Betterlife.com, LLC	Pacific Biologics	[Product not found on the Betterlife.com website.]
<i>Stephania delavaya</i> + <i>Stephania sinica</i>	Spes	Life Extension Vitamins	Botaniclab	[No product with the name “Spes” was found on the Life Extension Vitamins website; however, a product called “Chronofort” was identified on the website (see listing below).]
<i>Stephania pierrei</i>	Boh Ra Phet Pung Chang Capsule (Saboo Luerd)	Phuketherb Ltd.	[NA]	http://phuketherbs.velocall.com/pd1086802810.htm †
[<i>Stephania</i> sp.]	[Chronofort]	[the Life Extension Vitamins]	[NA]	[http://lifeextensionvitamins.stores.yahoo.net/chno-wwilu.html]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Stephania</i> sp.	Altera Tonic Herbal Supplement: Muscle and Joint Formula	Enk Natural [now called Enk Store]	Nature's Answer	[The original link redirects to the Enk Store homepage (http://www.enkueros.net/), but the product was not found in a search of that website.]
		Total Health Discount	Nature's Answer	http://www.totaldiscountvitamins.com/Templates/formTemplateM.asp?CatalogID=2949&SubfolderID=31 †
<i>Stephania</i> sp.	Basic Formulas Dragon Diet	InterNatural	Dragon Eggs Formulas	[Product not found on the Internatural.com website.] [The website- https://momentum98.com/dragon.html states that Dragon Eggs Formulas have been discontinued by the manufacturer.]
<i>Stephania</i> sp.	Ignite Your Life	NutritionStreet.com	[NA]	http://www.nutritionstreet.com/360facts.php †
		Healthynutritionaldiet.com	[NA]	[Retailer not found on the Internet.]
<i>Stephania</i> sp.	Ohco-Motion	NutritionBlvd.com DiscountBlvd.com	OHCO/Orient Herb Company	[Retailers not found on the Internet.]
<i>Stephania</i> sp.	Over-Eater's Diet	HerbsMD	Alive Energy	http://www.herbsmd.com/shop/xq/asp/pid.7716/qx/productdetail.htm †
<i>Stephania</i> sp.	Physical Transformation Formulas Over Eater's	InterNatural	Alive Energy	[Product not found on the Internatural.com website.]
<i>Stephania</i> sp.	Stephania & Astragalus Tea Pills	Morningstar Health	Plum Flower	http://www.morningstarhealth.com/store/product172.html †
<i>Stephania</i> sp.	Stephania Astragalus	Kang Le So	[NA]	[Retailer no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
<i>Stephania</i> sp.	Triphala Herbal Diet Program	Herbal Advisor	[NA]	[Link automatically relocates to iHerb.com website (http://www.iherb.com/) but the product was not found in a search of website.]
[<i>Stephania</i> sp.]	[Bai Yao Zi Cepharantha Tuber (<i>Stephaniae</i> Cepharanthae Tuber); Dioscorea Root (<i>Dioscoreae</i> Rhizoma)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcm-treatment.com/images/wholesale/herb-price/herb.index.htm] [Product available in wholesale price list of Chinese herbs.]
<i>Stephania</i> sp. + <i>Clematis</i> sp.	Clematis & Stephania Formula	Spanda- Product found at manufacturer's site- Golden Flower Chinese Herbs	Golden Flower Chinese Herbs	[http://www.spanda.com/catalog/GFHERB.html] [Product ingredients list includes Clematis Root (Wei Ling Xian) and <i>Stephaniae Tetrandrae</i> root (Han Fang Ji).]
<i>Stephania tetandra</i> [<i>tetrandra</i>]	Stephania and Astragalus / Fang Ji Huang Qi Wan	Herbswest, LLC	[NA]	http://www.herbswest.net/items/13341.shtml †
<i>Stephania tetandra</i> [<i>tetrandra</i>]	Weight Loss	Alterna-Med, Inc.	Samra	[The link automatically relocates to - http://www.vitaminproshop.com/ , but no product with <i>Stephania</i> was found in a search of that website.]

Source: Gold and Sloan 2003a.

Table A-3. Botanical products for oral use, available as of March 4, 2003 on the web, that have no Latin name but are likely to be *Asarum* species

Species	Medicinal name	Retailer	Manufacturer	Web page with AA Product
Chinese wild ginger	Bio-Nutritional Formulas Intestinalis	Nutritional Ecological Environmental Delivery System (NEEDS)	NA	[Product not found on the needs.com website.]
Chinese wild ginger	Medicated Oil	Solstice Medicine Company	Bee Brand	http://www.sosusaco.com/product/productDetail.asp?iProductID=150 †
Chinese wild ginger	Mullein Lung Complex with Ephedra	iHerb.com	Planetary Formulas	[http://www.iherb.com/ProductDetails.aspx?c=1&pid=1577&at=0] [Product ingredients list includes “ginger root,” but Chinese wild ginger was not specified.]
		Seacoast Natural Foods	NA	[The only information on ginger on the http://www.seacoastvitamins.com website referred to <i>Zingiber officinale</i> and not to Chinese wild ginger.]
[Chinese wild ginger]	[999 Zheng Tian Wan]	[Opone.com]	NA	[http://opone.stores.yahoo.net/headzhentian.html] [Product ingredients list includes Chinese wild ginger.]
		[PlazaQ.com]	NA	[http://plusq.stores.yahoo.net/head999zhent.html] [Product ingredients list includes Chinese wild ginger.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA Product
[Chinese wild ginger]	[Headache Aid Tea: Magic Herb Tea #4: OS]	[Opone.com]	NA	http://opane.stores.yahoo.net/heidteamah.html [Product ingredients list includes Chinese wild ginger, but website notes that the product is temporarily out of stock.]
[Chinese wild ginger]	[Bao Zhen Gao (K154)]	[Opone.com] [PlazaQ.com]	[NA] [NA]	http://opane.stores.yahoo.net/painbaozheng.html [Product ingredients list includes Chinese wild ginger.] http://plusq.stores.yahoo.net/painbaozheng.html [Product ingredients list includes Chinese wild ginger.]
Wild ginger	Chinese Specific Cold Pills	TMC Alternatives	NA	http://members.fortunecity.com/davidpilling/html/body_chcoldpills.htm †
Wild ginger	Energy Formula	God's Remedy Natural Products	NA	http://godsremedy.com/hepatitis/energy.htm †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA Product
Wild ginger	Expellin Extract	PlazaQ.com Opane.com	Lanzhou Traditional Herbs Lanzhou Traditional Herbs	[The PlazaQ.com and Opane.com websites have other products with wild ginger (see below) and Chinese wild ginger (see above), but searches on those websites did not identify “Expellin Extract” as a product for sale.] [A company called Kingsway Trading was reported by the FDA on November 10, 2004 (http://www.fda.gov/oc/po/firmrecalls/kingsway11_04.html) to have recalled a product called Expellin Extract (Double Deers Formula) manufactured in China because it contained aristolochic acid. A second product called CardioFlex was also recalled at that time.]
[Wild ginger]	[Expellin Extract (Chuan Xiong Cha Tiao Wan)]	[CGCMall] [China-Herbs] [Wheatgrass for Your Health]	[Lanzhou Traditional Herbs] [Lanzhou Traditional Herbs] [Lanzhou Traditional Herbs]	[http://www.cgcmall.com/chuanxiong_mixture_p/hr00cxc1.htm] [Product ingredients list includes wild ginger.] [http://www.china-herbs.com/hr00cx1.html] [The same product containing wild ginger is available at this website. This site is also part of CGCMall.] http://www.wheatgrassforyourhealth.com/chineseherbs.html [The product is available at this website, but no ingredient list was found.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA Product
Wild ginger	First Aid Survival Kit	InterNatural	Turtle Island Herbs	<p>[Product was not found in a search of the Internatural.com website.]</p> <p>[Other products with wild ginger as an ingredient were found on InterNatural website (see below).]</p>
[Wild ginger]	[Four Elements Wild Ginger Flower Essence]	[InterNatural]	[Four Elements]	<p>[http://www.international-alternative-health.com/ingr/ingr231722.cfm]</p> <p>[Product ingredients list includes wild ginger.]</p>
Wild ginger	Mother Earth's Cough Syrup	Tao Herb Farm Vitanet	Heritage Products [Heritage Store brand]	<p>[Product not found in a search of the Taoherbfarm.com website.]</p> <p>[http://www.myvitanet.com/motear4ozher.html]</p> <p>[Product ingredients list includes wild ginger.]</p> <p>[Mother Earth's Cough Syrup is also available from other vendors. One website is- http://www.thewaytobalance.com/PRODUCTS/ecp-mecough.html. They list wild ginger as an ingredient. The label appears to be the same as the product above.]</p> <p>[The House of Nutrition Online (Heritage Products) website (http://hono.stores.yahoo.net/heritage-products.html) lists this product. They seem to be the manufacturer as well. Their ingredients list includes wild ginger elixir.]</p>

Species	Medicinal name	Retailer	Manufacturer	Web page with AA Product
Wild ginger	Tummy Soother	NutritionBlvd.com DiscountBlvd.com Kalyx	Nature's Gate NA	[Retailers no longer found on the Internet.] [Product not found on the Kalyx website.]
Wild ginger	URI-pH formula	PlazaQ.com	NA	http://store.yahoo.com/opane/urfork2.html †
Wild ginger	Wild ginger tincture	Healingalt.com	NA	[Retailer no longer found on the Internet.]
Wild ginger / xi xin	Du Huo & Loranthus Formula E.C.	Spanda	Golden Flower Chinese Herbs	[http://www.spanda.com/catalog/GFHERB.html] †
Xi xin	Allergy Tamer Elixir	Traditions of Tao	NA	[http://www.taofwellness.com/Merchant2/merchant.mvc?Screen=PROD&Store_Code=eshop&Product_Code=ALLLX] [Product confirmed but ingredients list does not contain wild ginger.]
[Wild Ginger]	[Eucommiae Musculoskeletal Support Pills: Du Zhong Zhuang Gu Wan]	[Opane.com] [PlazaQ.com]	NA NA	[http://opane.stores.yahoo.net/eucmussup100.html] † [Product ingredients list includes wild ginger, as well as Clematis root and Woolly Dutchmanspipe (<i>Aristolochia tomentosa</i>)] [http://plusq.stores.yahoo.net/eucmussup100.html] [Same ingredients listed as on the Opane.com website.]
[Wild Ginger]	[URI-pH Formula: Niao Suan Ping (K277)]	[Opane.com] [PlazaQ.com]	NA NA	[http://opane.stores.yahoo.net/urfork2.html] [Product ingredients list includes wild ginger.] [http://plusq.stores.yahoo.net/urfork2.html] [Product ingredients list includes wild ginger.]

Source: Gold and Slone 2003a.

Appendix B: Botanical Products Containing Aristolochic Acids

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Table B-1. Botanicals known or suspected to contain aristolochic acids

Botanical name*	Common or other names
<i>Aristolochia</i> species	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
<i>Aristolochia kaempferi</i> Willd. Syn. <i>Aristolochia chrysops</i> (Stapf) E.H. Wilson ex Rehder Syn. <i>Aristolochia feddei</i> H. Lévy Syn. <i>Aristolochia heterophylla</i> Hemsl Syn. <i>Aristolochia mollis</i> Dunn Syn. <i>Aristolochia setchuenensis</i> Franch. Syn. <i>Aristolochia shimadai</i> Hayata	yellowmouth Dutchman's pipe

Botanical name*	Common or other names
Syn. <i>Aristolochia thibetica</i> Franch. Syn. <i>Isotrema chrysops</i> Stapf Syn. <i>Isotrema heterophylla</i> (Hemsl.) Stapf Syn. <i>Isotrema lasiops</i> Stapf	
<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 siphon</i> L'Hér.	Dutchman's-pipe
<i>Aristolochia manshuriensis</i> [<i>manshuriensis</i>] Kom. Syn. <i>Hocquartia manshuriensis</i> (Kom.) Nakai Syn. <i>Isotrema manchuriensis</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 serpentary
<i>Aristolochia watsoni</i> Wooton & Standley or <i>Aristolochia watsonii</i> Wooton & Standley Syn. <i>Aristolochia porphyrophylla</i> Pfeifer	
<i>Aristolochia westlandii</i> Hemsl. Or <i>Aristolochia westlandi</i> Hemsl.	
<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.	
<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.	wild ginger Indian ginger Canada snakeroot false coltsfoot colic root heart snakeroot Vermont snakeroot

Botanical name*	Common or other names
Syn. <i>Asarum parvifolium</i> Raf. Syn. <i>Asarum reflexum</i> E.P. Bicknell Syn. <i>Asarum rubrocinctum</i> Peattie	southern snakeroot
<i>Asarum himalaicum</i> Hook. f. & Thomson ex Klotzsch or <i>Asarum himalaycum</i> Hook. f. & Thomson ex Klotzsch	tanyou-saishin (Japanese)
<i>Asarum splendens</i> (F. Maek.) C.Y. Cheng & C.S. Yang	do-saishin (Japanese)
<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 we will use the name as cited in J. Nat. Products 45:657-666 (1982)	

Source: FDA 2000.

Table B-2. Botanicals which may be adulterated with aristolochic acids

Botanical name*	Common or other names
<i>Akebia</i> species	akebia mu tong ku mu tong zi mutong bai mu tong mokutsu (Japanese) mokt'ong (Korean)
<i>Akebia quinata</i> (Houtt.) Decne. Syn. <i>Rajania quinata</i> Houtt.	chocolate vine fiveleaf akebia mu tong yu zhi zi mokutsu (japanese)
<i>Akebia trifoliata</i> (Thunb.) Koidz.	mu tong three leaf akebia yu zhi zi
<i>Asarum forbesii</i> Maxim.	batei-saishin (Japanese)
<i>Asarum heterotropoides</i> F. Schmidt Syn. <i>Asarum heterotropoides</i> F. Schmidt Syn. <i>Asiasarum heterotropoides</i> (F. Schmidt) F. Maek.	keirin-saishin (japanese) Chinese wild ginger Manchurian wild ginger bei xi xin xin xin
<i>Asarum sieboldii</i> Miq. Syn. <i>Asarum sieboldii</i> fo. <i>seoulense</i> (Nakai) C.Y. Cheng & C.S. Yang Syn. <i>Asarum sieboldii</i> var. <i>seoulensis</i> Nakai Syn. <i>Asiasarum heterotropoides</i> var. <i>seoulense</i> (Nakai) F. Maek. Syn. <i>Asiasarum sieboldii</i> (Miq.) F. Maek.	usuba-saishin (japanese) Chinese wild ginger xi xin hua xi xin manchurian wild ginger siebold's wild ginger

Botanical name*	Common or other names
<i>Clematis</i> species	clematis mufangji clematidis ireisen (japanese) wojoksum (korean)
<i>Clematis armandii</i> Franch. Syn. <i>Clematis armandii</i> fo. <i>farquhariana</i> (W.T. Wang) Rehder & E.H. Wilson Syn. <i>Clematis armandii</i> var. <i>biondiana</i> (Pavol.) Rehder Syn. <i>Clematis biondiana</i> Pavol. Syn. <i>Clematis ornithopus</i> Ulbr.	armand's clematis chuan mu tong (stem) xiao mu tong armand's virgin bower
<i>Clematis chinensis</i> Osbeck.	chinese clematis wei ling xian (root)
<i>Clematis hexapetala</i> Pall.	
<i>Clematis montana</i> Buch.-Ham. ex DC. Syn. <i>Clematis insulari-alpina</i> Hayata	
<i>Clematis uncinata</i> Champ. ex Benth. Syn. <i>Clematis alsomitriifolia</i> Hayata Syn. <i>Clematis chinensis</i> var. <i>uncinata</i> (Champ. ex Benth.) Kuntze Syn. <i>Clematis drakeana</i> H. Lév. & Vaniot Syn. <i>Clematis floribunda</i> (Hayata) Yamam. Syn. <i>Clematis gagnepainiana</i> H. Lév. & Vaniot Syn. <i>Clematis leiocarpa</i> Oliv. Syn. <i>Clematis ovatifolia</i> T. Ito ex Maxim. Syn. <i>Clematis uncinata</i> var. <i>biternata</i> W.T. Wang Syn. <i>Clematis uncinata</i> var. <i>coriacea</i> Pamp. Syn. <i>Clematis uncinata</i> var. <i>floribunda</i> Hayata Syn. <i>Clematis uncinata</i> var. <i>ovatifolia</i> (T. Ito ex Maxim.) Ohwi ex Tamura Syn. <i>Clematis uncinata</i> var. <i>taitongensis</i> Y.C. Liu & C.H. Ou	
<i>Cocculus</i> species	cocculus
<i>Cocculus carolinus</i> (L.) DC. Syn. <i>Cebatha carolina</i> Britton Syn. <i>Epibaterium carolinum</i> (L.) Britton Syn. <i>Menispermum carolinum</i> L.	
<i>Cocculus diversifolius</i> DC. Syn. <i>Cocculus madagascariensis</i> Diels	
<i>Cocculus hirsutus</i> (L.) Diels Syn. <i>Cocculus villosus</i> DC. Syn. <i>Menispermum hirsutum</i> L.	
<i>Cocculus indicus</i> Royle Syn. <i>Anamirta paniculata</i> Colebr.	indian cockle
<i>Cocculus laurifolius</i> DC.	

Botanical name*	Common or other names
Syn. <i>Cinnamomum esquirolii</i> H. Lév.	
<i>Cocculus leaebe</i> DC.	
<i>Cocculus madagascariensis</i> Diels Syn. <i>Cocculus diversifolius</i> DC.	
<i>Cocculus orbiculatus</i> DC. Syn. <i>Cissampelos pareira</i> Linn.	
<i>Cocculus orbiculatus</i> (L.) DC. Syn. <i>Cocculus cuneatus</i> Benth. Syn. <i>Cocculus sarmentosus</i> (Lour.) Diels Syn. <i>Cocculus sarmentosus</i> var. <i>linearis</i> Yamam. Syn. <i>Cocculus sarmentosus</i> var. <i>pauciflorus</i> Y.C. Wu Syn. <i>Cocculus sarmentosus</i> var. <i>stenophyllus</i> Merr. Syn. <i>Cocculus thunbergii</i> DC. Syn. <i>Cocculus trilobus</i> (Thunb.) DC. Syn. <i>Menispermum orbiculatus</i> L. Syn. <i>Menispermum trilobum</i> Thunb. Syn. <i>Nephroia sarmentosa</i> Lour.	moku-boui (Japanese)
<i>Cocculus palmatus</i> (Lam.) DC.	columba columbo
<i>Cocculus pendulus</i> Diels Syn. <i>Cebatha pendula</i> (J.R. & C. Forst.) Kuntze Syn. <i>Epibaterium pendulus</i> Forst. f. Syn. <i>Cocculus Epibaterium</i> DC.	
<i>Cocculus pendulus</i> (Forst. & Forst.) Diels	
<i>Cocculus palmatus</i> Hook. Syn. <i>Jateorhiza miersii</i> Oliver	colombo
<i>Cocculus thunbergii</i> DC.	
<i>Diploclisia affinis</i> (Oliv.) Diels Syn. <i>Diploclisia chinensis</i> Merr. Syn. <i>Cocculus affinis</i> Oliv.	
<i>Diploclisia chinensis</i> Merrill	xiangfangchi
<i>Menispermum dauricum</i>	
<i>Saussurea lappa</i> (Decne.) Sch. Bip.	mokkou (Japanese)
<i>Sinomenium acutum</i> (Thunb.) Rehder & E.H. Wilson Syn. <i>Cocculus diversifolius</i> var. <i>cinereus</i> Diels Syn. <i>Cocculus heterophyllus</i> Hemsl. & E.H. Wilson Syn. <i>Menispermum acutum</i> Thunb. Syn. <i>Sinomenium acutum</i> (Thunb.) Rehder & E.H. Wilson var. <i>cinereum</i> (Diels) Rehder & E.H. Wilson Syn. <i>Sinomenium diversifolium</i> (Diels) Diels	orientvine xunfengteng dafengteng daqingmuxinag zhuigusan da ye qingshener mufangji hanfangji

Botanical name*	Common or other names
	tuteng zhuigufeng maofangji
<i>Stephania</i> species	stephania
<i>Stephania tetrandra</i> S. Moore	fen fang ji , fang ji fang ji (root) han fang ji kanboi (Japanese) hanbanggi (Korean) fun-boui (Japanese)
<i>Vladimiria souliei</i> (Franch.) Ling	sen-mokkou

Source: FDA 2000.

Table B-3. Mu tong and fang ji are declared ingredients in the following products:

Source: FDA 2000.

- Ba Zheng Wan
- Chun Yang Zheng Ji Wan
- Da Huang Qing Wei Wan
- Dang Gui Si Ni Wan
- Dao Chi Wan
- Dieda Wan
- Fu Ke Fen Qing Wan
- Guan Xin Su He Wan
- Ji Sheng Ju He Wan
- Kat Kit Wan
- Long Dan Xie Gan Wan
- Quell Fire
- Shi Xiang Fan Shen Wan
- Xin Yi Wan

Table B-4. Botanical products determined by FDA to contain aristolochic acids

Product name	Responsible firm
Rheumixx	PharmaBotanixx, Irvine, CA (Distributor), Sun Ten Laboratories, Inc., Irvine, CA (Manufacturer)
BioSlim Doctor's Natural Weight Loss System Slim Tone Formula	Thane International, LaQuinta, CA (Distributor)
Prostatin	Herbal Doctor Remedies, Monterey Park, CA (Distributor)
Fang Ji Stephania	Lotus Herbs Inc., LaPuente, CA (Distributor)
Mu Tong <i>Clematis armandi</i>	Lotus Herbs Inc., LaPuente, CA (Distributor)
Temple of Heaven Chinese Herbs Radix aristolochiae	Mayway Corporation, Oakland, CA (Distributor) and Almira Alchemy, Alachua, FL (Distributor)
Meridian Circulation	East Earth Herb Inc. (Brand name Jade Pharmacy), Eugene, OR
Qualiherb Chinese Herbal Formulas Dianthus Formulas Ba Zheng San	QualiHerb (Division of Finemost), Cerritos, CA (Distributor)
Clematis & Carthamus Formula 21280 (2 samples)	QualiHerb (Division of Finemost), Cerritos, CA (Distributor)
Virginia Snake Root, Cut <i>Aristolochia serpentaria</i> (2 samples)	Penn Herb Co., Philadelphia, PA (Manufacturer)
Green Kingdom Akebia Extract	Green Kingdom Herbs, Bay City, MI (Manufacturer) Ava Health, Grove City, OH (Distributor)
Green Kingdom Stephania Extract	Green Kingdom Herbs, Bay City, MI Ava Health (Distributor)
Neo Concept Aller Relief	BMK International, Inc., Wellesley, MA (Distributor), Sun Ten Labs, Irvine, CA (Manufacturer)
Mu Tong <i>Clematis armandi</i>	Botanicum.com, Winnipeg, Canada and Pembina, ND
Fang Ji Stephania	Botanicum.com, Winnipeg, Canada and Pembina, ND
Stephania tetrandra, roots, whole ^a	Ethnobotanical, Racine, WI

Source: FDA 2001b

^aProduct labeling states "Not for human consumption."

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Appendix C: Recalls of Products Containing Aristolochic Acids

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Table C-1. Recalls of products containing aristolochic acid as reported by the U.S. Food and Drug Administration

Products recalled prior to April 9, 2001 are reported in Table B-4, above. Products recalled after that date are reported here based on FDA News or FDA Recalls.

Date	Company	Product(s) [manufacturers]	Reason for recall	Reference
5/21/2001	Vital Nutrients/RHG & Co., Middletown Connecticut	Vital Nutrients Joint Ease; Verified Quality Brand Joint Comfort	<i>Clematis chinensis</i> extract determined to contain aristolochic acid	FDA 2001h (Recalls) http://www.fda.gov/bbs/topics/ENFORCE/2001/ENF00724.html
6/20/2001	Blue Light, Inc., Ithaca, NY- Products sold under “Treasure of the East” label	<p><i>Single ingredient</i></p> <p>Guan Mu Tong Ma Dou Ling</p> <p><i>Herbal combinations including Guan Mu Tong as an ingredient</i></p> <p>Ba Zheng San Dang Gui Si Ni Tang Dao Chi San Fu Fang Di Hu Tang Gan Lu Xiao Du Dan Kou Yan Ning Long Dan Xie Gan Tang Pai Shi Tang Xiao Ji Yin Zi Xin Yi San Yang Yin Xiao Yan Tang</p> <p>[Tianjiang Pharmaceutical Co. Ltd., China]</p>	Products contained aristolochic acid	FDA 2001i (FDA News) http://www.fda.gov/bbs/topics/ENFORCE/2001/ENF00715.html
7/31/2001	Pacific Biologic Co., Clayton, CA	Herb- <i>Akebia Trifoliata Caulis</i> (Mu Tong) Herb- <i>Asarum Sieboldii Herba cum Radix</i>	Herbs contained aristolochic acid	FDA 2001j (Recalls)

Date	Company	Product(s) [manufacturers]	Reason for recall	Reference
		(Xi Xin) <i>Brands-</i> Herbal Masters Arpanex B Herbal Masters Cys Herbal Masters Koms A Balance & Harmony Artiflex B Balance & Harmony Gentiana Combination Balance & Harmony Allerhay Pacific Biologic Orthoflex		http://www.fda.gov/oc/po/firmrecalls/pacificbio8_01.html
11/10/2004	Kingsway Trading, Inc., Brooklyn, NY	a) Double Deers Formula brand Expellin Extract (Concentrated), dietary herbal supplement, Chuan Xiong Cha Tiao Wan b) Cardioflex (Guan Xin Su He Wan) dietary supplement [Shanghai Chinese Herbal Co., Ltd., Shanghai, People's Republic of China]	Products contained aristolochic acid	FDA 2005 (Recalls) http://www.fda.gov/bbs/topics/enforce/2005/ENF00915.html
4/10/2008	Herbal Science International, Inc., City of Industry, CA	Tou Tong San (Headache Formula); Du Huo Ji Sheng Tang (Du Huo Joint Relief)	Products contained aristolochic acid	FDA 2008 (Recalls) http://www.fda.gov/oc/po/firmrecalls/herbalscience04_08.html

Sources: FDA 2001h, 2001i, 2001j, 2005, 2008