FINAL

Report on Carcinogens Background Document for

Aristolochic Acids

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

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FOREWORD

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

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chemical and physical properties, analytical methods, production, use, and occurrence may come from published and/or unpublished sources.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

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

CONTRIBUTORS

Project Managers, Authors, and Principal Reviewers

National Toxicology Program (NTP) and National Institute of Environmental Health Sciences (NIEHS)

Ruth Lunn, Dr.P.H.	Director, Report on Carcinogens Office
C.W. Jameson, Ph.D.	Report on Carcinogens Office (former Director; currently at CWJ Consulting, LLC)
Gloria D. Jahnke, D.V.M.	Report on Carcinogens Office

Constella Group, LLC (Support provided through NIEHS Contract Number NO1-ES-35505)

Sanford Garner, Ph.D.	Principal Investigator
Stanley Atwood, M.S., DABT	
Greg Carter, M.E.M.	

Consultants

Jean-Pierre Cosyns, M.D., D.S.M.	Université Catholique de Louvain, Brussels, Belgium
Dan Levy, Ph.D.	U.S. Food and Drug Administration

Administrative Support

Shawn Jeter, B.S.	NTP/ Report on Carcinogens Office
Anna Lee Sabella	Kelly Services
Ella Darden, B.S.	Constella Group, LLC
Tracy Saunders, B.S.	Constella Group, LLC

Editorial Support

Susan Dakin, Ph.D.	Independent Consultant in Technical & Scientific Writing & Editing

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:

Arthur P. Grollman, M.D. (Chair)
Department of Pharmacology
State University of New York at Stony Brook

A. Morrie Craig, Ph.D.
College of Veterinary Medicine
Oregon State University

Patricia E. Ganey, Ph.D.
Department of Pharmacology and Toxicology
Michigan State University

Yanze Liu, Ph.D.
Bio-organic and Natural Products
Research Laboratory
McLean Hospital

Albert B. Lowenfels, M.D.
Department of Community and Preventive Medicine
New York Medical College

Joëlle L. Nortier, M.D.
Department of Nephrology
Erasme Hospital
Université Libre de Bruxelles

Brian T. Schaneberg, Ph.D.
Technical Services
ChromaDex, Inc.

Bryan L. Stegelmeir, D.V.M., Ph.D.
Poisonous Plant Research Laboratory
U.S. Department of Agriculture

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

Introduction

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

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

Human Exposure

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

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

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

Human Cancer Studies

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

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

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

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

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

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

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

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

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

Studies in Experimental Animals

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

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

Absorption, Distribution, Metabolism, and Excretion

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

Toxicity

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

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

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

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

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

Genetic Damage and Mechanistic Data

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

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

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

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

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

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

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

A possible mechanism for the dose-dependent urothelial proliferation induced in rats fed an aristolochic acid mixture has been proposed based on altered expression and phosphorylation of cell-cycle proteins. The aristolochic acid mixture induced expression of cyclin D/cdk4 and cyclin E/cdk2, increased phosphorylation of the retinoblastoma (Rb) tumor suppressor protein, and decreased Rb/E2F complexes, thus freeing E2F to 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-N^6-yl)-aristolactam I

dA-AAII: 7-(deoxyadenosin-N^6-yl)-aristolactam II

dAMP: deoxyadenosine monophosphate

DAD: photodiode array detector

dCMP: deoxycytidine monophosphate

dG-AAI: 7-(deoxyguanosin-N^2-yl)-aristolactam I

dG-AAII: 7-(deoxyguanosin-N^2-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

IC_{50}: 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

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

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

1.1 Chemical identification

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

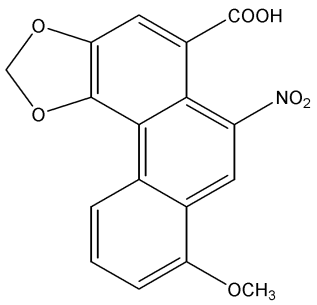
examples), and aristolochic acids are present in the roots, stems, leaves, and fruit (EMEA 2000, IARC 2002).

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

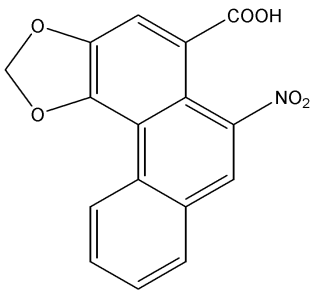
Table 1-1. Examples of *Aristolochia* species used in botanical products

Aristolochia species (location)	Parts used in herbal medicine	Aristolochic acid components
A. fangchi (China)	root	AA I, II, IIIa
A. manshuriensis (China)	stem	AA I, II, IIIa, IV, IVa; aristolic acid II
A. contorta (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
A. debilis (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
A. clematitis (Europe)	herb, root	AA I, II, III, IIIa, IV, IVa
A. indica (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.



Aristolochic acid I



Aristolochic acid II

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-*d*]-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-*d*]-1,3-dioxole-5-carboxylic acid
CAS Registry number	475-80-9
Molecular formula	$C_{16}H_9NO_6$
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.

1.2 Physical-chemical properties

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

the physical or chemical properties of aristolochic acid II other than its molecular weight 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

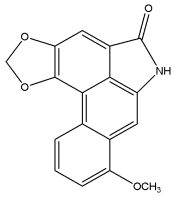
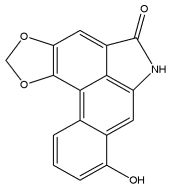
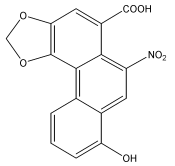
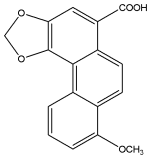
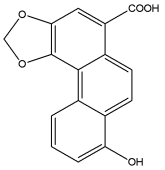
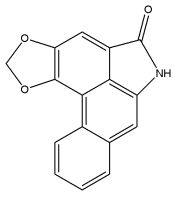
Source: IARC 2002.
NF = not found.

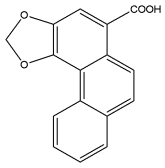
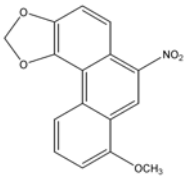
1.3 Metabolites

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

Only aristolactam I and aristolactam II were identified in urine samples collected from 6 healthy human volunteers given a mixture of aristolochic acids I and II over several days (Krumbiegel *et al.* 1987). More information on metabolites and metabolism is provided 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	
Aristolic acid I	296.1	
3,4-Methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid	282.1	
Aristolactam II	263.3	

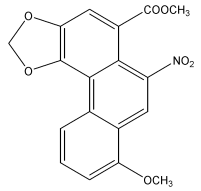
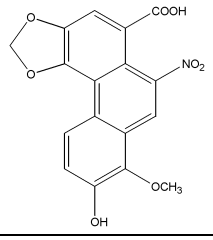
Metabolite	Molecular weight	Structure
3,4-Methylenedioxy-1-phenanthrenecarboxylic acid	266.3	
Decarboxylated metabolite	297.3	

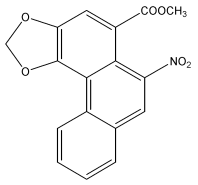
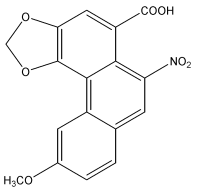
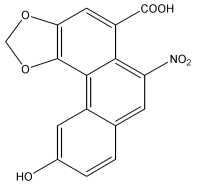
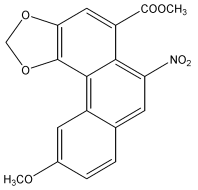
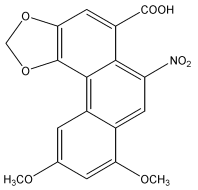
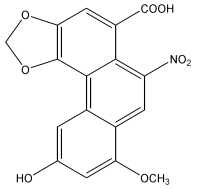
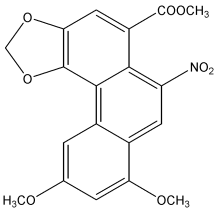
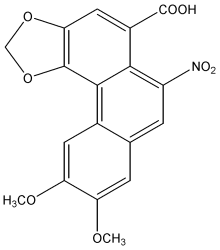
Source: Chan *et al.* 2007a, Krumbiegel *et al.* 1987.

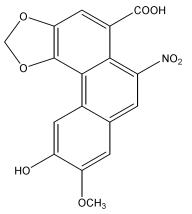
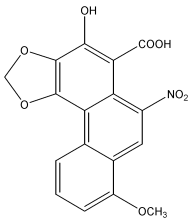
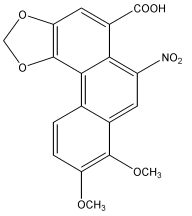
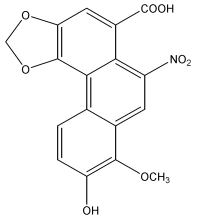
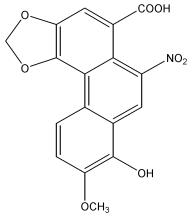
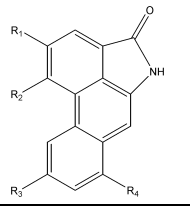
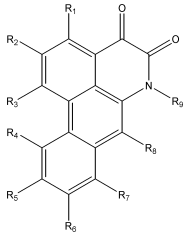
1.4 Aristolochic acid analogues

As mentioned above, aristolochic acids are a complex mixture of nitrophenanthrene carboxylic acids that are primarily found in plants in the family Aristolochiaceae. In addition to aristolochic acids, other chemically related compounds found in these plants include aristolactams and dioxoaporphines. The dioxoaporphines are thought to function as intermediates in the biosynthesis of aristolactams, which are precursors of aristolochic acids (Kumar *et al.* 2003). The structures of aristolochic acids I and II are shown in Figure 1-1 (above), and examples of the structures of aristolactams are shown in Table 1-5 (above). The general structure of the aristolactams is shown in Table 1-6, which also shows the structures of some other aristolochic acids and the basic structure of 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 R_1 = –OH or $–OCH_3$, R_2 = $–OCH_3$, $R_1 + R_2$ = $–OCH_2O–$, and R_3 and R_4.= –H, –OH, or $–OCH_3$.

[c] Where $R_1–R_9$ = –H, –OH, $–OCH_3$, or $–CH_3$ and $R_2 + R_3$ = $–OCH_2O–$.

2 Human Exposure

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

2.1 Use

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

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

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

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

parts of China. Chuan Mu Tong (*C. armandii* stem) is used mainly for the treatment of urethritis, to relieve pain, and to promote lactation. Some Aristolochiaceous plants, such as the roots of *A. fangchi* (Guang Fang Ji) and *A. heterophylla* (Han Zhong Fang Ji), are also used as source plants of Fang Ji, which was originally obtained from the Menispermaceous plant *Stephania tetrandra* and used for the treatment of edema. [The overlapping use of different plants as one crude drug or one plant used as different crude 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
A. clematitis	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
A. contorta	ma dou ling	E. Asia	as an antiseptic, or sedative; to treat hemorrhoids, cough, asthma, epigastric pain, arthralgia, or edema
A. debilis	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
A. elegans	elegant Dutchman's pipe	South America to Mexico	as an antiseptic, antipyretic, or emmenagogue; to treat snakebite or scorpion stings
A. fangchi	guang fang ji	E. Asia	as a diuretic, antipyretic, or analgesic; to treat lung disorders or rheumatic arthritis
A. indica	Indian birthwort	S. Asia	as an emmenagogue, abortifacient, or antipyretic; to treat snakebite or diarrhea
A. kaempferi	yellowmouth Dutchman's pipe	E. Asia	to treat lung ailments, hemorrhoids, or ascites
A. macrophylla	pipevine	E. United States	as an antiseptic; to treat swelling of the feet or legs
A. molissima	xun gu feng	E. Asia	as a diuretic or anti-inflammatory; to treat arthralgia or pain
A. manshuriensis	Manchurian birthwort	E. Asia	as an anti-inflammatory, diuretic, emmenagogue, or galactagogue
A. reticulata	Texas Dutchman's pipe	S.W. United States	as a stimulant or to promote sweating; to treat stomach disorders,
A. rotunda	snakeroot	Europe	as an abortifacient, diuretic, emmenagogue, or antihelminthic; to treat cough or wounds
A. serpentaria	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
Asarum canadense	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.

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

In addition to use in studies of toxicity and carcinogenicity, aristolochic acids are used in biochemical studies as relatively selective inhibitors of phospholipase A_2 (see Section 5.2.3).

2.2 Production

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

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

2.3 Measurements of Exposure

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

2.3.1 Analysis methods

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

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

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

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

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

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

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

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

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

2.3.2 Biological indices of exposure

Aristolochic acid–DNA (AA-DNA) adducts have been identified in the kidneys of patients with Chinese herb nephropathy using ^{32}P-post-labeling analysis (Arlt *et al.* 2001b, Arlt *et al.* 2001a, Cosyns 2003, Gillerot *et al.* 2001). These adducts are specific markers of exposure to aristolochic acids I and II (Bieler *et al.* 1997). See Section 5 for further discussion of AA-DNA adducts. Grollman *et al.* (2007) described the use of liquid chromatography electrospray ionization/multistage mass spectrometry (LC-ESI/MS/MS^3) as a means of specifically confirming the chemical identity of the dA-AL-I and dA-AL-II adducts using synthetic adduct standards.

2.4 Occurrence and Exposure

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

2.4.1 Occurrence in plants

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

Geographical distribution

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

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

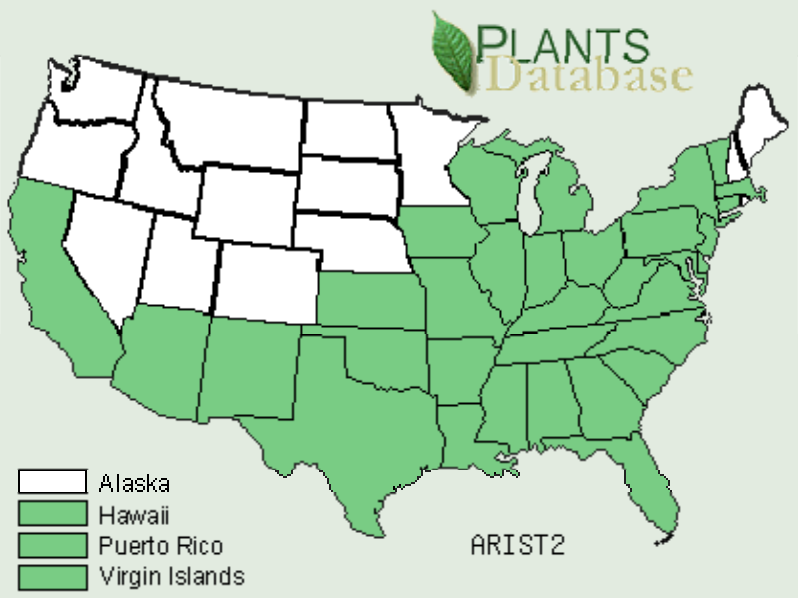


Figure 2-1. Distribution of *Aristolochia* species within the United States
Source: USDA 2005, Plants occur in states colored green.

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

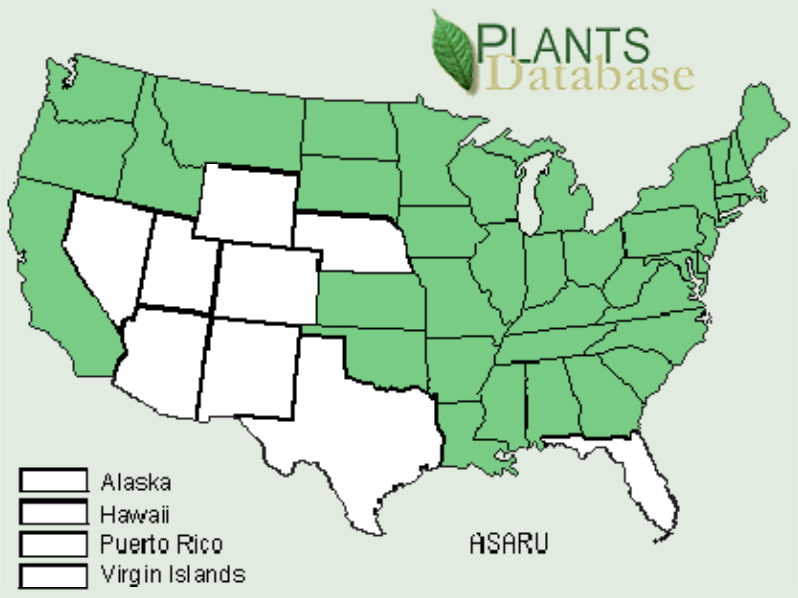


Figure 2-2. Distribution of *Asarum* species within the United States
Source: USDA 2005, Plants occur in states colored green.

Concentrations in plants

A number of studies have reported concentrations of aristolochic acids I and II in medicinal plants as summarized in Table 2-2. The plants in which aristolochic acids were analyzed include several species of plants used in traditional Chinese medicine (Hashimoto *et al.* 1999, Lee *et al.* 2001, Jong *et al.* 2003, Zhai *et al.* 2006, Zhang *et al.* 2006b, Zhou *et al.* 2006, Wu *et al.* 2007a, Yuan *et al.* 2007). Samples were obtained from medicinal plant stores (seeds or roots) or from preserved laboratory plant materials, or were collected specifically for some studies. Aristolochic acids were found in almost all samples from *Aristolochia* species; however, considerable variability in the aristolochic acid content was reported. Li *et al.* (2004a,b) also demonstrated that the aristolochic acids (AA I and AA II) content of *A. fangchi* and *A. manshuriensis* varied by geographic region. Furthermore, *Aristolochia* plants collected from several areas in one province did not contain detectable levels of aristolochic acids.

Only trace levels were reported in samples from *Asarum* in the report by Hashimoto *et al.*; however, Jong *et al.* analyzed additional *Asarum* species for aristolochic acid I and reported the highest level in *A. crispulatum*. Aristolochic acids were found only in species belonging to the family Aristolochiaceae, and were not detected in medicinal

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

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

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

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

ND = not detected.

Aristolochic acids also occur in North American species of Aristolochiaceae (Schaneberg *et al.* 2002,McMillin *et al.* 2003). Results from these two studies are summarized in Table 2-3. The Schaneberg *et al.* study reported what the authors described as unexpectedly high levels of aristolochic acids in *Hexastylis arifolia* (common name, littlebrownjug). No current medicinal uses for this plant were identified, but Schaneberg *et al.* observed that this and other species of *Hexastylis* had traditional uses that did pose some health hazard. However, they also noted that *Hexastylis* is probably not collected 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	
Aristolochia macrophylla	3,900	6,600	Schaneberg *et al.* 2002
Aristolochia serpentaria	1,300	97	
Hexastylis arifolia	2,100	6,600	
Asarum canadense	BQ–370	ND	
Asarum caudatum	BQ	ND	
Asarum wagneri	ND	ND	
Asarum canadense (dry root)	6–18.4[a]	NR	McMillin *et al.* 2003
Essence of wild ginger	0.048[a]	NR	

BQ = detected below the limit of quantitation; ND = not detected; NR = not reported.
[a]Results were reported as aristolochic acid.

2.4.2 Occurrence in foods or insects

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

2.4.3 Occurrence and concentrations in botanical products

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

Occurrence as a contaminant in herbal preparations

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

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

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

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

A multiple-to-one category describes multiple herbs derived from different parts of the same species of plant. The three herbs ma dou ling, qing mu xiang, and tian xian teng are derived, respectively, from the fruit, root, and stem of *A. debilis* or *A. contorta*. (3) A one-to-multiple category describes one herb that refers to multiple plant species. The herb fang ji refers to the root of either *A. fangchi* (guang fang ji), *Stephania tetrandra* (han fang ji), *Cocculus trilobus*, or *C. orbiculatus* (mu fang ji) (EMEA 2000, IARC 2002). *A. fangchi* belongs to the Aristolochiaceae family, while the latter three belong to the Menispermaceae family and do not contain aristolochic acids. [The first and third categories described by Wu *et al.* have the greatest potential to contribute to the unintended substitution of botanical material containing aristolochic acids for material that does not contain it.] Possible substitutions for "fang ji," "mu tong," "mu xiang," and "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	*Stephania tetrandra*	root
	guang fang ji	*Aristolochia fangchi*	
	mu fang ji	*Cocculus trilobus* *Cocculus orbiculatus*	
Mu tong	guan mu tong	*Aristolochia manshuriensis*	stem
	chuan mu tong	*Clematis armandii* *Clematis montana*	
	bai mu tong	*Akebia quinata* *Akebia trifoliata*	
Mu xiang	qing mu xiang	*Aristolochia debilis*	root
	mu xiang	*Aucklandia lappa*	
	guang mu xiang	*Saussurea lappa*	
	tu mu xiang	*Inula helenium* *Inula racemosa*	
	chuan mu xiang	*Vladimiria souliei* *Vladimiria souliei* var. *cinerea*	
Ma dou ling	ma dou ling	*Aristolochia contorta* *Aristolochia debilis*	fruit
	gua lou	*Trichosanthis kirilowii*	

Sources: EMEA 2000, IARC 2002, Zhu 2002.

Substitutions arising because of name confusion have also been reported between botanicals used in Japanese herbal medicines and botanicals with similar names used in Chinese herbal medicines. In a study of an outbreak of Chinese herb nephropathy in Japan (see Section 3.1.2), Tanaka *et al.* (2001) suggested that plant species in Japanese preparations of Chinese herbal medicines could have been substituted because similar Japanese and Chinese names refer to different plants in Japan and China (see Table 2-5). Confusion may also occur among Japanese names that are similar but refer to different herbal medicines; "sei-mokkou" refers to *Aristolochia debilis* (supplied as "qing mu xiang" in Chinese herbal medicines, see Table 2-5), while the Japanese names "mokkou" 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 *Akebia quinata*	kan-mokutsu *Aristolochia manshuriensis*	toki-shigyaku-ka-gosyuyu-syokyo-to toki-shigyaku-to gorin-san kami-gedoku-to sho-hu-san tu-do-san ryutan-syakan-to
Boui *Sinomenium acutum*	kou-boui *Aristolochia fangchi* kanchu-boui *Aristolochia heterophylla*	boui-ougi-to boui-bukuryo-to sokei-kakketsu-to

Source: Tanaka *et al.* 2001.

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

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

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

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

Occurrence and concentrations in botanical preparations

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

detected in 13 of 14 batches. Aristolochic acids also were found in samples of a Chinese herbal medicine taken by patients presenting with renal complications in Japan (Tanaka *et al.* 2000a). Gillerot *et al.* (2001) analyzed pills from a Chinese herbal preparation purchased in Shanghai, China. These pills were used by a 46-year-old woman for 6 months before she developed severe anemia and subacute renal failure. The aristolochic acids content of the herbal pills was determined to be about 0.07%. Lee *et al*. (2001) analyzed weight-loss powders and pills used in Taiwan. Five weight-loss pills and 11 weight-loss powders were collected directly from patients admitted to a hospital in Taipei because of slight renal failure. Aristolochic acids were found in 3 of 5 pills and 9 of 11 powders. Samples of 42 commercial Chinese plant mixtures sold for use in weight-loss regimens in Switzerland were analyzed for aristolochic acid I (Ioset *et al.* 2003). Four of the preparations were confirmed to contain aristolochic acid I by TLC and HPLC/UV/MS, and the presence of aristolochic acid I was suspected in two additional preparations. Aristolochic acid I was quantified by UV and MS methods in two samples of powder reported to consist of either a single herb (han fang ji, i.e., *Stephania tetrandra* root) or a mixture of 8 herbs (ba zheng san). The single herb preparation contained 0.044% [440 ppm] by UV and 0.040% [400 ppm] by MS, while the mixture of 8 herbs contained 0.009% [90 ppm] by UV and 0.014% [140 ppm] by MS. Over-the-counter Chinese prepared medicines purchased at a local store in Taiwan between January and September 2001 were analyzed for aristolochic acids I and II by Ho *et al.* (2006) using HPLC and UV detection. Aristolochic acid I was quantified in 8 out of 11 and aristolochic acid II in 5 out of 11 samples (neither aristolochic acid I nor II was detectable 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 powder	NR NR	NR NR	< 20–1,560 1,800–2,900	Vanhaelen *et al.* 1994[a]
China	pill	700	NR	0.3 mg/pill	Gillerot *et al.* 2001[b]
Taiwan	pill powder	< 1–39 < 1–598	< 1–124 < 1–148	< 1–163 < 1–694	Lee *et al.* 2001[c]
Japan	NS	1.1–6.7	1.3–6.7	3.1–15.1	Tanaka *et al.* 2000a[d]
Switzerland	powder	90–440	NR	NR	Ioset *et al.* 2003[e]
Taiwan	NS	ND–19.97 nmol/g	ND–3.95 nmol/g	NR	Ho *et al.* 2006[f]
Australia	NS	8, 40	8, 210	NR	Cheung *et al.* 2006[g]

AA I = aristolochic acid I; AA II = aristolochic acid II; NR = not reported; NS = not specified.
[a]Range of values reported from 12 (upper row) and 2 (lower row) batches of *S. tetrandra* powders distributed in Belgium from 1990 to 1992.
[b]Sample of a Chinese herbal preparation purchased in Shanghai for "waste discharging and youth keeping" purposes.
[c]Range of values from 5 weight-loss pills and 11 weight-loss powders collected from renal-failure patients treated in Taipei.
[d]Samples of the same herbal medicine collected from two patients with glycosuria.
[e] Range of values from 2 weight-loss powders purchased in Switzerland.
[f]Range 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.
[g] Values for 2 manufactured herbal products marketed under the Chinese proprietary names "Dao Chi Pian" and "Chuan Xiong Cha Tiao San."

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

aristolochic acids [one sample had 8 ppm of aristolochic acids I and II, and the other sample had 40 ppm of aristolochic acid I and 210 ppm of aristolochic acid II]. No aristolochic acids were detected in 21 samples of Chinese raw herbs purchased at the same time. Recalls of products containing aristolochic acids have been reported by the U.S. Food and Drug Administration beginning in 2000 and continuing with the report of a recall of two products in 2008 (see Appendix B, Table B-4 and Appendix C, Table C-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
Aristolochia root	280	0.64	140	0.32

Source: Schaneberg and Khan 2004.
N/A = not applicable; ND = not detected.

Exposure from using botanical products

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

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

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

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

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

2.4.4 Occupational exposure

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

2.5 Regulations and guidelines

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

2.5.1 United States

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

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

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

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

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

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

2.5.2 Other countries

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

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

2.6 Summary

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

herbs. Nevertheless, botanical products potentially containing aristolochic acids are still 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.

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

3.1 Studies on herbal medicine nephropathy or AAN

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

3.1.1 Belgian epidemic

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

and *Magnolia officinalis*. The Chinese name for *S. tetrandra* is "fang ji," which is similar to the name for *Aristolochia fangchi* ("guang fang ji") (see Section 2.4.3 and Table 2-4). A subsequent publication showed that most of the herb powders delivered to the Belgian clinic under the name *S. tetrandra* from 1990 to 1992 contained aristolochic acids but not tetrandrine, a compound expected to be present in a preparation made from *S. tetrandra,* suggesting that *A. fangchi* was used in place of *S. tetrandra* (Vanhaelen *et al.* 1994). Aristolochic acids are known nephrotoxic agents that cause acute renal failure and tubular lesions in experimental animals and humans (as reviewed by Cosyns 2003).

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

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

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

3.1.2 Worldwide cases of herbal medicine nephropathy or AAN

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

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

[1]Arlt *et al.* 2002b cited the following publications in French: Pourrat *et al.* (1994) and Stengel and Jones (1998).
[2]Arlt *et al.* 2002b also cited the following publications in Chinese: Chen *et al.* (2001) and Li *et al.* (2001).
[3]Arlt *et al.* 2002b also cited the following two publications in Japanese: Tanaka *et al.* (1997a,b).

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

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

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

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

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

Fanconi syndrome in some cases (N = 7), and (3) chronic AAN (N = 47). Fanconi syndrome is characterized by proximal tubular dysfunction and slowly progressive renal dysfunction reported to be reversible when exposure to aristolochic acids ceased (Lee *et al.* 2004). Reported cases of Fanconi syndrome (Izumotani *et al.* (1993), Ubara *et al.* (1999), Krumme *et al.* (2001), Lee *et al.* (2004) and Tsai *et al.* (2005)) improved when exposure to herbal medicines containing aristolochic acids was interrupted; however, this improvement was temporary for some patients even though they did not resume use of the herbal medicine (Lee *et al.*). [It should be noted that an acute, limited phase of intoxication will not necessarily be followed by a chronic phase, but only recovery from a chronic phase could be interpreted as true reversibility of AAN.] Although this presentation has mostly been reported from Asian countries, the case reported by Krumme *et al.* (2001) was that of a Caucasian man in Germany. Hypokalemia with paralysis has been reported in 2 AAN patients with Fanconi syndrome (Yang *et al.* 2002a, Tsai *et al.* 2005), and cases of AAN with Fanconi syndrome that rapidly progressed to renal failure have been documented (Lee *et al.* 2004, Hong *et al.* 2006). Almost all of the reported cases were in adults, but the case reported by Hong *et al.* (2006) occurred in a 10-year-old boy. Reasons for the slower and possibly reversible progression of symptoms have been the subject of speculation (Tanaka *et al.* 2000a, Tanaka *et al.* 2001), but no data have been presented to explain the differences. Tsai *et al.* (2005) stated that as of 2005, 24 cases of Fanconi syndrome secondary to AAN have been reported, mostly following consumption of the herb *A. manshuriensis*. In contrast, 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 *et al.* 2002b, Vanhaelen *et al.* 1994, Vanherweghem *et al.* 1993, Vanherweghem 1998	weight-loss	contained *A. fangchi*	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 *et al.* 2002b, Bieler *et al.* 1997, Cosyns *et al.* 1994a, Schmeiser *et al.* 1996, Cosyns *et al.* 1999, Kanaan *et al.* 2003	weight-loss	contained *A. fangchi*	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 *et al.* 2004b, Pourrat *et al.* 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 *et al.* 2001	hyperuricemia and prostatism	"Akebia 14"	I and II	NDT	1	Fanconi syndrome, reversible interstitial fibrosis
Spain	Pena *et al.* 1996	pain relief	*A. pistolochia* (taken as an infusion)	NDT	NDT	1	end-stage renal failure hypocellular interstitial fibrosis
Belgium	Vanherweghem *et al.* 1998	arthralgias	*Labelled as Stephania, but S. tetrandra not detected by chemical analysis*	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 *et al.* 1999	eczema	mu tong (*A. manshuriensis* or species of akebia or clematis) (taken as an infusion)	I and II	+	2	rapidly progressive renal failure interstitial fibrosis
United Kingdom	Cronin *et al.* 2002	hepatitis B	NR	I and II	NDT	1	renal failure and bone marrow suppression interstitial fibrosis
United States	Meyer *et al.* 2000	pain relief	NR	+	NDT	1	renal failure and bone marrow suppression interstitial fibrosis
United States	Grollman *et al.* 2007		Herbal remedy containing *Aristolochia*	NDT	+	1	end-stage renal failure
Cases from Asian countries							
China	Gillerot *et al.* 2001	health	various roots and leaves	I, II, and AR	+	1	rapidly progressive renal failure hypocellular interstitial fibrosis
China	Chen *et al.* 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 *et al.* 2001		mu tong *(A. manshuriensis)*	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 *et al.* 2004	tonic herbal remedy	mu tong (*A. manshuriensis*) and fang chi (*A. fangchi*)	I and II	NDT	1	acute renal failure (recovery) with underlying focal segmental glomerolosclerosis interstitial fibrosis
Hong Kong	Lo *et al.* 2005	Crohn's disease	pak mo tang (*A. mollissima*)	I	+	1	end-stage renal failure hypocellular interstitial fibrosis
Hong Kong	Laing *et al.* 2006	"liver enhancement"	longdan xieganwan (*A. manshuriensis*)	NDT	NDT	1	end-stage renal failure interstitial fibrosis
Japan	Izumotani *et al.* 1993	obesity	boui (*A. fangchi* and *A. heterophylla*)	NDT	NDT	1	Fanconi syndrome, somewhat reversible[e] no interstitial fibrosis
Japan	Tanaka *et al.* 1997b[b]	Chinese herbal remedy	NR	+	NDT	1	NA
Japan	Ubara *et al.* 1999	health promotion	various roots[f]	+	NDT	1	Fanconi syndrome, partly reversible hypocellular interstitial fibrosis
Japan	Nishimagi *et al.* 2001	edema	kan-mokutsu (*A. manshuriensis*)[g]	I	NDT	1	progressive renal failure interstitial fibrosis
Japan	Tanaka *et al.* 2001 also described in Tanaka *et al.* 2000a, Tanaka *et al.* 1997a	coldness of extremities, atopic dermatitis, nephrotic syndrome	kan-mokutsu (*A. manshuriensis*) and boui (*A. fangchi* and/or *A. heterophylla*)[g]	I, II and D	NDT	13[h]	Fanconi syndrome (N = 9) hypocellular, outer cortical interstitial fibrosis
Japan	Kazama *et al.* 2004	sterility	kan-mokutsu (*A. manshuriensis*)	NDT	NDT	1	Fanconi syndrome interstitial fibrosis
Korea	Lee *et al.* 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 *et al.* 2000	various purposes including weight-loss, nutritional supplement	NR	NDT	NDT	12	PAIN[i] most cases had rapid deterioration of renal function
Taiwan	Chang *et al.* 2001	nutritional supplement, weight loss, and treatment of non-renal disease	NR	NDT	NDT	20	PAIN[i] rapidly progressive renal failure
Taiwan	Yang *et al.* 2002a Yang *et al.* 2001	seizure and tonic encouragement leg edema	NR	I and II	NDT	2	1st patient: subacute renal failure, interstitial fibrosis 2nd patient: Fanconi syndrome with hypokalemic paralysis; hypocellular interstitial fibrosis
Taiwan	Tsai *et al.* 2005	leg edema	mu tong (*A. manshuriensis)*	I	NDT		Fanconi syndrome with hypokalemic paralysis, reversible No renal biopsy
Taiwan	Hong *et al.* 2006	health improvement	NR	I and II	NDT	1[j]	Fanconi syndrome with progressive renal failure and anemia interstitial fibrosis
Taiwan	Yang *et al.* 2006	lower back pain or nausea	duhuo tisheng tang, which contains xi xin (wild ginger, *Asarum* 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.

[a] Other 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.

[b] As 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.

3.2 Urothelial cancer

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

3.2.1 Case reports of urothelial cancer related to the Belgian epidemic

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

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

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

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

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

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

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

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

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

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

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

Urothelial cancer was found in 18 of the 39 patients (prevalence = 46%, 95% confidence interval [CI] = 29% to 62%), and 77 kidneys and 78 ureters were available for histologic evaluation (Nortier *et al.* 2000). One patient had a papillary bladder tumor, and the other 17 patients had carcinoma of the ureter, renal pelvis, or both. Mild to moderate urothelial atypia was found in 19 of the 21 patients without urothelial cancer. AA-DNA adducts were detected in the kidneys of the patients with herbal medicine nephropathy (samples were available from 38 of the 39 patients, and total adduct levels ranged from 1.7 to 175 per 10^9 nucleotides) but not in 8 patients (controls) with end-stage renal failure unrelated to herbal medicine nephropathy. Adduct levels did not differ between the patients with and without urothelial cancer. Tissue samples from 25 kidney specimens with a diagnosis of neoplasia (12 specimens), dysplasia (7 specimens), or no abnormalities (6 specimens) were also analyzed for adducts of the mycotoxin ochratoxin A (OTA) with DNA. Low levels of OTA-related DNA adducts (1.3 to 6.8 per 10^9 nucleotides) were detected in tissue from 2 of 12 patients with cancer and 2 of 7 with dysplasia; no adducts were detected in the control patients. The cumulative doses of *A. fangchi*, *M. officinalis*, and acetazolamide were significantly higher in patients with urothelial cancer than in patients without cancer; these compounds were almost always prescribed together. The prevalence of urothelial cancer was significantly higher ($P = 0.05$) in the 15 patients who received a total dose of *A. fangchi* greater than 201 g (10 cases) than in the 24 patients who ingested less than 200 g (8 cases). Patients with and without urothelial cancer did not

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

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

3.2.3 Case reports of urothelial cancer outside Belgium

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

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

Lord *et al.* (2001) reported invasive TCC in the renal pelvis and ureter of a 49-year-old woman who had developed end-stage renal failure after taking an herbal remedy containing aristolochic acid. [This case is 1 of 2 cases of AAN that were reported in an earlier publication [Lord *et al.* 1999] and are summarized in Table 3-2.] AA-DNA adducts were detected in both ureteral (40 per 10^9 nucleotides) and renal tissues (3.8 per 10^9 nucleotides). The authors stated that the woman did not have any confounding factors at the time of presentation with AAN; she was a nonsmoker and was not taking any other medicine.

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

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

Another case report from the United Kingdom described the occurrence of TCC in the urinary bladder of a 30-year-old Chinese man who had consumed the Chinese herb Longdan Xieganwan for at least 5 years to "enhance" his liver (Laing *et al.* 2006). The authors reported that Longdan Xieganwan contained (prior to 2002) *A. manshuriensis* root (*caulis*); however, the authors did not analyze the product for aristolochic acids or the patient's tissues for AA-DNA adducts. The patient presented with symptoms of renal toxicity, and renal biopsy showed that he had interstitial fibrosis consistent with CHN. The patient progressed to end-stage renal failure after the diagnosis of bladder cancer. 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 *et al.* 1994b	Consumption of weight-loss agent containing *A. fangchi* AA-DNA adducts detected in the kidney Schmeiser *et al.* 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 *et al.* 1995	Consumption of weight-loss agent containing *A. fangchi*	42-yr-old woman with rapidly progressive renal failure	1	papillary TCC of renal pelvis and mutlifocal *in situ* TCC of adjacent urothelial epithelium	Patient also used analgesics
Reginster *et al.* 1997	Consumption of weight-loss agent containing *A. fangchi* AA-DNA adducts were detected in the renal tissues of 5 patients (Schmeiser *et al.* 1996)	15 women aged 27–59 with CHN[a]	2	1st patient: papillary TCC of bladder and microinvasive urothelial carcinoma of ureter 2nd patient: *in situ* 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 *et al.* 2003	Probably consumption of weight-loss agent AA-DNA adducts detected in the right kidney (patient 8, Table 1 in Arlt *et al.* 2001b)	53-year-old woman	1	TCC *in situ* 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 Clinques St. Luc (reviews by Arlt *et al.* 2002b)
Nortier *et al.* 2003	Consumption of weight-loss agent containing *A. fangchi* 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 *et al.* 1999 Cliniques Universitaires St.-Luc	Consumption of weight-loss agent containing *A. fangchi*; 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 *in situ* of the renal pelvis and ureter	Includes patient described by Cosyns *et al.* 1994a and Reginster *et al.* 1997 Arlt *et al.* 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 *et al.* 2000, Nortier and Vanherweghem 2002 Hospital Erasme	Consumption of weight-loss agent containing *A. fangchi*; 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	*Tumor description* 1 urinary bladder tumor; the rest of the tumors in renal pelvis and ureter *Other effects* comparison of cumulative dose of herbal remedy in patients with and without cancer Ingredient — *P*-value *A. fangchi* — 0.035 *M. officinalis* — 0.026 acetazolamide — 0.012 fenfluramine — 0.130 diethylpropion — 0.200 prevalence of urothelial cancer vs. total dose of *A. fangchi* Dose — Prevalence > 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 *A. fangchi* (substituted for *S. tetrandra*), *M. officinalis*, acetazolamide, fenfluramine, and diethylpropion, the first three of which were almost always prescribed together

Reference	Exposure	Study population or description of patients	Number (prevalence) of cancer cases	Effects: Tumor location and characterization or other effects	Comments
Lemy *et al.* 2008 Update (15 year follow-up after exposure) of Nortier and colleagues Hospital Erasme	Consumption of weight-loss agent containing *A. fangchi*; 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)	*Tumor description* 17 cases of upper tract urtothelial cancer (renal or ureter) Follow-up: 15 cases developed bladder cancer *Other effects* Mean cumulative dose (g) of ingested *Aristolochia* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 2001 United Kingdom	Consumption of herbal remedy containing mu tong (*A. manshuriensis* or *Akebia* or *Clematis* 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 *et al.* 1999 (see Table 3-1)
Arlt *et al.* 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 *et al.* 2005 Hong Kong	Consumption of pak mo tang (*A. mollissima*) 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 *et al.* 2006 United Kingdom	Consumption of *Longdan Xieganwan* (*A. manshuriensis* root [*caulis*]; [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-Leszkowicz *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).

3.3 Clinical studies of urothelial cancer and consumption of Chinese herbs

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

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

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

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

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

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

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

3.4 Balkan endemic nephropathy and associated urothelial cancer

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

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

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

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

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

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

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

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

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

3.5 Discussion

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

3.5.1 Association between botanical products containing aristolochic acids and nephropathy

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

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

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

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

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

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

Although the fraction of patients who developed AAN from exposure to botanical products containing aristolochic acids was about 5% (see Section 3.1.1), urothelial cancer occurred at a high prevalence (40% and 46% in two studies, see Table 3-2) among patients in the Belgian epidemic with end-stage renal failure associated with AAN. Although renal disease or dialysis is a risk factor for urothelial cancer, the prevalence of cancer in AAN patients appears to be higher than that observed among patients with end-stage renal disease in general. However, the prevalence studies of AAN patients were very small and were conducted specifically to look for urothelial cancer. Wu *et al*. (2004c) summarized the data from several large studies of kidney-transplant patients and reported that the prevalence of cancer (at all sites) ranged from 4% to 18%, with an average of 6%. In Western nations, the predominant cancers in transplant patients were squamous-cell carcinoma of the skin and virus-related tumors. In contrast, TCC was the most common cancer in the Taiwanese study, with a prevalence of 4.1% in 730 kidney-

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

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

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

3.5.3 Localization of the TCC tumors

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

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

3.5.4 Strengths and weaknesses of the studies

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

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

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

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

3.6 Summary

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

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

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

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

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

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

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

In summary, exposure to aristolochic acids has been associated with a progressive interstitial renal fibrosis in several populations (primarily in Belgium, the Balkans, and China). An increased incidence or prevalence of upper urothelial tumors has been detected in individuals with aristolochic acids–associated end-stage renal failure. In some studies, AA-DNA adducts have been detected in urothelial tissues from the cancer patients, demonstrating exposure to aristolochic acids. Studies of renal-transplant or dialysis patients have reported elevated risks for urothelial cancer associated with 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.
[a]End of treatment period.

4.2 Rats

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

4.2.1 Acute exposure

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

approaching normal values by month 1, and was nearly restored at 3 to 6 months after treatment. Tubular lesions showed gradual recovery after 1 month and were nearly resolved at 3 and 6 months. However, at 6 months, renal preneoplastic lesions and extrarenal tumors were observed in all dosed groups, and renal tumors (including 4 renal mesenchymal tumors and 1 nephroblastoma) were observed with the two higher doses (Table 4-2). Extrarenal tumors included skin (appendage epithelial), thyroid gland (follicular epithelial), and mammary gland (ductal epithelial) tumors. These lesions were 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.

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

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

4.2.2 Subchronic to chronic exposure

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

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

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

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

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

unknown type (not determined morphologically due to small size) occurred in 2 additional rats. No tumors were detected in the control group. No statistical analyses were 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.
[a]No statistical analysis reported.
[b]Includes one metastatic tumor.

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

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

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

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

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

Body weight was not affected by exposure to the herbal mixture. Forestomach papillomas and squamous-cell carcinomas occurred in male rats given the weight-loss regimen but not in controls. One female rat exposed to the herbal mixture developed a forestomach 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.
[a]Included 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.
[b]Malignant tumor of unclear histogenesis

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

Hwang *et al.* (2006) investigated the subchronic toxicity of aristolochic acids and aqueous extracts of dried fruits from *A. contorta* (described as *A. fructus* by the authors) in male and female Sprague-Dawley rats (4 weeks old). Ten rats per sex per group were administered daily doses of extracts of *A. fructus* at 0, 21.35, 213.5, or 2,135 mg/kg by gavage for 90 days and were killed at the end of the treatment period. These doses were equivalent to 0.05, 0.5, and 5 mg/kg of aristolochic acids. Other groups were dosed with a mixture of aristolochic acids (44% aristolochic acid I and 56% aristolochic acid II) at 0, 0.05, 0.5, and 5 mg/kg for 90 days. There were significant decreases in body-weight gain in the high-dose groups compared with controls. No excess mortality was reported in the treatment groups, and clinical signs, hematology, and serum biochemistry in the treatment groups and controls were similar. Two male rats in the *A. fructus* high-dose group and one male rat in the aristolochic acids high-dose group developed carcinoma of the transitional epithelium of the renal pelvis. Forestomach papillomas and carcinoma 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
A. fructus 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.
[a]Statistics not provided

4.3 Two-stage study

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

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

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

4.4 Rabbits

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

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

4.5 Summary

4.5.1 Studies using aristolochic acids

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

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

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

4.5.2 Studies using extracts from Aristolochia *species*

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

most prevalent findings following oral administration. One study also reported tumors of the mammary gland, thyroid gland, and skin. Injection site polymorphocellular sarcomas also were reported in one study. Table 4-12 presents results for studies that used *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
[a] The number of animals with these tumors were not reported

4.5.3 Studies using botanical products containing aristolochic acids

Forestomach papillomas and squamous-cell carcinomas occurred in male rats given a weight-loss regimen of herbal ingredients that contained aristolochic acids but not in controls. One female rate exposed to the herbal mixture developed a forestomach 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

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

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

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

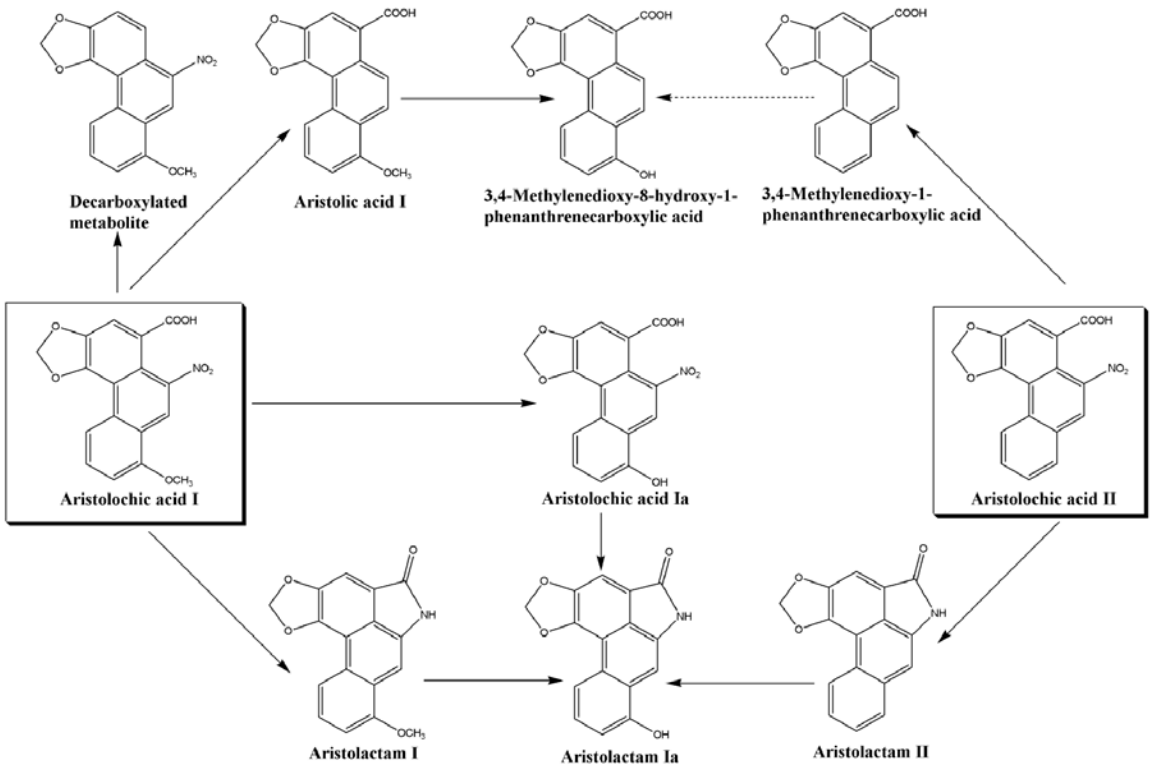

Figure 5-1. Phase I metabolism of aristolochic acids I and II in mammals

Source: Krumbiegel *et al.* 1987.
The dashed arrow indicates that the metabolite was found only after administration of the corresponding precusor.

Metabolites of aristolochic acids are excreted in the urine and feces (Krumbiegel *et al.* 1987). The primary metabolite of aristolochic acid I was aristolactam Ia; the average proportion of the dose in rats was about 46% in the urine (mostly in a conjugated form) and 37% in feces. Several other minor metabolites of aristolochic acid I (generally occurring at trace levels to less than 5% of the administered dose) were identified (Table 5-1). Aristolactam II was the primary metabolite of aristolochic acid II, with 4.6% recovered in the urine and 8.9% in the feces. In rats, metabolites of aristolochic acid I were excreted within 24 hours, while the metabolites of aristolochic acid II were still measurable in urine at 48 to 72 hours. Quantitative measurements of the metabolites in 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	+	+	–	–	–
Aristolic 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.

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

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

5.2 Toxicity

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

5.2.1 Renal toxicity in humans

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

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

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

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

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

5.2.2 Toxicity in experimental animals

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

General toxicity

Mengs (1987) determined LD_{50} values for rats and mice exposed to aristolochic acids by either oral or intravenous administration. The LD_{50} value for aristolochic acids in Wistar rats for oral administration was reported to be 203.4 mg/kg b.w. in males and 183.9 mg/kg b.w. in females, while the values for intravenous administration were 82.5 mg/kg b.w. in males and 74.0 mg/kg b.w. in females. The LD_{50} for aristolochic acids in NMRI mice for oral administration was 55.9 mg/kg b.w. in males and 106.1 mg/kg b.w. in females, while the values for intravenous administration were 38.4 mg/kg b.w. in males and 70.1 mg/kg b.w. in females. Mengs noted that the results suggested that aristolochic acids were slightly more toxic to mice than to rats.

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

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

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

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

Renal toxicity

As in humans exposed to aristolochic acids (see Section 5.2.1), renal toxicity is the most pronounced effect in experimental animals or livestock. Dumić (1954, cited in Grollman *et al.* 2007) and Martinčić (1957) reported cases of aristolochic acids nephropathy in horses that were fed hay contaminated with *A. clematitis*. Martinčić (1957) examined kidneys from 26 horses that were poisoned by ingesting contaminated hay, and from 2 horses that were experimentally poisoned. Diffuse nephritic alterations of the cortical tubules were reported while the glomeruli were unaffected. Inflammatory processes in the interstitial tissue lead to cirrhosis of both kidneys. The renal toxicity studies in laboratory animals, including details on study design and summaries of renal toxicity, are described in Table 5-2. The major findings from these studies are summarized after the table. (See 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 *et al.* 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 ≥ 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 *et al.* 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 *et al.* 1998	Rats Wistar- M (11, 4-7/group)/ F (9, 4-5/group)	weight-loss regimen with *S. tetrandra* [gavage (in olive oil)]	0.15 (70 mg *S. tetrandra* 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 *et al.* 2000	Sprague-Dawley- F (100, 30-40/group)	*A. manshuriensis* 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 *et al.* 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 *et al.* 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 *A. manshuriensis*, 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 *et al.* 2005	Sprague-Dawley- F (44, 3-14/group)	aristolochic acid I (95% purity) extracted from *A. manshuriensis* [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 *et al.* 2006	Wistar-F (54, ≥ 8/group)	decoction of *A. manshuriensis* [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 *et al.* 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 *et al.* 2006	Sprague-Dawley- M,F (80, 10 each sex/group)	extract of fruit of A. contorta [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 *et al.* 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,	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 *et al.* 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 AAII-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 *et al.* 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 *et al.* 2004	NIH- M (64, 8/group)/ F (64, 8/group)	*A. manshuriensis* from 3 Chinese counties or provinces aristolochic acid contents of *A. manshuriensis*: 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 *et al.* 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 ubular damage was progressive and dose-dependent
Cosyns *et al.* 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	*Aristolochia* 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).

[a]Experimental 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.]

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Metabonomic studies

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

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

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

5.2.3 Toxicity to kidney or urinary tract cells in vitro

Balachandran *et al.* (2005) examined the structure-activity relationships of aristolochic acid analogues based on cytotoxicity as assessed by the neutral red assay *in vitro*. This study tested both cultured proximal tubular cells from pig kidney (LLC-PK_1) and a human epithelial breast cell line (BT-549). More than 20 compounds were tested, including aristolochic acids I, Ia, 7-OH I, II, III, IVa, VIIIa, C (IIIa), and D (V), aristolic acid, and seven aristolactam derivatives. Aristolochic acid I was by far the most toxic to LLC-PK_1 cells, followed by aristolochic acids VIIIa, II, and Ia. None of the other compounds were toxic to LLC-PK_1 cells. Aristolochic acids were not toxic to BT-549 cells, which the authors interpreted as indicating that the cytotoxic action is specific to the kidney. They also concluded that the ring structures, side chains, and location of the side chains are critical determinants of toxicity and that the nitro group ($–NO_3$) and the methoxy group ($–OCH_3$) in the locations that they occupy in the aristolochic acid I molecule are associated with maximum toxicity. The authors concluded that any additions, deletions, substitutions, or replacement of the positions of the side chains drastically reduced toxicity.

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

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

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

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

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

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

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

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

(half-maximal inhibitory concentration [IC_{50}] = 40 μM) of phospholipase-dependent release of arachidonate from phosphatidyl choline or phosphatidyl inositol in human neutrophils *in vitro* (Rosenthal *et al.* 1989). Studies of the ability of aristolochic acids to block the arachidonic acid response to inflammation led to the observation that the compound is more acutely toxic to macrophages (IC_{50} = 2.5 μM) than to neutrophils (IC_{50} = 100 μM) (Glaser *et al.* 1995). Aristolochic acids also have been shown to inhibit phospholipase A_2–mediated effects of snake venom on local edema *in vivo* (Vishwanath and Gowda 1987) and on neutrophil motility *in vitro* (Sundell *et al.* 2003), effects that might be related to the use of *Aristolochia* species in traditional medical treatments for snakebite.

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

5.3 Genetic damage and related effects

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

5.3.1 DNA adduct formation

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

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

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

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

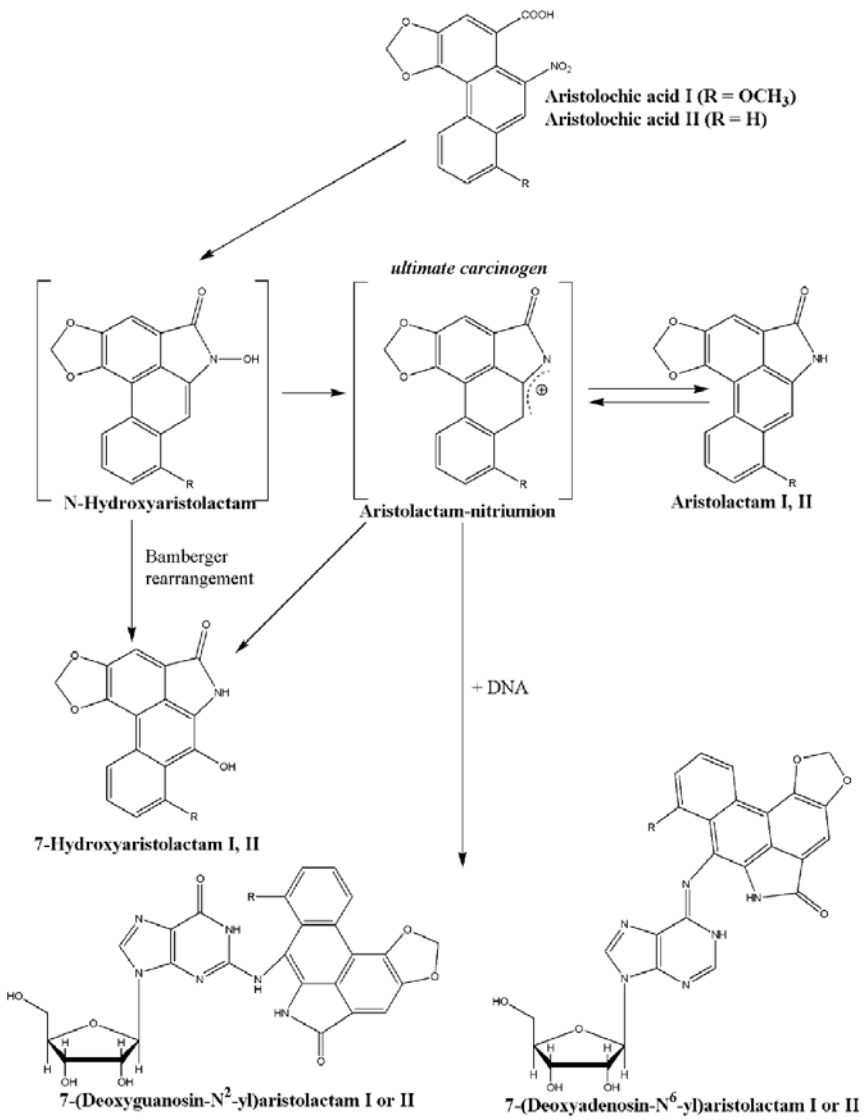

Figure 5-2. Metabolic activation of aristolochic acids and adduct formation

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

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

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

Studies in humans with AAN or BEN

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

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

samples contained the highest individual levels reported in that paper. In contrast to Arlt *et al.* (2004b), Pfohl-Leszkowicz *et al.* (2007) did not detect AA-DNA adducts in French and Belgian patients who had been exposed to slimming regimens that contained aristolochic acids (see sections 3.2 for AAN-related studies and Section 3.4 for findings related to BEN). Pfohl-Leszkowicz *et al.* did not offer an explanation for the discrepancy between their findings and those of others who clearly demonstrated aristolochic acid adducts in tissues of AAN patients. Pfohl-Leszkowicz *et al.* and Arlt *et al.* used slightly different chromatographic conditions (mainly differences in molarity and pH of some of the developing solutions) in their analyses of DNA adducts. Arlt *et al.* (2001b) [Pfohl-Leszkowicz was a coauthor for this paper] (see below) reported that analysis of OTA adducts required different chromatographic conditions than routinely used for detecting aristolochic acid adducts; therefore, these authors used the conditions suitable for OTA-related adducts and demonstrated that aristolochic adducts could be detected with this method. Pfohl-Leszkowicz *et al.* (2007) did not report any results for simultaneous determination of AA-DNA and OTA-related adducts on the same chromatographic plate. Pfohl-Leszkowicz *et al.* did detect aristolochic acid adducts *in vitro* [albeit at lower levels, and for shorter duration than another *in vitro* study (although there were 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 *A. fangchi* (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 *et al.* 1996 (Bieler *et al.* 1997 (Belgium)
Ureter (1)	weight-loss preparation containing *A. fangchi* (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 *et al.* 1997 Arlt *et al.* 2001b (Belgium)
Kidney (38)	weight-loss preparation containing *A. fangchi* (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 *et al.* 2000 (Belgium)
Ureter (11)		dA-AAI dG-AAI dA-AAII	0.22–3.4 NR NR	
Kidney (2)[c]	weight-loss preparation containing *A. fangchi* (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 *et al.* 2001b (Belgium)
Kidney (1)	various roots and leaves (108 mg AA) [6]	dA-AAI	1.8	Gillerot *et al.* 2001 (China)
Kidney (1) Liver (1) Pancreas (1) Lymph nodes (1) Stomach (1) Lung (1) Bladder (1)	weight-loss preparation containing *A. fangchi* (189 g of herb) [14]	dA-AAI	8.1 0.87 0.8 0.5 1.9 0.16 ND	Nortier *et al.* 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 (*A. manshuriensis*) (NR) [24]	dA-AAI	3.8 40 20 1.0 1.0 16	Lord *et al.* 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 *et al.* 2004b[e] (France)
Kidney (1)	*A. mollissima* (800 g of herb) [6]	dA-AAI	NR	Lo *et al.* 2005 (Hong Kong)
Kidney (1)	Herbal remedy containing *Aristolochia* (NR) [NR]	dA-AAI + -AAII dG-AAI	11–34 0.2–1	Grollman *et al.* 2007 (U.S.)

dA-AAI = 7-(deoxyadenosin-N^6-yl)-aristolactam I; dA-AAII = 7-(deoxyadenosin-N^6-yl)-aristolactam II; dG-AAI = 7-(deoxyguanosin-N^2-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.

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

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

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

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

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

Studies in experimental animals

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

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

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

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

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

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

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

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

Mei *et al.* (2006) investigated DNA adduct formation in rat kidney and liver. Groups of six male Big Blue rats were administered oral doses of aristolochic acids (mixture, 40% aristolochic acid I, 56% aristolochic acid II) at 0, 0.1, 1.0, and 10 mg/kg b.w. 5 days/week for 3 months. Rats were sacrificed the day after the final treatment. Three major adducts were identified (dA-AAI, dA-AAII, and dG-AAI), and there was a strong linear dose response. Although DNA adducts were detected in both the kidneys and livers of rats exposed to aristolochic acids, the kidneys ($4{,}598 \pm 148 \times 10^{-8}$ nucleotides) had about twice the level of DNA adducts observed in the liver ($1{,}967 \pm 468 \times 10^{-8}$ nucleotides) at 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 1997
Wistar rats (M/F)	Weight-loss *(S. tetrandra)* 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 *et al.* 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 dG-AAI dA-AAII	911.4 1,676.6 2,010.3	Mei *et al.* 2006
		liver	dA-AAI dG-AAI dA-AAII	684.1 720.9 561.8	

AA I = aristolochic acid I; AA II = aristolochic acid II; dA-AAI = 7-(deoxyadenosin-N^6-yl)-aristolactam I; dA-AAII = 7-(deoxyadenosin-N^6-yl)-aristolactam II; dG-AAI = 7-(deoxyguanosin-N^2-yl)-aristolactam I; dG-AAII = 7-(deoxyguanosin-N^2-yl)-aristolactam II; NI = specific molecular forms of adducts were not identified; total adduct levels are given.
[a]The first value is for nuclease P1 extraction and the second for *n*-butanol extraction.
[b]Initial adduct level/level at 36 weeks as reported by IARC 2002.
[c]Level in males/level in females.

Dong *et al.* (2006) exposed 3 male Wistar rats to aristolochic acid I or II or aristolactam I at 5 mg/kg b.w. per day for 7 days by gavage. Nine different tissues were collected. The highest adduct levels were detected in intestine, kidney, and liver of rats exposed to aristolochic acid I and in kidney, bladder, and intestine of rats exposed to aristolochic acid II (Table 5-5). Rats exposed to aristolochic acid II had the highest adduct levels; however, other studies found higher adduct levels in rats exposed to aristolochic acid I. Levels of adducts in rats exposed to aristolactam I were much lower, ranging from 2 to 24 adducts per 10^8 nucleotides.

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

	No. of adducts per 10^8 nucleotides ± SD					
	aristolochic acid I		aristolochic acid II		aristolactam I	
DNA source	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-N^6-yl)-aristolactam I; dA-AA II = 7-(deoxyadenosin-N^6-yl)-aristolactam II; dG-AAI = 7-(deoxyguanosin-N^2-yl)-aristolactam I; dG-AAII = 7-(deoxyguanosin-N^2-yl)-aristolactam II.

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

In vitro *studies in cell-free systems*

The affinity of aristolochic acids I and II to form adducts at the first adenine of codon 61 (CAA) in the H-*ras* gene was assessed in *in vitro* studies using a polymerase arrest assay in a plasmid (pNPR) containing exon 2 of the mouse H-*ras* gene (Arlt *et al.* 2000). Aristolochic acids I and II modified by chemical reduction with zinc were incubated with the pNPR plasmid, and the sites of polymerase arrest 3' to the bulky aristolochic acid adducts were determined. Both aristolochic acids showed a preference for adduct formation and arrest sites at purine bases; however, the polymerase arrest spectra differed for the two molecules. Aristolochic acid I preferentially formed adducts at guanine residues, but polymerase arrest sites were primarily at adenine residues. Conversely, aristolochic acid II reacted preferentially to form adducts with adenine residues, but polymerase arrest occurred relatively equally at guanine, adenine, and cytosine residues. Neighboring bases affected adduct formation for both aristolochic acids I and II, with flanking pyrimidine residues favoring binding. The differences in adduct formation sites and polymerase arrest sites were suggested to result from structural characteristics of the DNA adducts formed by the two aristolochic acid molecules. The authors also suggested that the mutation "hot spot" at the first adenine of codon 61 of H-*ras* did not result from initial adduct formation but could be due to non-random action of DNA repair processes, because analysis of adducts by ^{32}P-postlabeling showed formation of adducts at both adenines in codon 61.

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

5.3.2 Prokaryotic systems

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

Salmonella typhimurium

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

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

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

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

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

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

Escherichia coli

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

response was much greater without activation. Aristolochic acid II also was considered to 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					
S. typhimurium TA100, TA1537	reverse mutation	50 (mixture)	+	+	Robisch *et al.* 1982
S. typhimurium TA1535,TA1538, TA98	reverse mutation	200 (mixture)	–	–	Robisch *et al.* 1982
E. coli PQ37	DNA damage (SOS chromotest)	0.38 µg/assay (plant extract)	+[a]	+	Kevekordes *et al.* 1999
Aristolochic acid I					
S. typhimurium TA100, TA1537	reverse mutation	100	+	+	Schmeiser *et al.* 1984
S. typhimurium TA100NR[b]	reverse mutation	200	–	–	Schmeiser *et al.* 1984
S. typhimurium TA100, TA98, TA1535	reverse mutation	50	+	+	Chakrabarty *et al.* 1987
S. typhimurium TA1978, *hisG46*, *hisD3052*	reverse mutation	1,000	–	–	Chakrabarty *et al.* 1987
S. typhimurium TA100, TA102, TA1537	reverse mutation	100	+	NT	Pezzuto *et al.* 1988
S. typhimurium TA98NR[b], TA100NR[b]	reverse mutation	200	–	NT	Pezzuto *et al.* 1988
S. typhimurium TM677	forward mutation (*hprt* locus)	8.5 µg/mL	+	NT	Pezzuto *et al.* 1988
S. typhimurium TA98, YG1020, YG1021	reverse mutation	170	(+)	NT	Götzl and Schimmer 1993
S. typhimurium TA1537	reverse mutation	85	+	NT	Götzl and Schimmer 1993
S. typhimurium YG1024, TA100, YG1025, YG1026, YG1029	reverse mutation	34	+	NT	Götzl and Schimmer 1993
S. typhimurium TA98, TA100	reverse mutation	100	+	+	Zhang *et al.* 2004
E. coli PQ37	DNA damage (SOS chromotest)	0.17 µg/assay	+[a]	+	Kevekordes *et al.* 1999
Aristolochic acid II					
S. typhimurium TA98, YG1020, YG1021	reverse mutation	78	(+)	NT	Götzl and Schimmer 1993
S. typhimurium YG1021, YG1024, TA100, YG1025, YG1026, YG1029	reverse mutation	[31][c]	+	NT	Götzl and Schimmer 1993
S. typhimurium 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
E. coli PQ37	DNA damage (SOS chromotest)	0.16 µg/assay	+	(+)	Kevekordes *et al.* 1999
Aristolochic acid IV					
S. typhimurium TA100	reverse mutation	100	(+)	–	Pistelli *et al.* 1993
Aristolactams					
S. typhimurium TA100, TA1537	reverse mutation	50 (AL I, II)	–	+	Schmeiser *et al.* 1984
S. typhimurium TA100, TA98, TA1535, TA1978, and strains carrying *hisG46* or *hisD3052*	reverse mutation	1,000 (AL I)	–	–	Chakrabarty *et al.* 1987
Aristolic acid					
S. typhimurium TA100, TA98, TA1535, TA1978, and strains carrying *hisG46* or *hisD3052*	reverse mutation	1,000	–	–	Chakrabarty *et al.* 1987
S. typhimurium TA98, TA100, YG1021, YG1026	reverse mutation	276	–	NT	Götzl and Schimmer 1993
S. typhimurium 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.
[a]The response was much greater without metabolic activation.
[b]Nitroreductase-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.]

5.3.3 Lower eukaryotes

Exposure of *Drosophila melanogaster* to aristolochic acids (composition not specified) caused sex-linked recessive lethal mutation, chromosome damage in the sex-chromosome loss test, and recombinogenic damage in the somatic mutation and recombination test (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.
[a]The 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].

5.3.4 In vitro *studies in mammalian cells*

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

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

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

at the tk locus in a concentration-dependent manner (at concentrations ≥ 25 μg/mL) with or without metabolic activation.

Liu *et al.* (2004) used embryonic fibroblast cells from a human *p53* knock-in (Hupki) mouse strain to generate human *p53* DNA-binding domain mutations. Fibroblasts were harvested from 13.5-day-old embryos homozygous for the humanized *p53* allele. Twenty-four cultures of the primary Hupki cells were exposed to 100 μM aristolochic acid I for 48 hours and then passaged for 8 to 10 weeks. Ten of the 24 cultures were established (defined as having acquired a uniform morphology and a population-doubling time of 72 hours or less) within this timeframe and were analyzed for *p53* mutations. Six base substitutions were identified in five of the established cultures. Four of the substitutions were A:T → T:A transversions on the nontranscribed strand, and two were C:G → G:C transversions. The authors noted that A:T → T:A transversions are relatively rare in spontaneous or UV-induced mutations, but are a hallmark of mutations induced by aristolochic acid I. Feldmeyer *et al.* (2006) reported similar results in a study with the same cell line exposed to 50 μM aristolochic acid I. Eighteen immortalized cultures were examined for *p53* mutations, and six cell lines were found with base changes, five of which were A:T → T:A transversions. Feldmeyer *et al.* also found that one of the mutations in their cell lines was at the same location (codon 139) that was reported in an aristolochic acid-exposed patient (Nortier *et al.* 2000).

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

following 4 hours or 23 hours of exposure to the test agent. Wu *et al.* (2007b) (see above) also found that aristolochic acids (12.5 to 50 μM.) increased the frequency of micronuclei 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 *et al.* 1986[a]
Porcine proximal tubular epithelial cells	AA I	0.32	DNA damage	+	NT	Li *et al.* 2006a
Human hepatoma (HepG2) cells	AA mixture	25 μM	DNA damage	+	NT	Wu *et al.* 2007b
Rat fibroblast-like cells	AA I, AA II	20	mutation at *hprt* locus	+	NT	Maier *et al.* 1987[a]
CHO cells	AA I	18.2	mutation at *hprt* locus	+	NT	Pezzuto *et al.* 1988[a]
Mouse lymphoma cells	AA I	25	forward mutation	+	+	Zhang *et al.* 2004
Hupki mouse fibroblasts (human *p53* knock-in strain)	AA I	100 μM	*p53* DNA-binding domain mutation	+	NT	Liu *et al.* 2004
Hupki mouse fibroblasts (human *p53* knock-in strain)	AA I	50 μM	*p53* DNA-binding domain mutation	+	NT	Feldmeyer *et al.* 2006
Human lymphocytes	AA mixture	1	chromosomal aberrations	+	NT	Abel and Schimmer 1983[a]
CHO cells	AA I	25–50	chromosomal aberrations	+	+	Zhang *et al.* 2004
Human lymphocytes and hepatoma cells	AA mixture	17	micronucleus induction	+	+	Kevekordes *et al.* 2001[a]
CHO cells	AA I	25–50	micronucleus induction	+	+	Zhang *et al.* 2004
Human hepatoma (HepG2) cells	AA mixture	12.5 μM	micronucleus induction	+	NT	Wu *et al.* 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.
[a]Cited in IARC 2002.

5.3.5 In vivo *studies*

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

Mutation in rodents

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

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

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

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

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 40 μg 160 μg 320 μg	3.7 10.7* 172.5* 305.3*	Maier *et al.* 1985
Sprague-Dawley rats (M) 6-TG resistance	AA mixture gavage	s.c. granulation tissue[b]	45 mg/kg b.w. 90 mg/kg b.w.	18.1* 54.5*	Maier *et al.* 1985
Sprague-Dawley rats (M) 6-TG resistance	AA I s.c. injection	s.c. granulation tissue[b]	control (5%) control (19%) 80 μg (5%) 80 μg (19%)	3.4 4.0 68.5* 59.5*	Maier *et al.* 1987
Sprague-Dawley rats (M) 6-TG resistance	AA II s.c. injection	s.c. granulation tissue[b]	control (5%) control (19%) 320 μg (5%) 320 μg (19%)	3.4 4.0 17.3* 17.5*	Maier *et al.* 1987
Muta mice (M) *lacZ* mutation	AA mixture (56% I, 40% II) 4 intragastric injections	forestomach	control 15 mg/kg b.w.	33 1,129*[c]	Kohara *et al.* 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) *cII* gene	AA mixture (40% AAI, 56% AAII) gavage	kidney	control 0.1 mg/kg b.w. 1.0 mg/kg b.w. 10 mg/kg b.w.	29 78*** 242*** 1,319***	Chen *et al.* 2006b, Mei *et al.* 2006
Big Blue rats (M) *cII* gene	AA mixture (40% AAI, 56% AAII) gavage	liver	control 0.1 mg/kg b.w. 1.0 mg/kg b.w. 10 mg/kg b.w.	28 37 113*** 666***	Mei *et al.* 2006

*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.
[a]The value in parentheses is the oxygen tension of the cell cultures.
[b]Rats were exposed *in vivo*, but cells were harvested and cultured *in vitro*.
[c]The *P*-value was not reported by the authors.

Mutational spectra in tumors from animals or humans

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

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

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

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

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

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

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

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

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

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

Several publications have concluded that OTA does not play a role in BEN or its associated upper urothelial cancer (Grollman and Jelakovic 2007, Grollman *et al.* 2007, de Jonge and Vanrenterghem 2008) based on the findings of the A:T → T:A transversions in urinary tumors from patients with probable BEN. However, some recent reviews still consider OTA to be a potential risk factor for BEN (Peraica *et al.* 2008, Stefanovic and 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 *et al.* 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 *et al.* 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 *et al.* 2006
	kidney	ND	NR	
	ureter	ND	NR	
	bladder	ND	NR	
	liver	ND	NR	
Human AAN patient (F)	bladder	*p53* 230 ACC→ CCC	1/1	Cosyns 2003
	bladder	*p53* 248 CGG→ CAG	1/1	
Human AAN patient (F)	ureter	*p53* 139 AAG→TAG	1/1	Lord *et al.* 2004
	breast	*p53* 245 GGC→GAC	1/1	
	liver	*p53* 245 GGC→GAC	1/1	
Human BEN patients (N = 11) with urothelial cancer[b]	renal pelvis and/or ureter or bladder	*P53* (19 base substitution mutations) at A:T pairs	89%	Grollman *et al.* 2007
		A:T → T:A transversions	15/19	

ND = not detected; NR = not reported.
[a]Includes 1 metastatic tumor.
[b]Exposure to aristolochic acids was presumed by the authors but not documented.

Unscheduled DNA synthesis, DNA damage, and micronucleus induction in rodents

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

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

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

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

5.4 Mechanistic studies and considerations

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

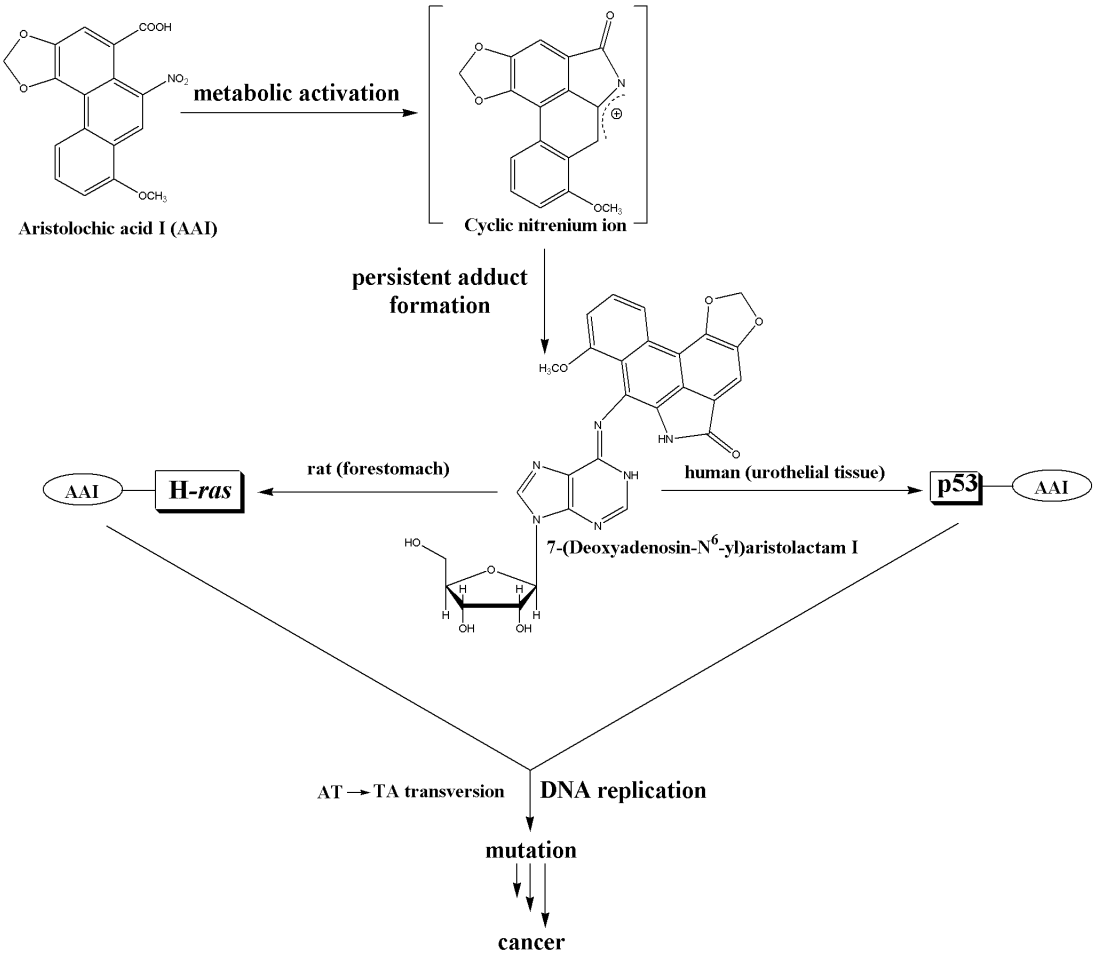

Figure 5-3. Proposed mechanism for aristolochic acids-induced carcinogenesis

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

5.4.1 Renal toxicity

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

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

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

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

5.4.2 Carcinogenesis in animals

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

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

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

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

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

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

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

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

Chang *et al.* (2006) investigated the possible role of activation of cell-cycle progression via cyclin D_1/cdk4 and cyclin E/cdk2 in the induction of the urothelial proliferation in male Wistar rats exposed to an aristolochic acids mixture (41% aristolochic acid I; 56% aristolochic acid II) at either 5 or 10 mg/kg b.w. per day. The authors reported that dose-dependent urothelial proliferation was detected histologically, and at doses of 5 and 10 mg/kg, respectively, induction of cyclin D_1/cdk4 increased 1.57- and 1.95-fold, and induction of cyclin E/cdk2 increased 1.46- and 1.62-fold. Phosphorylation of the retinoblastoma tumor suppressor protein (Rb) also increased 1.75-fold at the low dose and 2.07-fold at the high dose, while Rb/E2F complexes were reduced to 0.65 of the control level at the low dose and 0.24 of the control level at the high dose. The authors suggested that induction of cyclin-cdk complexes could result in phosphorylation of Rb and release of E2F from Rb, resulting in promotion by E2F of cell-cycle transition from the G1 to the S phase, which could cause urothelial proliferation as a pro-carcinogenic phenomenon in tumorigenesis.

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

5.4.3 Metabolic activation and toxic effects in humans

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

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

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

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

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

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

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

5.5 Summary

5.5.1 Absorption, distribution, metabolism, and excretion

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

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

5.5.2 Toxicity

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

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

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

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

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

Aristolochic acids and their aristolactam derivatives are cytotoxic to cells growing in culture, including kidney cells and human epithelial breast cells. The cytotoxic effects of aristolochic acids may be linked to a rapid increase in intracellular calcium that promotes apoptosis. Other studies reported that aristolochic acids disrupted mitochondrial permeability transition in human renal tubular epithelial cells, an effect that may be involved in renal injury, and one study reported cell-cycle arrest in human urinary tract epithelial cells. Aristolochic acids are also specific inhibitors of phospholipase A_2 and may have other specific biochemical targets that explain its renal toxicity and its widespread use in traditional plant-based medical therapies throughout the world.

5.5.3 Genetic damage and related effects

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

5.5.4 Mechanistic studies and considerations

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

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

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6 References

1. Abel G, Schimmer O. 1983. Induction of structural chromosome aberrations and sister chromatid exchanges in human lymphocytes in vitro by aristolochic acid. Hum Genet 64(2): 131-133. (Support not reported. Authors affiliated with Institut für Botanik und Pharmazeutische Biologie der Universität Erlangen-Nürnberg, Germany.)

2. AOAC. 2007. Method for Aristolochic Acid Granted AOAC Official Method Status. AOAC International. http://www.aoac.org/ILM/jul_aug_07/aristolochic_acid.htm. Last accessed on 11/09/07.

3. Arlt VM, Wiessler M, Schmeiser HH. 2000. Using polymerase arrest to detect DNA binding specificity of aristolochic acid in the mouse H-ras gene. Carcinogenesis 21(2): 235-242. (Support not reported. Authors affiliated with German Cancer Research Center, Germany.)

4. Arlt VM, Schmeiser HH, Pfeifer GP. 2001a. Sequence-specific detection of aristolochic acid-DNA adducts in the human p53 gene by terminal transferase-dependent PCR. Carcinogenesis 22(1): 133-140. (Supported by the Boehringer Ingelheim Fonds. Authors affiliated with German Cancer Research Center, Germany; Beckham Research Institute of the City of Hope, USA.)

5. Arlt VM, Pfohl-Leszkowicz A, Cosyns J, Schmeiser HH. 2001b. Analyses of DNA adducts formed by ochratoxin A and aristolochic acid in patients with Chinese herbs nephropathy. Mutat Res 494(1-2): 143-150. (Support not reported. Authors affiliated with German Cancer Research Centre, Germany; Ecole Nationale Supérieure Agronomique de Toulouse, France; Université Catholique de Louvain, Belgium.)

6. Arlt VM, Ferluga D, Stiborova M, Pfohl-Leszkowicz A, Vukelic M, Ceovic S, Schmeiser HH, Cosyns JP. 2002a. Is aristolochic acid a risk factor for Balkan endemic nephropathy-associated urothelial cancer? Int J Cancer 101(5): 500-502. (Supported by Cancer Research, UK and the Grant Agency of the Czech Republic. Authors affiliated with Institute of Cancer Research, UK; University of Ljubljana, Slovenia; Charles University, Czech Republic; Laboratoire de Toxicologie et Sécurité Alimentaire, France; General Hospital, Croatia; German Cancer Research Center, Germany; Université Catholique de Louvain, Belgium.)

7. Arlt VM, Stiborova M, Schmeiser HH. 2002b. Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. Mutagenesis 17(4): 265-277. (Supported by Cancer Research, UK and the Grant Agency of the Czech Republic. Authors affiliated with Institute of Cancer Research, UK; Charles University, Czech Republic; German Cancer Research Center, Germany.)

8. Arlt VM, Alunni-Perret V, Quatrehomme G, Ohayon P, Albano L, Gaïd H, Michiels JF, Meyrier A, Cassuto E, Wiessler M, Schmeiser HH, Cosyns JP. 2004b. Aristolochic acid (AA)-DNA adduct as marker of AA exposure and risk factor for AA nephropathy-associated cancer. Int J Cancer 111(6): 977-980. (Support not reported. Authors affiliated with Institute of Cancer Research, UK; Hôpital Pasteur, France; Hôpital Européen Georges Pompidou, France; German Cancer Research Center, Germany; Université Catholique de Louvain Medical School, Belgium.)

9. Arlt VM, Stiborova M, Brocke JV, Simoes ML, Lord GM, Nortier JL, Hollstein M, Phillips DH, Schmeiser HH. 2007. Aristolochic acid mutagenesis: molecular clues to the aetiology of Balkan endemic nephropathy-associated urothelial cancer. Carcinogenesis. (Supported by the Association for International Cancer Research and a Waltraud-Lewenz phD student grant. Authors affiliated with Institute of Cancer Research, UK; Charles University, Czech Republic; German Cancer Research Center, Germany; Guys Hospital and Kings College, UK; Academic Erasme Hospital, Belgium.)

10. Balachandran P, Wei F, Lin RC, Khan IA, Pasco DS. 2005. Structure activity relationships of aristolochic acid analogues: toxicity in cultured renal epithelial cells. Kidney Int 67(5): 1797-1805. (Supported by the Food and Drug Administration. Authors affiliated with University of Mississippi; State Food and Drug Administration, China.)

11. Barnes PM, Powell-Griner E, McFann K, Nahin RL. 2004. Complementary and Alternative Medicine Use Among Adults: United States, 2002. Advance data from vital health and statistics; no. 343. U.S. Department of Health and Human Services, CDC, National Center for Health Statistics. http://www.cdc.gov/nchs/data/ad/ad343.pdf.

12. Bent S, Ko R. 2004. Commonly used herbal medicines in the United States: a review. Am J Med 116(7): 478-485. (Supported by the National Center for Complementary and Alternative Medicine. Authors affiliated with University of California; San Francisco VA Medical Center; California Department of Health Services.)

13. Bieler CA, Stiborova M, Wiessler M, Cosyns JP, van Ypersele de Strihou C, Schmeiser HH. 1997. ^{32}P-post-labelling analysis of DNA adducts formed by aristolochic acid in tissues from patients with Chinese herbs nephropathy. Carcinogenesis 18(5): 1063-1067. (Support not reported. Authors affiliated with German Cancer Research Center, Germany; Charles University, Czech Republic; University of Louvain Medical School, Belgium.)

14. Broschard TH, Wiessler M, von der Lieth CW, Schmeiser HH. 1994. Translesional synthesis on DNA templates containing site-specifically placed deoxyadenosine and deoxyguanosine adducts formed by the plant carcinogen

aristolochic acid. Carcinogenesis 15(10): 2331-2340. (Support not reported. Authors affiliated with German Cancer Research Center, Germany.)

15. Broschard TH, Wiessler M, Schmeiser HH. 1995. Effect of site-specifically located aristolochic acid DNA adducts on in vitro DNA synthesis by human DNA polymerase a. Cancer Lett 98(1): 47-56. (Supported by the Deutsche Forschungsgemeinschaft. Authors affiliated with German Cancer Research Center, Germany.)

16. Burlinson B. 1989. An in vivo unscheduled DNA synthesis (UDS) assay in the rat gastric mucosa: preliminary development. Carcinogenesis 10(8): 1425-1428. (Support not reported. Authors affiliated with Glaxo Group Research Ltd., UK.)

17. Cavin C, Delatour T, Marin-Kuan M, Holzhauser D, Higgins L, Bezencon C, Guignard G, Junod S, Richoz-Payot J, Gremaud E, Hayes JD, Nestler S, Mantle P, Schilter B. 2007. Reduction in antioxidant defenses may contribute to ochratoxin A toxicity and carcinogenicity. Toxicol Sci 96(1): 30-9. (Supported by the EU. Authors affiliated with Nestle Research Center, Switzerland; University of Dundee, UK; University College London, UK; Imperial College London, UK.)

18. CFR. 2003. Subpart F - Flavoring agents and related substances. Code Fed Reg 21(Ch. 1): 172.510-172.515.

19. Chakrabarty SN, Pakrashi A, Siddiqui AI. 1987. Aristolic acid: an infertility agent is non-mutagenic to bacterial system. Indian J Pharmac 19: 26-31. (Support not reported. Authors affiliated with Indian Institute of Chemical Biology, Calcutta.)

20. Chan W, Cui L, Xu G, Cai Z. 2006a. Study of the phase I and phase II metabolism of nephrotoxin aristolochic acid by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 20(11): 1755-60. (Supported by Hong Kong Baptist University and the Research Grant Council, China. Authors affiliated with Hong Kong Baptist University, China; Chinese Academy of Sciences, China.)

21. Chan W, Luo HB, Zheng Y, Cheng YK, Cai Z. 2007a. Investigation of the metabolism and reductive activation of carcinogenic aristolochic acids in rats. Drug Metab Dispos 35(6): 866-74. (Supported by the Hong Kong Baptist University, Research Grant Council and the University Grants Committee of Hong Kong. Authors affiliated with Hong Kong Baptist University, China.)

22. Chan W, Lee KC, Liu N, Cai Z. 2007b. A sensitivity enhanced high-performance liquid chromatography fluorescence method for the detection of nephrotoxic and carcinogenic aristolochic acid in herbal medicines. J Chromatogr A 1164(1-2): 113-9. (Supported by the Research Grant Council,

University Grants Committe of Hong Kong. Authors affiliated with Hong Kong Baptist University, China.)

23. Chang CH, Wang YM, Yang AH, Chiang SS. 2001. Rapidly progressive interstitial renal fibrosis associated with Chinese herbal medications. Am J Nephrol 21(6): 441-448. (Support not reported. Authors affiliated with Shin-Kong Wu Ho-Su Memorial Hospital, Taiwan; Veterans General Hospital, Taiwan.)

24. Chang CH, Yang CM, Yang AH. 2007a. Renal diagnosis of chronic hemodialysis patients with urinary tract transitional cell carcinoma in Taiwan. Cancer 109(8): 1487-92. (Support not reported. Authors affiliated with Shin-Kong Wu Ho-Su Memorial Hospital, Taiwan; Fu Jen Catholic University, Taiwan; Veterans General Hospital - Taipei, Taiwan.)

25. Chang HR, Lian JD, Lo CW, Chang YC, Yang MY, Wang CJ. 2006. Induction of urothelial proliferation in rats by aristolochic acid through cell cycle progression via activation of cyclin D1/cdk4 and cyclin E/cdk2. Food Chem Toxicol 44(1): 28-35. (Supported by Chung Shan Medical University Research Fund. Authors afilliated with Chung Shan Medical University Hospital, Taiwan; Chung Shan Medical University, Taiwan.)

26. Chang HR, Lian JD, Lo CW, Huang HP, Wang CJ. 2007b. Aristolochic acid-induced cell cycle G1 arrest in human urothelium SV-HUC-1 cells. Food Chem Toxicol 45(3): 396-402. (Supported by Chung Shan Medical University Research Fund. Authors affiliated with Chung-Shan Medical University Hospital, Taiwan.)

27. ChemIDPlus. 2004a. Aristolochic Acid. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "aristolochic acid I"). Last accessed: 1/06/04.

28. ChemIDPlus. 2004b. Aristolochic Acid II. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "aristolochic acid II"). Last accessed: 1/06/04.

29. ChemSources. 2006. Aristolochic acid. Chemical Sources International, Inc. http://www.chemsources.com. Last accessed: 3/9/04.

30. Chen JK, Chen TT. 2004b. Chinese Medical Herbology and Pharmacology, City of Industry, CA: Art of Medicine Press, Inc. (Support and author affiliations not reported.)

31. Chen L, Mei N, Yao L, Chen T. 2006b. Mutations induced by carcinogenic doses of aristolochic acid in kidney of Big Blue transgenic rats. Toxicol Lett 165(3): 250-6. (Supported by U.S. Department of Energy; U.S. Food and Drug Administration and National Natural Science Foundation of China. Authors

affiliated with U.S. Food and Drug Administration; Shanghai Jiao Tong University, China.)

32. Chen M, Su M, Zhao L, Jiang J, Liu P, Cheng J, Lai Y, Liu Y, Jia W. 2006a. Metabonomic study of aristolochic acid-induced nephrotoxicity in rats. J Proteome Res 5(4): 995-1002. (Supported by Shanghai Leading Academic Discipline Project. Authors affiliated with Shanghai Jiao Tong University, China; Shanghai University of Traditional Chinese Medicine, China; Shaghai Institute for Systems Biology, China.)

33. Chen SM, Fan MY, Tseng CC, Ho Y, Hsu KY. 2007a. Pharmacokinetics and nephrotoxicity of aristolochic acid in rabbits. Toxicon 50(2): 180-8. (Support not reported. Authors affiliated with Taipei Medical University, Taiwan.)

34. Chen T, Guo L, Zhang L, Shi L, Fang H, Sun Y, Fuscoe JC, Mei N. 2006c. Gene Expression Profiles Distinguish the Carcinogenic Effects of Aristolochic Acid in Target (Kidney) and Non-target (Liver) Tissues in Rats. BMC Bioinformatics 7 Suppl 2: S20. (Support not reported. Authors affiliated with U.S. Food and Drug Administration; Applied Biosystems, USA; Z-Tech Corporation, USA; Solexa, Inc., USA.)

35. Chen W, Chen Y, Li A. 2001. [The clinical and pathological manifestations of aristolochic acid nephropathy--the report of 58 cases]. Zhonghua Yi Xue Za Zhi 81(18): 1101-1105. (Support not reported. Authors affiliated with China-Japan Friendship Hospital, China.)

36. Cheng CL, Chen KJ, Shih PH, Lu LY, Hung CF, Lin WC, Yesong Gu J. 2006. Chronic renal failure rats are highly sensitive to aristolochic acids, which are nephrotoxic and carcinogenic agents. Cancer Lett 232(2): 236-42. (Supported by Tunghai University and Taichung Veterans General Hospital, China. Authors affiliated with Tunghai University, China; Taichung Veterans General Hospital, China.)

37. Cheung TP, Xue C, Leung K, Chan K, Li CG. 2006. Aristolochic acids detected in some raw chinese medicinal herbs and manufactured herbal products - a consequence of inappropriate nomenclature and imprecise labelling? Clin Toxicol (Phila) 44(4): 371-8. (Support not reported. Authors affiliated with RMIT University, Australia; Hong Kong Baptist University, China.)

38. Cosyns JP, Jadoul M, Squifflet JP, De Plaen JF, Ferluga D, van Ypersele de Strihou C. 1994a. Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? Kidney Int 45(6): 1680-1688. (Support not reported. Authors affiliated with University of Louvain Medical School, Belgium; University of Ljubljana, Slovenia.)

39. Cosyns JP, Jadoul M, Squifflet JP, Van Cangh PJ, van Ypersele de Strihou C. 1994b. Urothelial malignancy in nephropathy due to Chinese herbs. Lancet

344(8916): 188. (Support not reported. Authors affiliated with University of Louvain Medical School, Belgium.)

40. Cosyns JP, Goebbels RM, Liberton V, Schmeiser HH, Bieler CA, Bernard AM. 1998. Chinese herbs nephropathy-associated slimming regimen induces tumours in the forestomach but no interstitial nephropathy in rats. Arch Toxicol 72(11): 738-743. (Support not reported. Authors affiliated with Catholic University of Louvain Medical School, Belgium; German Cancer Research Center, Germany.)

41. Cosyns JP, Jadoul M, Squifflet JP, Wese FX, van Ypersele de Strihou C. 1999. Urothelial lesions in Chinese-herb nephropathy. Am J Kidney Dis 33(6): 1011-1017. (Support not reported. Authors affiliated with Université Catholique de Louvain, Belgium.)

42. Cosyns JP, Dehoux JP, Guiot Y, Goebbels RM, Robert A, Bernard AM, van Ypersele de Strihou C. 2001. Chronic aristolochic acid toxicity in rabbits: a model of Chinese herbs nephropathy? Kidney Int 59(6): 2164-2173. (Support not reported. Authors affiliated with Université Catholique de Louvain, Belgium.)

43. Cosyns JP. 2002a. When is "aristolochic acid nephropathy" more accurate than "Chinese herbs nephropathy"? Kidney Int 61(3): 1178. (Support not reported. Authors affiliated with Université Catholique de Louvain, Belgium.)

44. Cosyns JP. 2003. Aristolochic acid and 'Chinese herbs nephropathy': a review of the evidence to date. Drug Saf 26(1): 33-48. (The author stated that the research was not supported by public or private grants. Authors affiliated with Université Catholique de Louvain, Belgium.)

45. CR. 2004. Dangerous Supplements - Still at Large. Consumer Reports. http://www.consumerreports.org/cro/health-fitness/drugs-supplements/dangerous-supplements-504/overview/. Last accessed on 11/09/07.

46. Cronin AJ, Maidment G, Cook T, Kite GC, Simmonds MS, Pusey CD, Lord GM. 2002. Aristolochic acid as a causative factor in a case of Chinese herbal nephropathy. Nephrol Dial Transplant 17(3): 524-525. (Supported by The Medical Research Council, UK. Authors affiliated with Wexham Park Hospital, UK; Hammersmith Hospital, UK; Royal Botanic Gardens, UK.)

47. Cui M, Liu ZH, Qiu Q, Li H, Li LS. 2005. Tumour induction in rats following exposure to short-term high dose aristolochic acid I. Mutagenesis (Pre-publication). (Support not reported. Authors affiliated with Nanjing University School of Medicine, China.)

48. de Jonge H, Vanrenterghem Y. 2008. Aristolochic acid: the common culprit of Chinese herbs nephropathy and Balkan endemic nephropathy. Nephrol Dial Transplant 23(1): 39-41. (Support not reported. Authors affiliated with University Hospital Gasthuisberg, Belgium.)

49. Debelle F, Nortier J, Arlt VM, De Prez E, Vienne A, Salmon I, Phillips DH, Deschodt-Lanckman M, Vanherweghem JL. 2003. Effects of dexfenfluramine on aristolochic acid nephrotoxicity in a rat model for Chinese-herb nephropathy. Arch Toxicol 77(4): 218-226. (Supported by the Groupement pour l'Etude, le Traitement et la Réhabilitation Sociale des Insuffisants Rénaux Chroniques, the Fonds de la Recherche Scientifique Médicale (Belgium), the Fondation Erasme (Hôpital Erasme, Brussels, Belgium) and the Cancer Research UK. Authors affiliated with Université Libre de Bruxelles, Belgium; Institute of Cancer Research, UK.)

50. Debelle FD, Nortier JL, De Prez EG, Garbar CH, Vienne AR, Salmon IJ, Deschodt-Lanckman MM, Vanherweghem JL. 2002. Aristolochic acids induce chronic renal failure with interstitial fibrosis in salt-depleted rats. J Am Soc Nephrol 13(2): 431-436. (Supported by the Groupement pour l'Etude, le Traitement et la Réhabilitation Sociale des Insuffisants Rénaux Chroniques, the Fonds de la Recherche Scientifique Médicale (Belgium), the Fondation Erasme (Hôpital Erasme, Brussels, Belgium) and the Cancer Research UK. Authors affiliated with Université Libre de Bruxelles, Belgium.)

51. Debelle FD, Nortier JL, Husson CP, De Prez EG, Vienne AR, Rombaut K, Salmon IJ, Deschodt-Lanckman MM, Vanherweghem JL. 2004. The renin-angiotensin system blockade does not prevent renal interstitial fibrosis induced by aristolochic acids. Kidney Int 66(5): 1815-1825. (Supported by the Groupement pour l'Etude, le Traitement et la Réhabilitation Sociale des Insuffisants Rénaux Chroniques, the Fonds de la Recherche Scientifique Médicale (Belgium), the Fondation Erasme (Hôpital Erasme, Brussels, Belgium) and the Cancer Research UK. Authors affiliated with Université Libre de Bruxelles, Belgium.)

52. Debelle FD, Vanherweghem JL, Nortier JL. 2008. Aristolochic acid nephropathy: a worldwide problem. Kidney Int 74(2): 158-69. (Supported by the Groupment pour l'Etrude, le Traitment et la Réhabilitation Sociale des Insuffisants Rénaux Chroniques, the Fonds de la Recherche Scientifique Médicale and the Fondation Erasme. Authors affiliated with Erasme Hospital, Belgium; Université Libre de Bruxelles, Belgium.)

53. Depierreux M, Van Damme B, Vanden Houte K, Vanherweghem JL. 1994. Pathologic aspects of a newly described nephropathy related to the prolonged use of Chinese herbs. Am J Kidney Dis 24(2): 172-180. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium; Katholieke Universiteit, Belgium; Universitair Ziekenhuis Brugmann, Belgium.)

54. Dharmananda S. 2001. Are Aristolochia Plants Dangerous? Institute for Traditional Medicine. http://www.itmonline.org/arts/aristolochia.htm. Last accessed: 3/14/06. (Support not reported. Authors affiliated with Institute for Traditional Medicine, USA.)

55. Dong H, Suzuki N, Torres MC, Bonala RR, Johnson F, Grollman AP, Shibutani S. 2006. Quantitative determination of aristolochic Acid-derived DNA adducts in rats using 32p-postlabeling/polyacrylamide gel electrophoresis analysis. Drug Metab Dispos 34(7): 1122-7. (Supported by School of Medicine, SUNY Stone Brook and NIEHS. Authors affiliated with State University of New York at Stony Brook.)

56. Dumic A. 1954. Horse Poisoning with Aristolochia clematitis, Zagreb: Izdanje Vojno tehnickog glasnika. p. 3-45. (Support and affiliation unknown. As cited in Grollman *et al.* 2007)

57. EFSA. 2006. Opinion of the Scientific Panel on contaminants in the Food Chain on a Request from the Commission Related to Ochratoxin A in Food. Question No. EFSA-Q-2005-154. 56 pp.

58. EMEA. 2000. Position paper on the risks associated with the use of herbal products containing Aristolochia species. European Agency for the Evaluation of Medicinal Products. http://www.emea.eu.int/pdfs/human/hmpwp/002300en.pdf. Last accessed: 3/9/04.

59. FDA. 1995. Dietary Supplement Health and Education Act of 1994. U.S. Food and Drug Administration. http://www.cfsan.fda.gov/~dms/dietsupp.html. Last accessed: 7/1/06.

60. FDA. 2000. Letter to health care professionals - FDA concerned about botanical products, including dietary supplements, containing aristolochic acid. U.S. Food and Drug Administration. Last updated: 4/1/01. http://vm.cfsan.fda.gov/~dms/ds-botl2.html. Last accessed: 5/18/04.

61. FDA. 2001a. Letter to industry associations regarding safety concerns related to the use of botanical products containing aristolochic acid. U.S. Food and Drug Administration. Last updated: 8/3/01. http://www.cfsan.fda.gov/~dms/ds-botl4.html. Last accessed: 5/18/04.

62. FDA. 2001b. Listing of Botanical Ingredients of Concern. U.S. Food and Drug Administration. Last updated 4/9/01. http://www.cfsan.fda.gov/~dms/ds-bot2.html. Last accessed: 5/5/05.

63. FDA. 2001c. FDA Warns Consumers to Discontinue Use of Botanical Products that Contain Aristolochic Acid. U.S. Food and Drug Administration. http://www.cfsan.fda.gov/~dms/addsbot.html. Last accessed: 10/25/05.

64. FDA. 2001h. Enforcement Report: Recalls and Field Corrections: Foods -- Class I. U.S. Food and Drug Administration. http://www.fda.gov/bbs/topics/ENFORCE/2001/ENF00724.html. Accessed on 8/27/08.

65. FDA. 2001i. Enforcement Report: Recalls and Field Corrections - Class I. U.S. Food and Drug Administration. http://www.fda.gov/bbs/topics/ENFORCE/2001/ENF00715.html. Accessed 8/27/08.

66. FDA. 2001j. Pacific Biologic Recalls Seven Herbal Products That Contain the Herb Akebia Trifoliata Caulis (Mu Tong) and the Herb Asarum Sieboldii Herba Cum Radix (Xi Xin) Because of a Possible Health Risk. U.S. Food and Drug Administration. http://www.fda.gov/oc/po/firmrecalls/pacificbio8_01.html.

67. FDA. 2005. Enforcement Report: Recalls And Field Corrections: Foods And Cosmetics -- Class U.S. Food and Drug Administration. http://www.fda.gov/bbs/topics/enforce/2005/ENF00915.html. Accessed on 8/27/08.

68. FDA. 2008. Herbal Science International, Inc. Recalls Twelve Dietary Herbal Supplements Nationwide Because of Possible Health Risk Associated with Ephedra, Aristolochic Acid and Human Placenta. U.S. Food and Drug Administration. http://www.fda.gov/oc/po/firmrecalls/herbalscience04_08.html. Accessed on 8/27/08.

69. Feldmeyer N, Schmeiser HH, Muehlbauer KR, Belharazem D, Knyazev Y, Nedelko T, Hollstein M. 2006. Further studies with a cell immortalization assay to investigate the mutation signature of aristolochic acid in human p53 sequences. Mutat Res 608(2): 163-8. (Supported by the Association for International Cancer Research, UK. Authors affiliated with German Cancer Research Center, Germany.)

70. Fernando RC, Schmeiser HH, Nicklas W, Wiessler M. 1992. Detection and quantitation of dG-AAI and dA-AAI adducts by ^{32}P-postlabeling methods in urothelium and exfoliated cells in urine of rats treated with aristolochic acid I. Carcinogenesis 13(10): 1835-1839. (Support not reported. Authors affiliated with German Cancer Research Center, Germany.)

71. Fernando RC, Schmeiser HH, Scherf HR, Wiessler M. 1993. Formation and persistence of specific purine DNA adducts by ^{32}P-postlabelling in target and non-target organs of rats treated with aristolochic acid I. In Postlabelling Methods for Detection of DNA Adducts, IARC Scientific Publications No. 124. Phillips DH, Castegnaro M, Bartsch H, eds. Lyon, France: International Agency for Research on Cancer. p. 167-71. (Support not reported. Authors affiliated with German Cancer Research Center, Germany.)

72. Flurer RA, Jones MB, Vela N, Ciolino LA, Wolnick KA. 2001. Determination of Aristolochic Acid in Traditional Chinese Medicines and Dietary Supplements. DFS/ORO/ORA No. 4212. U. S. Food and Drug Administration. 13 pp.

73. Frei H, Würgler FE, Juon H, Hall CB, Graf U. 1985. Aristolochic acid is mutagenic and recombinogenic in Drosophila genotoxicity tests. Arch Toxicol 56(3): 158-166. (Supported by Swiss National Science Foundation and the Julius Klaus Stiftung. Authors affiliated with Swiss Federal Institute of Technology, Switzerland; University of Zurich, Switzerland.)

74. Furihata C, Yamawaki Y, Jin SS, Moriya H, Kodama K, Matsushima T, Ishikawa T, Takayama S, Nakadate M. 1984. Induction of unscheduled DNA synthesis in rat stomach mucosa by glandular stomach carcinogens. J Natl Cancer Inst 72(6): 1327-1334. (Supported by the Ministry of Education, Science and Culture, Japan; Ministry of Health and Welfare, Japan; and the Society for Promotion of Cancer Research. Authors affiliated with University of Tokyo, Japan; Tientsin Institute of Materia Medica, China; Cancer Institute, Japan; National Institute of Hygienic Sciences, Japan.)

75. Gautier J, Richoz J, Welti DH, Markovic J, Gremaud E, Guengerich FP, Turesky RJ. 2001. Metabolism of ochratoxin A: absence of formation of genotoxic derivatives by human and rat enzymes. Chem Res Toxicol 14(1): 34-45. (Support not reported. Authors affiliated with Nestec Ltd., Switzerland; Vanderbilt University School of Medicine, TN.)

76. Genega EM, Porter CR. 2002. Urothelial neoplasms of the kidney and ureter. An epidemiologic, pathologic, and clinical review. Am J Clin Pathol 117 Suppl: S36-48. (Support not reported. Authors affiliated with University of Pennsylvania Medical Center, PA; Stony Brook University Hospital and Medical Center, NY.)

77. Gillerot G, Jadoul M, Arlt VM, van Ypersele De Strihou C, Schmeiser HH, But PPH, Bieler CA, Cosyns JP. 2001. Aristolochic acid nephropathy in a Chinese patient: time to abandon the term "Chinese herbs nephropathy"? Am J Kidney Dis 38(5): E26. (Support not reported. Authors affiliated with Université Catholique de Louvain, Belgium; German Cancer Research Center, Germany; Chinese University of Hong Kong, China.)

78. Glaser KB, Sung ML, Hartman DA, Lock YW, Bauer J, Walter T, Carlson RP. 1995. Cellular and topical in vivo inflammatory murine models in the evaluation of inhibitors of phospholipase A2. Skin Pharmacol 8(6): 300-8. (Support not reported. Authors affiliated with Wyeth-Ayerst Research, NJ; Abbott Labs, IL.)

79. Gold LS. 2003. L.S. Gold, Carcinogenic Potency Project, University of California, letter to Dr. C. L. Taylor and Dr. Robert Moore, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition, College Park, MD dated June 24, 2003. http://potency.berkeley.edu/pdfs/FDAAAJuneLetter.pdf.

80. Gold LS, Slone TH. 2003a. L.S. Gold, Carcinogenic Potency Project, University of California, letter to Dr. C. L. Taylor, Director, Office of Nutritional Products,

Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition, College Park, MD dated March 4, 2003. http://potency.berkeley.edu/pdfs/FDAAristolochicAcidLetter.pdf.

81. Gold LS, Slone TH. 2003b. Aristolochic acid, an herbal carcinogen, sold on the Web after FDA alert. N Engl J Med 349(16): 1576-1577. (Support not reported. Authors affiliated with University of California, Berkeley.)

82. Götzl E, Schimmer O. 1993. Mutagenicity of aristolochic acids (I, II) and aristolic acid I in new YG strains in Salmonella typhimurium highly sensitive to certain mutagenic nitroarenes. Mutagenesis 8(1): 17-22. (Support not reported. Authors affiliated with Universität Erlangen-Nürnberg, FRG.)

83. Grollman AP, Jelakovic B. 2007. Role of environmental toxins in endemic (Balkan) nephropathy. October 2006, Zagreb, Croatia. J Am Soc Nephrol 18(11): 2817-23. (Supported by NIEHS, Fogarty International Center and the Croatian Ministries of Science and Health. Authors affiliated with Stony Brook University, NY; Zagreb University School of Medicine and University Hospital Center, Croatia.)

84. Grollman AP, Shibutani S, Moriya M, Miller F, Wu L, Moll U, Suzuki N, Fernandes A, Rosenquist T, Medverec Z, Jakovina K, Brdar B, Slade N, Turesky RJ, Goodenough AK, Rieger R, Vukelic M, Jelakovic B. 2007. Aristolochic acid and the etiology of endemic (Balkan) nephropathy. Proc Natl Acad Sci U S A 104(29): 12129-34. (Supported by NIEHS, Fogarty International Center and the Croatian Ministry of Science. Authors affiliated with Stony Brook University, NY; Josip Benčević General Hospital, Croatia; Roche Molecular Systems, CA; Institute Rudjer Bošković, Croatia; New York State Department of Health; Zagreb University School of Medicine, Croatia; Croatian Center for Endemic Nephropathy, Croatia.)

85. Hadjiolov D, Fernando RC, Schmeiser HH, Wießler M, Hadjiolov N, Pirajnov G. 1993. Effect of diallyl sulfide on aristolochic acid-induced forestomach carcinogenesis in rats. Carcinogenesis 14(3): 407-410. (Support not reported. Authors affiliated with National Center of Oncology, Bulgaria; German Cancer Research Center, Germany.)

86. Hanna GM. 2004. NMR regulatory analysis: determination and characterization of Chinese-herb aristolochic acids. Pharmazie 59(3): 170-174. (Support not reported. Authors affiliated with the U.S. Food and Drug Administration.)

87. Hashimoto K, Higuchi M, Makino B, Sakakibara I, Kubo M, Komatsu Y, Maruno M, Okada M. 1999. Quantitative analysis of aristolochic acids, toxic compounds, contained in some medicinal plants. J Ethnopharm 64: 185-189. (Support not reported. Authors affiliated with Tsumura and Company, Japan; Toyama Medical and Pharmaceutical University, Japan.)

88. Ho JAA, Kuo HY, Wang YT, Yang CS. 2006. Occurrence of aristolochic acids in over-the-counter Chinese prepared medicines. Asian J Chem 18(1): 253-258. (Supported by the Taichung Veterans General Hospital and the National Chi-Nan University Joint Research Program. Authors affiliated with National Chi-Nan University, Taiwan; Taichung Veterans General Hospital, Taiwan; National Tsing Hua University, Taiwan.)

89. Hong L, Sakagami Y, Marumo S, Xinmin C. 1994. Eleven aristolochic acid derivatives from Aristolochia cinnabarina. Phytochemistry 37(1): 237-239. (Support not reported. Authors affiliated with Nagoya University, Japan; Chengdu Institute of Biology, China.)

90. Hong YT, Fu LS, Chung LH, Hung SC, Huang YT, Chi CS. 2006. Fanconi's syndrome, interstitial fibrosis and renal failure by aristolochic acid in Chinese herbs. Pediatr Nephrol 21(4): 577-579. (Support not reported. Authors affiliated with Taichung Veterans General Hospital, Taiwan; Chi Mei Hospital, Taiwan.)

91. Hranjec T, Kovac A, Kos J, Mao W, Chen JJ, Grollman AP, Jelakovic B. 2005. Endemic nephropathy: the case for chronic poisoning by aristolochia. Croat Med J 46(1): 116-125. (Supported by the NIEHS and Stony Brook University. Authors affiliated with State University of Stony Brook, USA; Zagreb University School of Medicine, Croatia.)

92. Hsieh SC, Huang MF, Lin BS, Chang HT. 2006. Determination of aristolochic acid in Chinese herbal medicine by capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr A 1105(1-2): 127-34. (Supported by Shin Kong Wu Ho-Su Memorial Hospital and the National Science Council of the Republic of China. Authors affiliated with Shin Kong Wu Ho-Su Memorial Hospital, Taiwan; Taipei medical University, Taiwan; National Taiwan University, Taiwan; National Yang-Ming University, Taiwan.)

93. Hsin YH, Cheng CH, Tzen JT, Wu MJ, Shu KH, Chen HC. 2006. Effect of aristolochic acid on intracellular calcium concentration and its links with apoptosis in renal tubular cells. Apoptosis 11(12): 2167-77. (Supported by Taichuing Veterans General Hospital and the National Chung Hsing University. Authors affiliated with National Chung Hsing University and Taichuing Veterans General Hospital, Taiwan.)

94. Hu SL, Zhang HQ, Chan K, Mei QX. 2004. Studies on the toxicity of Aristolochia manshuriensis (Guanmuton). Toxicology 198(1-3): 195-201. (Supported by Hong Kong Baptist University. Authors affiliated with China Academy of Traditional Chinese Medicine, China; Hong Kong Baptist University, China; Zhongshan Hospital of Traditional Chinese Medicine, China.)

95. Hwang MS, Park MS, Moon JY, Lee JS, Yum YN, Yoon E, Lee H, Nam KT, Lee BM, Kim SH, Yang KH. 2006. Subchronic toxicity studies of the aqueous

extract of Aristolochiae fructus in Sprague-Dawley rats. J Toxicol Environ Health A 69(24): 2157-65. (Support not reported. Authors affiliated with Korea Food and Drug Administration; Sungkyunkwan University, South Korea.)

96. IARC. 1993. Some Naturally Occurring Substances; Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. vol. 56, Lyon, France: International Agency for Research on Cancer. 599 pp.

97. IARC. 2002. Some Traditional Herbal Medicines, Some Mycotoxins, Napthalene and Styrene, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. vol. 82, Lyon, France: International Agency for Research on Cancer. p. 69-128.

98. Ioset JR, Raoelison GE, Hostettmann K. 2003. Detection of aristolochic acid in Chinese phytomedicines and dietary supplements used as slimming regimens. Food Chem Toxicol 41(1): 29-36. (Supported by the Swiss National Science Foundation. Authors affiliated with Université de Lausanne, Switzerland; Université d'Antananarivo, Madagascar.)

99. Ivic M. 1970. The problem of etiology of endemic nephropathy. Acta Fac Med Naiss 1: 29-37. (Supported by the Union of Medical Scientific Institutions of SR Serbia. Authors affiliated with the Institute of Histology, Niš.)

100. Izumotani T, Ishimura E, Tsumura K, Goto K, Nishizawa Y, Morii H. 1993. An adult case of Fanconi syndrome due to a mixture of Chinese crude drugs. Nephron 65(1): 137-140. (Support not reported. Authors affiliated with Osaka City University Medical School, Japan.)

101. Jackson L, Kofman S, Weiss A, Brodovsky H. 1964. Aristolochic acid (NSC-50413): Phase I clinical study. Cancer Chemother Rep 42: 35-37. (Support not reported. Authors affiliated with Jefferson Medical College Hospital and Presbyterian-St. Luke's Hospital, USA.)

102. Jiménez-Ferrer JE, Pérez-Terán YY, Román-Ramos R, Tortoriello J. 2005. Antitoxin activity of plants used in Mexican traditional medicine against scorpion poisoning. Phytomedicine 12(1-2): 116-122. (Supported by Coordinación de Investigación en Salud, IMSS and CONACyT. Authors affiliated with Centro de Investigación Biomédica del Sur and Universidad Autónoma Metropolitana, Mexico.)

103. Jong TT, Lee MR, Hsiao SS, Hsai JL, Wu TS, Chiang ST, Cai SQ. 2003. Analysis of aristolochic acid in nine sources of Xixin, a traditional Chinese medicine, by liquid chromatography/atmospheric pressure chemical ionization/tandem mass spectrometry. J Pharm Biomed Anal 33(4): 831-837. (Supported by the National Science Council, ROC. Authors affiliated with the National Chung-Hsin University, Taiwan; National Cheng Kung University,

Taiwan; Chuang Song Zong Pharmaceutical Co. Ltd., Taiwan; Beijing University, China.)

104. Kamp HG, Eisenbrand G, Janzowski C, Kiossev J, Latendresse JR, Schlatter J, Turesky RJ. 2005. Ochratoxin A induces oxidative DNA damage in liver and kidney after oral dosing to rats. Mol Nutr Food Res 49(12): 1160-7. (Supported by the Swiss Federal Office of Public Health. Authors affiliated with University of Kaiserslauten, Germany; FDA; Toxicologic Pathology Associates, AR; Swiss Federal Office of Public Health, Switzerland.)

105. Kanaan N, Cosyns JP, Jadoul M, Goffin E. 2003. The importance of a histology-based diagnosis of interstitial nephropathy in two patients with renal insufficiency. Nephrol Dial Transplant 18(2): 440-2. (Support not reported. Authors affiliated with Université Catholique de Louvain, Belgium.)

106. Karmaus W, Dimitrov P, Simeonov V, Tsolova S, Bonev A, Georgieva R. 2008. Metals and kidney markers in adult offspring of endemic nephropathy patients and controls: a two-year follow-up study. Environ Health 7: 11. (Supported by NIH, the Fogarty International Center and NIEHS. Authors affiliated with University of South Carolina, SC; National Center of Public Health Protection, Bulgaria; Vratza District Hospital, Bulgaria.)

107. Kazama I, Matsubara M, Michimata M, Suzuki M, Hatano R, Sato H, Ito S. 2004. Adult onset Fanconi syndrome: extensive tubulo-interstitial lesions and glomerulopathy in the early stage of Chinese herbs nephropathy. Clin Exp Nephrol 8(3): 283-287. (Support not reported. Authors affiliated with Tohoku University Graduate School of Medicine, Japan.)

108. Kessler DA. 2000. Cancer and herbs. N Engl J Med 342(23): 1742-1743. (Support not reported. Authors affiliated with Yale University School of Medicine.)

109. Kevekordes S, Mersch-Sundermann V, Burghaus CM, Spielberger J, Schmeiser HH, Arlt VM, Dunkelberg H. 1999. SOS induction of selected naturally occurring substances in Escherichia coli (SOS chromotest). Mutat Res 445(1): 81-91. (Support not reported. Authors affiliated with University of Göttingen, Germany; University of Heidelberg, Germany; German Cancer Research Center, Germany.)

110. Kevekordes S, Spielberger J, Burghaus CM, Birkenkamp P, Zietz B, Paufler P, Diez M, Bolten C, Dunkelberg H. 2001. Micronucleus formation in human lymphocytes and in the metabolically competent human hepatoma cell line Hep-G2: results with 15 naturally occurring substances. Anticancer Res 21(1A): 461-469. (Support not reported. Authors affiliated with University of Göttingen, Germany.)

111. Kite GC, Yule MA, Leon C, Simmonds MS. 2002. Detecting aristolochic acids in herbal remedies by liquid chromatography/serial mass spectrometry. Rapid

Commun Mass Spectrom 16(6): 585-590. (Support not reported. Authors affiliated with Royal Botanic Gardens, UK and University of Greenwich, UK.)

112. Koh HL, Wang H, Zhou S, Chan E, Woo SO. 2006. Detection of aristolochic acid I, tetrandrine and fangchinoline in medicinal plants by high performance liquid chromatography and liquid chromatography/mass spectrometry. J Pharm Biomed Anal 40(3): 653-61. (Supported by the National University of Singapore Academic Research Fund and a research scholarship (WHS). Authors affiliated with National University of Singapore, Singapore; Universiti Tunku Abdul Rahman, Malaysia.)

113. Kohara A, Suzuki T, Honma M, Ohwada T, Hayashi M. 2002. Mutagenicity of aristolochic acid in the lambda/lacZ transgenic mouse (Muta^{TM}Mouse). Mutat Res 515(1-2): 63-72. (Support not reported. Authors affiliated with National Institute of Health Sciences, Japan; Nagoya City University, Japan; University of Tokyo, Japan.)

114. Krumbiegel G, Hallensleben J, Mennicke WH, Rittman N, Roth HJ. 1987. Studies on the metabolism of aristolochic acids I and II. Xenobiotica 17: 981-991. (Support not reported. Authors affiliated with Dr. Madaus GmbH & Co., Germany and University of Tübingen, Germany.)

115. Krumme B, Endmeir R, Vanhaelen M, Walb D. 2001. Reversible Fanconi syndrome after ingestion of a Chinese herbal 'remedy' containing aristolochic acid. Nephrol Dial Transplant 16(2): 400-402. (Support not reported. Authors affiliated with Deutsche Klinik für Diagnostik, Germany; Institute de Pharmacie, Belgium.)

116. Kumar V, Poonam, Prasad AK, Parmar VS. 2003. Naturally occurring aristolactams, aristolochic acids and dioxoaporphines and their biological activities. Nat Prod Rep 20(6): 565-583. (Supported by the Council of Scientific and Industrial Research. Authors affiliated with University of Delhi, India.)

117. Kupchan SM, Doskotch RW. 1962. Tumor inhibitors. I. Aristolochic acid, the active principle of Aristolochia indica. J Med Pharm Chem 91: 657-659. (Supported by the National Cancer Institute. Authors affiliated with University of Wisconsin.)

118. Laing C, Hamour S, Sheaff M, Miller R, Woolfson R. 2006. Chinese herbal uropathy and nephropathy. Lancet 368(9532): 338. (Supported by the Department of Health of Hong Kong. Authors affiliated with Hong Kong Baptist University, China.)

119. Lebeau C, Arlt VM, Schmeiser HH, Boom A, Verroust PJ, Devuyst O, Beauwens R. 2001. Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells. Kidney Int 60(4): 1332-1342. (Supported by the FRSM, FNRS, ARC and the Foundation Alphonse et Jean Forton, Belgium. Authors affiliated with University of Brussels Medical School, Belgium;

Institute of Cancer Research, UK; German Cancer Research Center, Germany; INSERM, France; Université Catholique de Louvain Medical School, Belgium.)

120. Lebeau C, Debelle FD, Arlt VM, Pozdzik A, De Prez EG, Phillips DH, Deschodt-Lanckman MM, Vanherweghem JL, Nortier JL. 2005. Early proximal tubule injury in experimental aristolochic acid nephropathy: functional and histological studies. Nephrol Dial Transplant 20(11): 2321-32. (Supported by Fonds de la Recherche Scientifique Médicale and the Banque Nationale, Belgium and Cancer Research, UK. Authors affiliated with Université Libre de Bruxelles, Belgium; Institute of Cancer Research, UK.)

121. Lee S, Lee T, Lee B, Choi H, Yang M, Ihm CG, Kim M. 2004. Fanconi's syndrome and subsequent progressive renal failure caused by a Chinese herb containing aristolochic acid. Nephrology (Carlton) 9(3): 126-129. (Support not reported. Authors affiliated with East-West Kidney Disease Research Institute and KyungHee University, Korea.)

122. Lee TY, Wu ML, Deng JF, Hwang DF. 2001. High-performance liquid chromatographic determination for aristolochic acid in medicinal plants and slimming products. J Chromatogr B Analyt Technol Biomed Life Sci 766(1): 169-174. (Supported by the Department of Health, Taiwan. Authors affiliated with National Taiwan Ocean University, Taiwan; Veterans Division of General Hospital, Taiwan; National Yang-Ming University, Taiwan.)

123. Lemy A, Wissing KM, Rorive S, Zlotta A, Roumeguere T, Muniz Martinez MC, Decaestecker C, Salmon I, Abramowicz D, Vanherweghem JL, Nortier J. 2008. Late onset of bladder urothelial carcinoma after kidney transplantation for end-stage aristolochic acid nephropathy: a case series with 15-year follow-up. Am J Kidney Dis 51(3): 471-7. (Support not reported. Authors affiliated with Universite Libre de Bruxelles, Belgium.)

124. Li W, Gong S, Wen D, Che B, Liao Y, Liu H, Feng X, Hu S. 2004a. Rapid determination of aristolochic acid I and II in Aristolochia plants from different regions by â-cyclodextrin-modified capillary zone electrophoresis. J Chromatogr A 17: 211-217. (Supported by NSFC. Authors affiliated with Peking University, China; Beijing Institute for Drug Control, China; China Academy of Traditional Chinese Medicine, China.)

125. Li W, Li R, Bo T, Liu H, Feng X, Hu S. 2004b. Rapid determination of aristolochic acids I and II in some medical plants by high performance liquid chromatography. Chromatographia 59: 233-236. (Supported by NSFC. Authors affiliated with Peking University, China; China Academy of Traditional Chinese Medicine, China.)

126. Li W, Chen Z, Liao Y, Liu H. 2005a. Separation methods for toxic components in Traditional Chinese Medicines. Anal Sci 21(9): 1019-29. (Supported by NSFC. Authors affiliated with Peking University, China.)

127. Li WH, Yang L, Su T, Song Y, Li XM. 2005b. [Influence of taking aristolochic acid-containing Chinese drugs on occurrence of urinary transitional cell cancer in uremic uremic patients undergoing dialysis]. Zhonghua Yi Xue Za Zhi 85(35): 2487-91. (Support unknown (non-English article). Authors affiliated with Peking University First Hospital, China.)

128. Li X, Yang L, Yu Y. 2001. [An analysis of the clinical and pathological characteristics of mu-tong (a Chinese herb) induced tubulointerstitial nephropathy]. Zhonghua Nei Ke Za Zhi 40(10): 681-687. (Support unknown (non-English article). Authors affiliated with Peking University First Hospital, China.)

129. Li X, Wang H. 2004. Aristolochic acid nephropathy: what we know and what we have to do. Nephrology (Carlton) 9(3): 109-111. (Support not reported. Authors affiliated with Peking University First Hospital, China.)

130. Li XB, Xing NZ, Wang Y, Hu XP, Yin H, Zhang XD. 2008. Transitional cell carcinoma in renal transplant recipients: A single center experience. Int J Urol 15(1): 53-7. (Support not reported. Authors affiliated with Capital University of Medical Sciences, China.)

131. Li Y, Liu Z, Guo X, Shu J, Chen Z, Li L. 2006a. Aristolochic acid I-induced DNA damage and cell cycle arrest in renal tubular epithelial cells in vitro. Arch Toxicol 80(8): 524-32. (Support not reported. Authors affiliated with Nanjing University School of Medicine and the Second Xiangya Hospital, China.)

132. Liang ZT, Jiang ZH, Leung KSY, Chan CL, Zhao ZZ. 2006. Authentication and differentiation of two easily confusable Chinese materia medica: Herba Solani Lyrati and Herba Aristolochiae Mollissimae. J Food Drug Anal 14(1): 36-43. (Supported by the Department of Health of Hong Kong. Authors affiliated with Hong Kong Baptist University, China.)

133. Ling Z, Jiang ZZ, Huang X, Zhang LY, Xu XY. 2007. A liquid chromatography-mass spectrometry method for the quantitation of aristololactam-I in rat plasma. Biomed Chromatogr 21(1): 10-4. (Support not reported. Authors affiliated with China Pharmaceutical University, China.)

134. Liu MC, Maruyama S, Mizuno M, Morita Y, Hanaki S, Yuzawa Y, Matsuo S. 2003. The nephrotoxicity of Aristolochia manshuriensis in rats is attributable to its aristolochic acids. Clin Exp Nephrol 7(3): 186-194. (Supported by the Aichi Kidney Foundation. Authors affiliated with Nagoya University Graduate School of Medicine, Japan; Kahya Co. Ltd., Japan.)

135. Liu Z, Hergenhahn M, Schmeiser HH, Wogan GN, Hong A, Hollstein M. 2004. Human tumor p53 mutations are selected for in mouse embryonic fibroblasts harboring a humanized p53 gene. Proc Natl Acad Sci U S A 101(9): 2963-2968. (Support not reported. Authors affiliated with German Cancer Research Center, Germany; Massachusetts Institute of Technology, USA.)

136. Lo SH, Mo KL, Wong KS, Poon SP, Chan CK, Lai CK, Chan A. 2004. Aristolochic acid nephropathy complicating a patient with focal segmental glomerulosclerosis. Nephrol Dial Transplant 19(7): 1913-1915. (The authors declared that they have no personal or financial conflict of interest in publishing this report. Authors affiliated with Pamela Youde Nethersole Eastern Hospital and Princess Margaret Hospital, China.)

137. Lo SH, Wong KS, Arlt VM, Phillips DH, Lai CK, Poon WT, Chan CK, Mo KL, Chan KW, Chan A. 2005. Detection of Herba Aristolochia Mollissemae in a patient with unexplained nephropathy. Am J Kidney Dis 45(2): 407-410. (Support not reported. Authors affiliated with Pamela Youde Nethersole Eastern Hospital, China; Queen Mary Hospital, China; Princess Margaret Hospital, China; Institute of Cancer Research, UK.)

138. Long DT, Voice TC. 2007. Role of exposure analysis in solving the mystery of Balkan endemic nephropathy. Croat Med J 48(3): 300-11. (Supported by NIH. Authors affiliated with Michigan State University.)

139. Lord GM, Tagore R, Cook T, Gower P, Pusey CD. 1999. Nephropathy caused by Chinese herbs in the UK. Lancet 354(9177): 481-482. (Support not reported. Authors affiliated with Hammersmith and Charing Cross Hospitals NHS, UK.)

140. Lord GM, Cook T, Arlt VM, Schmeiser HH, Williams G, Pusey CD. 2001. Urothelial malignant disease and Chinese herbal nephropathy. Lancet 358(9292): 1515-1516. (Support not reported. Authors affiliated with Hammersmith Hospitals, UK; Institute of Cancer Research, UK; German Cancer Research Center, Germany.)

141. Lord GM, Hollstein M, Arlt VM, Roufosse C, Pusey CD, Cook T, Schmeiser HH. 2004. DNA adducts and p53 mutations in a patient with aristolochic acid-associated nephropathy. Am J Kidney Dis 43(4): e11-17. (Supported by Cancer Research UK and the Medical Research Council, UK. Authors affiliated with Hammersmith Hospital NHS, UK; Institute of Cancer Research, UK; German Cancer Research Center, Germany; Harvard School of Public Health, USA.)

142. Maier P, Schawalder HP, Weibel B, Zbinden G. 1985. Aristolochic acid induces 6-thioguanine-resistant mutants in an extrahepatic tissue in rats after oral application. Mutat Res 143(3): 143-148. (Supported by the Swiss National Science Foundation. Authors affiliated with the Swiss Federal Institute of Technology and University of Zürich, Switzerland.)

143. Maier P, Schawalder H, Weibel B. 1987. Low oxygen tension, as found in tissues in vivo, alters the mutagenic activity of aristolochic acid I and II in primary fibroblast-like rat cells in vitro. Environ Mol Mutagen 10(3): 275-284. (Supported by the Swiss National Science Foundation. Authors affiliated with the Swiss Federal Institute of Technology and University of Zürich, switzerland.)

144. Mally A, Zepnik H, Wanek P, Eder E, Dingley K, Ihmels H, Volkel W, Dekant W. 2004. Ochratoxin A: lack of formation of covalent DNA adducts. Chem Res Toxicol 17(2): 234-42. (Supported by the Fifth RTD Framework Program of the European Union, the Physiological Effects of Coffee Committee in France, the U.S. Department of Energy, NIH, the State of Bavaria and the Deutsche Forschungsgemeinschaft. Authors affiliated with Universitat Wurzburg, Germany; Lawrence Livermore National Laboratory, CA.)

145. Mally A, Hard GC, Dekant W. 2007. Ochratoxin A as a potential etiologic factor in endemic nephropathy: Lessons from toxicity studies in rats. Food Chem Toxicol 45(11): 2254-60. (Support not reported. Authors affiliated with University of Würzburg, Germany.)

146. Marcus DM, Grollman AP. 2002. Botanical medicines--the need for new regulations. N Engl J Med 347(25): 2073-6. (Support not reported. Authors affiliated with Baylor College of Medicine, TX; State University of New York, NY.)

147. Marple JT, MacDougall M. 1993. Development of malignancy in the end-stage renal disease patient. Semin Nephrol 13(3): 306-314. (Support not reported. Authors affiliated with University of Kansas and Veterans Affairs Medical Center, USA.)

148. Martena MJ, van der Wielen JC, van de Laak LF, Konings EJ, de Groot HN, Rietjens IM. 2007. Enforcement of the ban on aristolochic acids in Chinese traditional herbal preparations on the Dutch market. Anal Bioanal Chem 389(1): 263-75. (Support not reported. Authors affiliated with Food and Consumer Product Safety Authority, Netherlands; Wageningen University, Netherlands.

149. Martincevic M. 1957. Toxic action of Aristolochia clematitis on the kidney of horses. Veterinarski Arhiv 27: 51-59. (Support and affiliations unknown. Foreign article.)

150. Martinez MC, Nortier J, Vereerstraeten P, Vanherweghem JL. 2002. Progression rate of Chinese herb nephropathy: impact of Aristolochia fangchi ingested dose. Nephrol Dial Transplant 17(3): 408-412. (Suport not reported. Authors affiliated with Hôpital Erasme, Belgium.)

151. MCA. 2002. Safety of Herbal Medicine Products. United Kingdom: Medicines Control Agency. 39 pp. http://www.mhra.gov.uk/home/groups/es-herbal/documents/websiteresources/con009293.pdf.

152. McMillin DL, Nelson CD, Richards DG, Mein EA. 2003. Determination of Aristolochic Acid in Asarum canadense (Wild Ginger). Meridian Institute. http://www.meridianinstitute.com/reports/wgreport.html. Last accessed: 5/18/04. (Support not reported. Authors affiliated with the Meridian Institute.)

153. Mehes J, Decsi L, Varga F, Kovacs S. 1958. [Selective chemical disconnection of the uriniferous tubes of the first order in rabbits.]. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol 234(6): 548-65. (Support and affiliations unknown (non-English article.))

154. Mei N, Arlt VM, Phillips DH, Heflich RH, Chen T. 2006. DNA adduct formation and mutation induction by aristolochic acid in rat kidney and liver. Mutat Res 602(1-2): 83-91. (Supported by Cancer Research, UK and the Association for International Cancer Research. Authors affiliated with U.S. FDA and the Institute of Cancer Research, UK.)

155. Meinl W, Pabel U, Osterloh-Quiroz M, Hengstler JG, Glatt H. 2006. Human sulphotransferases are involved in the activation of aristolochic acids and are expressed in renal target tissue. Int J Cancer 118(5): 1090-7. (Supported by the Commission of the European Communities. Authors affiliated with German Institute of Human Nutrition, Germany; Institute of Legal Medicine and Rudolf Boehm Institute of Pharmacology and Toxicology, Germany.)

156. Mengs U, Lang W, Poch J-A. 1982. The carcinogenic action of aristolochic acid in rats. Arch Toxicol 51: 107-119. (Support not reported. Authors affiliated with Dr. Madaus GmbH & Co, Germany; Laboratorios Madaus Cerafarm, Spain.)

157. Mengs U. 1983. On the histopathogenesis of rat forestomach carcinoma caused by aristolochic acid. Arch Toxicol 52(3): 209-220. (Support not reported. Authors affiliated with Dr. Madaus & Co., Germany.)

158. Mengs U. 1987. Acute toxicity of aristolochic acid in rodents. Arch Toxicol 59(5): 328-331. (Support not reported. Authors affiliated with Dr. Madaus GmbH & Co., Germany.)

159. Mengs U. 1988. Tumour induction in mice following exposure to aristolochic acid. Arch Toxicol 61(6): 504-505. (Support not reported. Authors affiliated with Dr. Madaus GmbH & Co., Germany.)

160. Mengs U, Klein M. 1988. Genotoxic effects of aristolochic acid in the mouse micronucleus test. Planta Med 54(6): 502-503. (Support not reported. Authors affiliated with Dr. Madaus GmbH & Co., Germany.)

161. Mengs U, Stotzem CD. 1992. Toxicity of aristolochic acid - a subacute study in male rats. Med Sci Res 20: 223-224. (Support not reported. Authors affiliated with Madaus AG, Germany.)

162. Mengs U, Stotzem CD. 1993. Renal toxicity of aristolochic acid in rats as an example of nephrotoxicity testing in routine toxicology. Arch Toxicol 67(5): 307-311. (Support not reported. Authors affiliated with Madaus AG, Germany.)

163. Meyer MM, Chen TP, Bennett WM. 2000. Chinese herb nephropathy. Proc (Bayl Univ Med Cent) 13(4): 334-337. (Support not reported. Authors affiliated with Oregon Health Sciences University; Kuala Lumpar Hospital, Malaysia; Good Samaritan Hospital, Oregon.)

164. Mix DB, Guinaudeau H, Shamma M. 1982. The aristolochic acids and aristolactams. J Nat Prod 45(6): 657-666. (Support not reported. Authors affiliated with Pennsylvania State University, PA; Universite de Limoges, France; Centre d'Etudes Pharmaceutiques, France.)

165. Nesslany F, Zennouche N, Simar-Meintieres S, Talahari I, Nkili-Mboui EN, Marzin D. 2007. In vivo Comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds. Mutat Res 630(1-2): 28-41. (Support not reported. Authors affiliated with Institut Pasteur de Lille, France; Université de LILLE II, France.)

166. Ni Y, Su M, Qiu Y, Chen M, Liu Y, Zhao A, Jia W. 2007. Metabolic profiling using combined GC-MS and LC-MS provides a systems understanding of aristolochic acid-induced nephrotoxicity in rat. FEBS Lett 581(4): 707-11. (Supported by Shanghai Leading Academic Discipline Project. Authors affiliated with Shanhai University of Traditional Chinese Medicine, China; Shanghai Jiao Tong University, China.)

167. Nikolic J. 2006. Epidemic Nephropathy and Upper Urothelial Tumors, Belgrade, Serbia: Izdavacko Preduzece Beograd, A.D. (Support not reported. Author affiliated with Institut za urologiju i nefrologiju, Serbia.)

168. Nishimagi E, Kawaguchi Y, Terai C, Kajiyama H, Hara M, Kamatani N. 2001. Progressive interstitial renal fibrosis due to Chinese herbs in a patient with calcinosis Raynaud esophageal sclerodactyly telangiectasia (CREST) syndrome. Intern Med 40(10): 1059-63. (Support not reported. Authors affiliated with Tokyo Women's Medical University School of Medicine, Japan.)

169. Nortier JL, Martinez MC, Schmeiser HH, Arlt VM, Bieler CA, Petein M, Depierreux MF, De Pauw L, Abramowicz D, Vereerstraeten P, Vanherweghem JL. 2000. Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi). N Engl J Med 342(23): 1686-1692. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium; German Cancer Research Center, Germany.)

170. Nortier JL, Vanherweghem JL. 2002. Renal interstitial fibrosis and urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi). Toxicology 181-182: 577-80. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium.)

171. Nortier JL, Schmeiser HH, Muniz Martinez MC, Arlt VM, Vervaet C, Garbar CH, Daelemans P, Vanherweghem JL. 2003. Invasive urothelial carcinoma after exposure to Chinese herbal medicine containing aristolochic acid may occur

without severe renal failure. Nephrol Dial Transplant 18(2): 426-428. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium; German Cancer Research Center, Germany; Institute of Cancer Research, UK.)

172. Nortier JL, Vanherweghem JL. 2007. For patients taking herbal therapy--lessons from aristolochic acid nephropathy. Nephrol Dial Transplant 22(6): 1512-7. (The authors declare no conflicts of interest. Authors affiliated with Université de Libre Bruxelles, Belgium.)

173. NTP. 2004. Ochratoxin A. In Report on Carcinogens. 11th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. p. III-209-III210. http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s140ochr.pdf.

174. O'Neil MJ, Heckelman PE, Koch CB, Roman KJ, eds. 2006. Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 14th ed. Whitehouse Station, NJ: Merck & Co., Inc. p. 129.

175. Ou JH, Pan CC, Lin JS, Tzai TS, Yang WH, Chang CC, Cheng HL, Lin YM, Tong YC. 2000. Transitional cell carcinoma in dialysis patients. Eur Urol 37(1): 90-94. (Support not reported. Authors affiliated with National Cheng-Kung University Hospital, Taiwan; Provincial Tainan General Hospital, Taiwan.)

176. Pailer M, Bergthaller P, Schaden G. 1965. Über die Isolierung und Charakterisierung von vier neuen Aristolochiasäuren (aus Aristolochia clematitis L.). Mh Chem 96: 863-883. (Supported by Osterreichischen Akademie der Wissenschaften. Authors affiliated with Aus dem Organisch-Chemischen Institut der Universität Wien.)

177. Palma N, Cinelli S, Sapora O, Wilson SH, Dogliotti E. 2007. Ochratoxin A-induced mutagenesis in mammalian cells is consistent with the production of oxidative stress. Chem Res Toxicol 20(7): 1031-7. (Supported by the Fifth RTD Framework Program of the European Union, NIH and NIEHS. Authors affiliated with Istituto Superiore di Sanita', Italy; Research Toxicology Centre, Italy; NIEHS, NC.)

178. Pena JM, Borras M, Ramos J, Montoliu J. 1996. Rapidly progressive interstitial renal fibrosis due to a chronic intake of a herb (Aristolochia pistolochia) infusion. Nephrol Dial Transplant 11(7): 1359-60. (Support not reported. Authors affiliated with Hospital Universitari Arnau de Vilanova, Spain; Universitat de Lleida, Spain.)

179. Peraica M, Domijan AM, Saric M. 2008. Mycotoxic and aristolochic acid theories of the development of endemic nephropathy. Arh Hig Rada Toksikol 59(1): 59-65. (Support not reported. Authors affiliated with Institute for Medical Research and Occupational Health, Croatia.)

180. Petronic VJ, Bukurov NS, Djokic MR, Milenkovic DZ, Vuksanovic AM, Avramovic AD, Nale DP. 1991. Balkan endemic nephropathy and papillary transitional cell tumors of the renal pelvis and ureters. Kidney Int Suppl 34: S77-9. (Support not reported. Authors affiliated with Institute for Urology and Nephrology, Yugoslavia.)

181. Pezzuto JM, Swanson SM, Mar W, Che CT, Cordell GA, Fong HH. 1988. Evaluation of the mutagenic and cytostatic potential of aristolochic acid (3,4-methylenedioxy-8-methoxy-10-nitrophenanthrene-1-carboxylic acid) and several of its derivatives. Mutat Res 206(4): 447-454. (Support not reported. Authors affiliated with University of Illinois at Chicago.)

182. PFAF. 2005. Plants for a Future Database. Plants for a Future. http://www.ibiblio.org/pfaf/D_search.html. Last accessed: 5/5/05.

183. Pfau W, Schmeiser HH, Wiessler M. 1990b. ^{32}P-postlabelling analysis of the DNA adducts formed by aristolochic acid I and II. Carcinogenesis 11(9): 1627-1633. (Support not reported. Authors affiliated with German Cancer Research Center, Germany; Institute of Cancer Research, UK.)

184. Pfohl-Leszkowicz A, Manderville RA. 2007. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Mol Nutr Food Res 51(1): 61-99. (Support not reported. Authors afffiliated with Laboratoire de Génie Chimique, France; University of Guelph, Canada.)

185. Pfohl-Leszkowicz A, Tozlovanu M, Manderville R, Peraica M, Castegnaro M, Stefanovic V. 2007. New molecular and field evidences for the implication of mycotoxins but not aristolochic acid in human nephropathy and urinary tract tumor. Mol Nutr Food Res. (Supported by ARC, AUF, 'Ligue Nationale Française contre le cancer,' European Union (EU), Région Midi-Pyrénées, and the Concerted Action 'Pavle Savic' France-Serbia. Authors affiliated with Laboratoire Génie chimique, France; University of Guelph, Canada; Institute for Medical Research and Occupational Health, Croatia; Institute of Nephrology, Serbia.)

186. Pistelli L, Nieri E, Bilia AR, Marsili A, Scarpato R. 1993. Chemical constituents of Aristolochia rigida and mutagenic activity of aristolochic acid IV. J Nat Prod 56(9): 1605-1608. (Supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST). Authors affiliated with Università di Pisa, Italy.)

187. Pool BL, Eisenbrand G, Preussmann R, Schlehofer JR, Schmezer P, Weber H, Wiessler M. 1986. Detection of mutations in bacteria and of DNA damage and amplified DNA sequences in mammalian cells as a systematic test strategy for elucidating biological activities of chemical carcinogens. Food Chem Toxicol 24(6-7): 685-91. (Support not reported. Authors affiliated with German Cancer Research Centre, Germany; University of Kaiserslautern, Germany.)

188. Pourrat J, Montastruc JL, Lacombe JL, Cisterne JM, Rascol O, Dumazer P. 1994. [Nephropathy associated with Chinese herbal drugs. 2 cases]. Presse Med 23(36): 1669. (Support not reported. Authors affiliated with Unité Fonctionnelle de Néphrologie, France; Service de Pharmacologie Clinique et Centre Midi-Pyrénées de Pharmacovigilance, France; Clinique Néphrologique Saint-Exupéry, France.)

189. Pozdzik AA, Salmon IJ, Debelle FD, Decaestecker C, Van den Branden C, Verbeelen D, Deschodt-Lanckman MM, Vanherweghem JL, Nortier JL. 2007. Aristolochic acid induces proximal tubule apoptosis and epithelial to mesenchymal transformation. Kidney Int 73(5): 595-607. (Supported by the Erasme Foundation, Fonds National de la Recherche Scientifique Médicale, Groupement pour l'Étude, le Traitement, et la Réhabilitation Sociale de l'Insuffisant Rénal Chronique and the Onderzoeksraad van de Vrije Universiteit Brussel. Authors affiliated with Université Libre de Bruxelles, Belgium; Vrije Universiteit Brussel, Belgium.)

190. Priestap HA. 1985a. Seven aristololactams from Aristolochia argentina. Phytochemistry 24(4): 849-852. (Support not reported. Authors affiliated with Universidad de Buenos Aires, Argentina.)

191. Priestap HA. 2008. Horation Priestap, University of Argentina, public comments to the National Toxicology Program regarding the background document for Aristolochic Acid. http://ntp.niehs.nih.gov/files/Priestap.pdf.

192. Qi X, Cai Y, Gong L, Liu L, Chen F, Xiao Y, Wu X, Li Y, Xue X, Ren J. 2007. Role of mitochondrial permeability transition in human renal tubular epithelial cell death induced by aristolochic acid. Toxicol Appl Pharmacol 222(1): 105-10. (Supported by the National Grand Fundamental Research 973 Program of China. Authors affiliated with Chinese Academy of Sciences, China; Graduate School of the Chinese Academy of Sciences, China.)

193. Qiu Q, Liu ZH, Chen HP, Yin HL, Li LS. 2000. Long-term outcome of acute renal injury induced by Aristolochia manshuriensis Kom in rats. Acta Pharmacol Sin 21(12): 1129-1135. (Support not reported. Authors affiliated with Nanjing University School of Medicine, China; Southeast University Medical College, China; Jinling Hospital, China.)

194. Radovanovic Z, Jankovic S, Jevremovic I. 1991. Incidence of tumors of urinary organs in a focus of Balkan endemic nephropathy. Kidney Int Suppl 34: S75-6. (Supported by the U.S. Department of Health and Human Services, the Scientific Community of Serbia, the Yugoslav-American Joint Fund and the Kuwait University. Authors affiliated with Insitute of Epidemiology, Yugoslavia.)

195. Rao KV, Tanrikut Y, Killion K. 1975. Fluorometric and GLC analyses of aristolochic acid. J Pharm Sci 64(2): 345-347. (Supported by the Regional

Medical Program Service, Department of Health, Education and Welfare. Authors affiliated with the University of Florida.)

196. Reginster F, Jadoul M, van Ypersele de Strihou C. 1997. Chinese herbs nephropathy presentation, natural history and fate after transplantation. Nephrol Dial Transplant 12(1): 81-86. (Support not reported. Authors affiliated with Cliniques Universitaires St-Luc, Belgium.)

197. Robisch G, Schimmer O, Göggelmann W. 1982. Aristolochic acid is a direct mutagen in Salmonella typhimurium. Mutat Res 105(4): 201-204. (Support not reported. Authors affiliated with Institut für Botanik und Pharmazeutische Biologie der Universität Erlangen-Nürnberg, Germany; Abteilung für Toxikologie, Germany.)

198. Rosenthal MD, Vishwanath BS, Franson RC. 1989. Effects of aristolochic acid on phospholipase A_2 activity and arachidonate metabolism of human neutrophils. Biochim Biophys Acta 1001(1): 1-8. (Supported by the American Heart Association and NIH. Authors affiliated with Eastern Virginia Medical School, VA; Medical College of Virginia, VA.)

199. Rossiello MR, Laconi E, Rao PM, Rajalakshmi S, Sarma DS. 1993. Induction of hepatic nodules in the rat by aristolochic acid. Cancer Lett 71(1-3): 83-87. (Supported by the National Cancer Institute of Canada, the Canadian Cancer Society, USPHS, and the Ministry of University and Scientific Technological Research, Italy. Authors affiliated with University of Toronto, Canada.)

200. Routledge MN, Orton TC, Lord PG, Garner RC. 1990. Effect of butylated hydroxyanisole on the level of DNA adduction by aristolochic acid in the rat forestomach and liver. Jpn J Cancer Res 81(3): 220-224. (Support not reported. Authors affiliated with University of York, UK; ICI Pharmaceuticals, UK.)

201. Sato N, Takahashi D, Chen SM, Tsuchiya R, Mukoyama T, Yamagata S, Ogawa M, Yoshida M, Kondo S, Satoh N, Ueda S. 2004. Acute nephrotoxicity of aristolochic acids in mice. J Pharm Pharmacol 56(2): 221-229. (Supported by the Organization for Pharmaceutical Society and Research in Japan. Authors affiliated with Chiba University, Japan; Taipei Medical University, Taiwan; Kotaro Pharmaceutical Co., Ltd. Japan.)

202. Schaneberg BT, Applequist WL, Khan IA. 2002. Determination of aristolochic acid I and II in North American species of Asarum and Aristolochia. Pharmazie 57(10): 686-689. (Supported by the U.S. Department of Agriculture, the ARS Specific Cooperative Agreement, the University of Missouri's Center for Phytonutrient and Phytochemical Studies and NIEHS. Authors affiliated with University of Mississippi and the Missouri Botanical Garden.)

203. Schaneberg BT, Khan IA. 2004. Analysis of products suspected of containing Aristolochia or Asarum species. J Ethnopharmacol 94(2-3): 245-249. (Supported by the FDA. Authors affiliated with the University of Mississippi.)

204. Schmeiser HH, Pool BL, Wiessler M. 1984. Mutagenicity of the two main components of commercially available carcinogenic aristolochic acid in Salmonella typhimurium. Cancer Lett 23(1): 97-101. (Support not reported. Authors affiliated with German Cancer Research Center, Germany.)

205. Schmeiser HH, Pool BL, Wiessler M. 1986. Identification and mutagenicity of metabolites of aristolochic acid formed by rat liver. Carcinogenesis 7(1): 59-63. (Support not reported. Authors affiliated with the German Cancer Research Center, Germany.)

206. Schmeiser HH, Schoepe KB, Wiessler M. 1988. DNA adduct formation of aristolochic acid I and II in vitro and in vivo. Carcinogenesis 9(2): 297-303. (Support not reported. Authors affiliated with Deutsches Krebsforschungszentrum, Germany.)

207. Schmeiser HH, Janssen JW, Lyons J, Scherf HR, Pfau W, Buchmann A, Bartram CR, Wiessler M. 1990. Aristolochic acid activates ras genes in rat tumors at deoxyadenosine residues. Cancer Res 50(17): 5464-5469. (Supported by the Deutsche Forschungsgemeinschaft. Costs of this article were defrayed in part by the payment of page charges. Authors affiliated with German Cancer Research Center, Germany; University of Ulm, Germany.)

208. Schmeiser HH, Scherf HR, Wiessler M. 1991. Activating mutations at codon 61 of the c-Ha-ras gene in thin-tissue sections of tumors induced by aristolochic acid in rats and mice. Cancer Lett 59(2): 139-143. (Support not reported. Authors affiliated with the German Cancer Research Center, Germany.)

209. Schmeiser HH, Bieler CA, Wiessler M, van Ypersele de Strihou C, Cosyns JP. 1996. Detection of DNA adducts formed by aristolochic acid in renal tissue from patients with Chinese herbs nephropathy. Cancer Res 56(9): 2025-2028. (Costs of publication were defrayed in part by the payment of page charges. Authors affiliated with the German Cancer Research Center, germany; Cliniques Universitaires St-Luc, Belgium.)

210. Schmeiser HH, Frei E, Wiessler M, Stiborová M. 1997. Comparison of DNA adduct formation by aristolochic acids in various in vitro activation systems by ^{32}P-post-labelling: evidence for reductive activation by peroxidases. Carcinogenesis 18(5): 1055-1062. (Support not reported. Authors affiliated with German Cancer Research Center, Germany; Charles University, Czech Republic.)

211. Shi S, Li W, Liao Y, Cai Z, Liu H. 2007. Online concentration of aristolochic acid I and II in Chinese medicine preparations by micellar electrokinetic chromatography. J Chromatogr A. (Supported by NSFC. Authors affiliated with Peking University, China; Hong Kong Baptist University, China.)

212. Shibutani S, Dong H, Suzuki N, Ueda S, Miller F, Grollman AP. 2007. Selective toxicity of aristolochic acids I and II. Drug Metab Dispos 35(7): 1217-22.

(Support not reported. Authors affiliated with State University of New York, NY; Chiba University, Japan.)

213. Solez K, Daugirdas J, Gregory MC, Frohnert PP, Bhowmik DM, Jha V, Cosyns JP. 2001. Is "Chinese herbs nephropathy" a prejudicial term? Am J Kidney Dis 38(5): 1141-2. (Support not reported. Author affiliations not reported.)

214. Sorenson WR, Sullivan D. 2007. Determination of aristolochic acid I in botanicals and dietary supplements potentially contaminated with aristolochic acid I using LC-UV with confirmation by LC/MS: collaborative study. J AOAC Int 90(4): 925-33. (Support not reported. Authors affiliated with Covance Laboratories, WI.)

215. Starr F, Starr K, Loope L. 2003. Plants of Hawai'i: Aristolochia littoralis. Hawaiian Ecosystems at Risk Project. http://www.hear.org/starr/hiplants/reports/pdf/aristolochia_littoralis.pdf. Last accessed: 10/19/05. (Support not reported. Authors affiliated with U.S. Geological Survey.)

216. Stefanovic V, Toncheva D, Atanasova S, Polenakovic M. 2006. Etiology of Balkan Endemic Nephropathy and Associated Urothelial Cancer. Am J Nephrol 26(1): 1-11. (Supported by INCO-Copernicus, Eurogendis, the Marie-Curie Fellowship Foundation, the Ministry of Education and Science of Bulgaria and the Ministry of Science and Environmental Protection Serbia. Authors affiliated with Institute of Nephrology and Hemodialysis, Serbia; Medical University, Bulgaria; Georg August University, Germany; Macedonian Academy of Sciences and Arts, Macedonia.)

217. Stefanovic V, Radovanovic Z. 2008. Balkan endemic nephropathy and associated urothelial cancer. Nat Clin Pract Urol 5(2): 105-12. (Support not reported. Authors affiliated with University of Nis, Serbia; Institute of Nephrology, Kuwait.)

218. Stemmer K, Ellinger-Ziegelbauer H, Ahr HJ, Dietrich DR. 2007. Carcinogen-specific gene expression profiles in short-term treated Eker and wild-type rats indicative of pathways involved in renal tumorigenesis. Cancer Res 67(9): 4052-68. (Supported by the Federal Ministry of Education and Research. The costs of this publication were defrayed in part by the payment of page charges. Authors affiliated with University of Knostanz, Germany; Bayer Healthcare AG, Germany.)

219. Stengel B, Jones E. 1998. [End-stage renal insufficiency associated with Chinese herbal consumption in France]. Nephrologie 19(1): 15-20. (Support unknown (non-English article). Authors affiliated with INSERM, France; ERA-ETDA Registry, UK.)

220. Stewart JH, Buccianti G, Agodoa L, Gellert R, McCredie MR, Lowenfels AB, Disney AP, Wolfe RA, Boyle P, Maisonneuve P. 2003. Cancers of the kidney

and urinary tract in patients on dialysis for end-stage renal disease: analysis of data from the United States, Europe, and Australia and New Zealand. J Am Soc Nephrol 14(1): 197-207. (Supported by the Italian Association for Cancer Research. Authors affiliated with University of Otago, New Zealand; Azienda Ospedaliera San Gerardo, Italy; National Institute of Diabetes, Maryland; Department of Internal Medicine and Nephrology, Poland; New York Medical College; Queen Elizabeth Hospital, Australia; University of Michigan; European Institute of Oncology, Italy.)

221. Stiborova M, Frei E, Arlt VM, Schmeiser HH. 2007. Metabolic activation of carcinogenic aristolochic acid, a risk factor for Balkan endemic nephropathy. Mutat Res. (Supported by the Grant Agency of the Czech Republic, the Ministry of Education of the Czech Republic, the German Cancer Research Center and the Association for International Cancer Research. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany; Institute of Cancer Research, UK.)

222. Stiborová M, Fernando RC, Schmeiser HH, Frei E, Pfau W, Wiessler M. 1994. Characterization of DNA adducts formed by aristolochic acids in the target organ (forestomach) of rats by ^{32}P-postlabelling analysis using different chromatographic procedures. Carcinogenesis 15(6): 1187-1192. (Support not reported. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany; University of Hamburg, Germany.)

223. Stiborová M, Frei E, Breuer A, Bieler CA, Schmeiser HH. 1999. Aristolactam I a metabolite of aristolochic acid I upon activation forms an adduct found in DNA of patients with Chinese herbs nephropathy. Exp Toxicol Pathol 51(4-5): 421-427. (Supported by the German Cancer Research Center and the Czech Ministry of Education. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany.)

224. Stiborová M, Hájek M, Frei E, Schmeiser HH. 2001a. Carcinogenic and nephrotoxic alkaloids aristolochic acids upon activation by NADPH : cytochrome P450 reductase form adducts found in DNA of patients with Chinese herbs nephropathy. Gen Physiol Biophys 20(4): 375-392. (Supported by the Grant Agency of the Czech Republic and the Ministry of Education of the Czech Republic. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany.)

225. Stiborová M, Frei E, Breuer A, Wiessler M, Schmeiser HH. 2001b. Evidence for reductive activation of carcinogenic aristolochic acids by prostaglandin H synthase -- ^{32}P-postlabeling analysis of DNA adduct formation. Mutat Res 493(1-2): 149-160. (Supported by the Grant Agency of Czech Republic and the Ministry of Education of the Czech Republic. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany.)

226. Stiborová M, Frei E, Wiessler M, Schmeiser HH. 2001c. Human enzymes involved in the metabolic activation of carcinogenic aristolochic acids: evidence for reductive activation by cytochromes P450 1A1 and 1A2. Chem Res Toxicol 14(8): 1128-1137. (Supported by the Grant Agency of the Czech Republic and the Ministry of Education of the Czech Republic. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany.)

227. Stiborová M, Frei E, Sopko B, Wiessler M, Schmeiser HH. 2002. Carcinogenic aristolochic acids upon activation by DT-diaphorase form adducts found in DNA of patients with Chinese herbs nephropathy. Carcinogenesis 23(4): 617-625. (Supported by the Grant Agency of the Czech Republic and the Ministry of Education of the Czech Republic. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany.)

228. Stiborová M, Frei E, Sopko B, Sopkova K, Markova V, Lankova M, Kumstyrova T, Wiessler M, Schmeiser HH. 2003. Human cytosolic enzymes involved in the metabolic activation of carcinogenic aristolochic acid: evidence for reductive activation by human NAD(P)H:quinone oxidoreductase. Carcinogenesis 24(10): 1695-703. (Supported by the Grant Agency of the Czech Republic and the Ministry of Education of the Czech Republic. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany.)

229. Stiborová M, Frei E, Hodek P, Wiessler M, Schmeiser HH. 2005a. Human hepatic and renal microsomes, cytochromes P450 1A1/2, NADPH:cytochrome P450 reductase and prostaglandin H synthase mediate the formation of aristolochic acid-DNA adducts found in patients with urothelial cancer. Int J Cancer 113(2): 189-197. (Support not reported. Authors affiliated with Charles University, Czech Republic; German Cancer Research Center, Germany.)

230. Straus SE. 2002. Herbal medicines--what's in the bottle? N Engl J Med 347(25): 1997-8. (Support not reported. Authors affiliated with National Center for Complementary and Alternative Medicine, MD.)

231. Sun D, Feng J, Dai C, Sun L, Jin T, Ma J, Wang L. 2006. Role of peritubular capillary loss and hypoxia in progressive tubulointerstitial fibrosis in a rat model of aristolochic acid nephropathy. Am J Nephrol 26(4): 363-71. (Support not reported. Authors affiliated with First Affiliated Hospital of China Medical University, China; Affiliated Hospital of Xuzhou Medical University, China.)

232. Sundell IB, Aziz KA, Zuzel M, Theakston RD. 2003. The role of phospholipases A2 in the stimulation of neutrophil motility by cobra venoms. Toxicon 41(4): 459-68. (Supported by the Wellcome Trust of Great Britain. Authors affiliated with the University of Liverpool, UK; Liverpool School of Tropical Medicine, UK.)

233. Tanaka A, Nishida R, Sawai K, Nagae T, Shinkai S, Ishikawa M, Maeda K, Murata M, Seta K, Okuda J, Yoshida T, Sugawara A, Kuwahara T. 1997a. [Traditional remedy-induced Chinese herbs nephropathy showing rapid deterioration of renal function]. Nippon Jinzo Gakkai Shi 39(8): 794-797. (Support unknown (non-English article). Authors affiliated with Saiseikai Nakatsu Hospital, Japan; Kyoto University, Japan.)

234. Tanaka A, Shinkai S, Kasuno K, Maeda K, Murata M, Seta K, Okuda J, Sugawara A, Yoshida T, Nishida R, Kuwahara T. 1997b. [Chinese herbs nephropathy in the Kansai area: a warning report]. Nippon Jinzo Gakkai Shi 39(4): 438-440. (Support unknown (non-English article). Authors affiliated with Saiseikai Nakatsu Hospital, Japan; Kyoto University, Japan.)

235. Tanaka A, Nishida R, Maeda K, Sugawara A, Kuwahara T. 2000a. Chinese herb nephropathy in Japan presents adult-onset Fanconi syndrome: could different components of aristolochic acids cause a different type of Chinese herb nephropathy? Clin Nephrol 53(4): 301-306. (Support not reported. Authors affiliated with Saiseikai Nakatsu Hospital, Japan; Kyoto University, Japan.)

236. Tanaka A, Nishida R, Yoshida T, Koshikawa M, Goto M, Kuwahara T. 2001. Outbreak of Chinese herb nephropathy in Japan: are there any differences from Belgium? Intern Med 40(4): 296-300. (Support not reported. Authors affiliated with Osaka Saiseikai Nakatsu Hospital, Japan; Kyoto University, Japan.)

237. Trujillo WA, Sorenson WR, La Luzerne P, Austad JW, Sullivan D. 2006. Determination of aristolochic acid in botanicals and dietary supplements by liquid chromatography with ultraviolet detection and by liquid chromatography/mass spectrometry: single laboratory validation confirmation. J AOAC Int 89(4): 942-59. (Support not reported. Authors affiliated with Covance Laboratories, WI.)

238. Tsai CS, Chen YC, Chen HH, Cheng CJ, Lin SH. 2005. An unusual cause of hypokalemic paralysis: aristolochic acid nephropathy with Fanconi syndrome. Am J Med Sci 330(3): 153-5. (Support not reported. Authors affiliated with Macky Memorial Hospital, China; Tri-service General Hospital, China.)

239. Turesky RJ. 2005. Perspective: ochratoxin A is not a genotoxic carcinogen. Chem Res Toxicol 18(7): 1082-90. (Supported by the National Center for Toxicological Research, AR and Nestec Ltd., Switzerland. Author affiliated with New York State Department of Health.)

240. Ubara Y, Hara S, Kobayashi M, Katori H, Tagami T, Yokota M, Kitamura Y, Yoshihara K, Matsushita Y, Yokoyama K, Takemoto F, Yamada A, Ishida A, Endo Y, Hara M. 1999. A case of Chinese herbs - induced renal interstitial fibrosis associated with fibrosis of salivary glands. Am J Kidney Dis 33(3): 1-5. (Support not reported. Authors affiliated with Toranomon Hospital, Japan.)

241. USDA. 2005. The Plants Database, Version 3.5. U.S. Department of Agriculture, National Resources Conservation Service, National Plant Data Center. http://plants.usda.gov. Last accessed: 10/18/05.

242. Vanhaelen M, Vanhaelen-Fastre R, But P, Vanherweghem JL. 1994. Identification of aristolochic acid in Chinese herbs. Lancet 343(8890): 174. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium; Chinese University of Hong Kong, China.)

243. Vanherweghem JL, Depierreux M, Tielemans C, Abramowicz D, Dratwa M, Jadoul M, Richard C, Vandervelde D, Verbeelen D, Vanhaelen-Fastre R, Vanhaelen M. 1993. Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. Lancet 341(8842): 387-391. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium; Vrije Universiteit Brussel, Belgium; Catholique de Louvain, Belgium.)

244. Vanherweghem JL, Tielemans C, Simon J, Depierreux M. 1995. Chinese herbs nephropathy and renal pelvic carcinoma. Nephrol Dial Transplant 10(2): 270-273. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium)

245. Vanherweghem JL, Cuykens JJ, Vandenbergh PH, Bouman KP, Hagers Y. 1998. Valvular heart disease and Chinese-herb nephropathy. Lancet 351: 991-992. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium; Algemeen Ziekenhuis Middelheim, Belgium.)

246. Vanherweghem LJ. 1998. Misuse of herbal remedies: the case of an outbreak of terminal renal failure in Belgium (Chinese herbs nephropathy). J Altern Complement Med 4(1): 9-13. (Support not reported. Authors affiliated with Université Libre de Bruxelles, Belgium)

247. Vishwanath BS, Gowda TV. 1987. Interaction of aristolochic acid with Vipera russelli phospholipase A_2: its effect on enzymatic and pathological activities. Toxicon 25(9): 929-37. (Supported by the University Grants Commission, India. Authors affiliated with University of Mysore, India; Virginia Commonwealth University, VA.)

248. Voice TC, Long DT, Radovanovic Z, Atkins JL, McElmurry SP, Niagolova ND, Dimitrov P, Petropoulos EA, Ganev VS. 2006. Critical evaluation of environmental exposure agents suspected in the etiology of Balkan endemic nephropathy. Int J Occup Environ Health 12(4): 369-76. (Support not reported. Authors affiliated with Michigan State University, MI; Kuwait University, Kuwait; Walter Reed Army Institute of Research, Washington, D.C.; National Center of Radiobiology and Radiation Protection, Bulgaria; Center of Public Health Protection, Bulgaria; Medical University of Sofia, Bulgaria.)

249. Wen YJ, Su T, Tang JW, Zhang CY, Wang X, Cai SQ, Li XM. 2006. Cytotoxicity of Phenanthrenes Extracted from Aristolochia contorta in Human Proximal Tubular Epithelial Cell Line. Nephron Exp Nephrol 103(3): e95-e102. (Supported by the Ministry of Education Foundation of China and NSFC. Authors affiliated with Peking University, China.)

250. Wu K, Jiang L, Cao J, Yang G, Geng C, Zhong L. 2007b. Genotoxic effect and nitrative DNA damage in HepG2 cells exposed to aristolochic acid. Mutat Res 630(1-2): 97-102. (Support not reported. Authors affiliated with Dalian Medical University, China.)

251. Wu KM, Farrelly JG, Upton R, Chen J. 2007a. Complexities of the herbal nomenclature system in traditional Chinese medicine (TCM): lessons learned from the misuse of Aristolochia-related species and the importance of the pharmaceutical name during botanical drug product development. Phytomedicine 14(4): 273-9. (Support not reported. Authors affiliated with FDA; American Herbal Pharmacopoeia, CA; Chengdu University of TCM, China.)

252. Wu MJ, Lian JD, Yang CR, Cheng CH, Chen CH, Lee WC, Shu KH, Tang MJ. 2004c. High cumulative incidence of urinary tract transitional cell carcinoma after kidney transplantation in Taiwan. Am J Kidney Dis 43(6): 1091-1097. (Support not reported. Authors affiliated with Taichung Veterans General Hospital, Taiwan; National Yang Ming University, Taiwan; Chung-Chan Medical University, Taiwan; National Cheng Kung University Medical College, Taiwan.)

253. Wu TS, Ou LF, Teng CM. 1994. Aristolochic acids, aristolactam alkaloids and amides from Aristolochia kankauensis. Phytochemistry 36(4): 1063-8. (Supported by the National Science Council of the Republic of China. Authors affiliated with the Natonal Cheng Kung University, Taiwan; National Taiwan University, Taiwan.)

254. Yang CS, Lin CH, Chang SH, Hsu HC. 2000. Rapidly progressive fibrosing interstitial nephritis associated with Chinese herbal drugs. Am J Kidney Dis 35(2): 313-318. (Support not reported. Authors affiliated with Cathay General Hospital, Taiwan; National Taiwan University Hospital, Taiwan.)

255. Yang HY, Lin JL, Chen KH, Yu CC, Hsu PY, Lin CL. 2006. Aristolochic acid-related nephropathy associated with the popular Chinese herb Xi Xin. J Nephrol 19(1): 111-114. (Support not reported. Authors affiliated with Chang Gung Memorial Hospital, Taiwan.)

256. Yang L, Li X, Wang H. 2007. Possible mechanisms explaining the tendency towards interstitial fibrosis in aristolochic acid-induced acute tubular necrosis. Nephrol Dial Transplant 22(2): 445-56. (Supported by Peking University. Authors affiliated with Peking University First Hospital Beijing, China.)

257. Yang MH, Chen KK, Yen CC, Wang WS, Chang YH, Huang WJ, Fan FS, Chiou TJ, Liu JH, Chen PM. 2002b. Unusually high incidence of upper urinary tract urothelial carcinoma in Taiwan. Urology 59(5): 681-7. (Supported by the Yen Tjing-Lin Medical Foundation. Authors affiliated with Taipei Veterans General Hospital, Taiwan; National Yang-Ming Universty School of Medicine, Taiwan.)

258. Yang SS, Chu P, Lin MS, Chen A, Lin YF. 2001. Two clinical variants of Chinese-herb nephropathy: case reports and review of the literature. J Med Sci 21(4): 217-224. (Support not reported. Authors affiliated with Tri-Service General Hospital, Taiwan; National Defense Medical Center, Taiwan; Taipei Medical University, Taiwan.)

259. Yang SS, Chu P, Lin YF, Chen A, Lin SH. 2002a. Aristolochic acid-induced Fanconi's syndrome and nephropathy presenting as hypokalemic paralysis. Am J Kidney Dis 39(3): E14. (Support not reported. Authors affiliated with Tri-Service General Hospital, Taiwan; National Defense Medical Center, Taiwan.)

260. Yu FY, Lin YH, Su CC. 2006. A sensitive enzyme-linked immunosorbent assay for detecting carcinogenic aristolochic acid in herbal remedies. J Agric Food Chem 54(7): 2496-501. (Supported by the National Science Council of the Republic of China. Authors affiliated with Chung Shan Medical University, Taiwan; Tian-Sheng Memorial Hospital, Taiwan.)

261. Yuan J, Liu Q, Wei G, Tang F, Ding L, Yao S. 2007. Characterization and determination of six aristolochic acids and three aristololactams in medicinal plants and their preparations by high-performance liquid chromatography-photodiode array detection/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 21(14): 2332-42. (Supported by the National Natural Science Foundation of China. Authors affiliated with Hunan University, China; Jiangxi University of Traditional Chinese Medicine, China.)

262. Yuan J, Nie L, Zeng D, Luo X, Tang F, Ding L, Liu Q, Guo M, Yao S. 2007b. Simultaneous determination of nine aristolochic acid analogues in medicinal plants and preparations by high-performance liquid chromatography. Talanta 73: 644-650. (Supported by the National Basic Research Program and the National Natural Science Foundation of China. Authors affiliated with Hunan University, China; Jiangxi University of Traditional Chinese Medicine, China; Cancer Hospital of Hunan Province, China; Nanchang Institute of Aeronautical Technology, China.)

263. Zhai ZD, Luo XP, Shi YP. 2006. Separation and determination of aristolochic acids in herbal medicines by microemulsion electrokinetic chromatography. Anal Chim Acta 561(1-2): 119-125. (Supported by the "Bairen Jihua" Foundation of Chinese Academy of Sciences and the National Natural Science Foundation of China. Authors affiliated with the Chinese Academy of Sciences, China.)

264. Zhang C, Wang X, Shang M, Yu J, Xu Y, Li Z, Lei L, Li X, Cai S, Namba T. 2006b. Simultaneous determination of five aristolochic acids and two aristololactams in Aristolochia plants by high-performance liquid chromatography. Biomed Chromatogr 20(4): 309-18. (Supported by National Natural Science Foundation of China and Peking University. Authors affiliated with Peking University, China; Henan Institute of Drug Control, China; Toyama Medical and Pharmaceutical University, Japan.)

265. Zhang CY, Wang X, Su T, Ma CM, Wen YJ, Shang MY, Li XM, Liu GX, Cai SQ. 2005b. New aristolochic acid, aristololactam and renal cytotoxic constituents from the stem and leaves of Aristolochia contorta. Pharmazie 60(10): 785-788. (Supported by the National Science Foundation of China and the 985 Project of Peking University. Authors affiliated with Peking University, China.)

266. Zhang H, Cifone MA, Murli H, Erexson GL, Mecchi MS, Lawlor TE. 2004. Application of simplified in vitro screening tests to detect genotoxicity of aristolochic acid. Food Chem Toxicol 42(12): 2021-2028. (Support not reported. Authors affiliated with Covance Laboratories, Inc., Virginia.)

267. Zhang L, Mu X, Fu J, Zhou Z. 2007. In vitro cytotoxicity assay with selected chemicals using human cells to predict target-organ toxicity of liver and kidney. Toxicol In Vitro 21(4): 734-40. (Supported by Peking University. Authors affiliated with Peking University.)

268. Zhang X, Wu H, Liao P, Li X, Ni J, Pei F. 2006a. NMR-based metabonomic study on the subacute toxicity of aristolochic acid in rats. Food Chem Toxicol 44(7): 1006-14. (Supported by the National Natural Science Foundation of China. Authors affiliated with Graduate School of Chinese Academy of Sciences, China.)

269. Zhou X, Zheng C, Sun J, You T. 2006. Analysis of nephroloxic and carcinogenic aristolochic acids in Aristolochia plants by capillary electrophoresis with electrochemical detection at a carbon fiber microdisk electrode. J Chromatogr A 1109(2): 152-159. (Supported by the National Natural Science Foundation of China, the State Key Laboratory of Electroanalytical Chemistry of Changchun Institute of Applied Chemistry, the Foundation of National Excellent Ph.D and the Scientific Foundation of Ph.D. of Heilongjiang University. Authors affiliated with Graduate School of the Chinese Academy of Sciences, China; Heilongjiang University, China.)

270. Zhu YP. 2002. Toxicity of the Chinese herb mu tong (Aristolochia manshuriensis). What history tells us. Adverse Drug React Toxicol Rev 21(4): 171-7. (The authors received no funding for this work. Authors affiliated with University of Groningen, Netherlands.)

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.

Mesotheraphy: 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 manshurensis* 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 pKa is the negative logarithm (to the base 10) of the acid dissociation constant (Ka); the lower the pKa, 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
Aristolochia clematis	PMS-Ease	InnerLife Wellness Center	Växa	[Product not found on the Innerwellness.com website.]
Aristolochia fangji	Tong Xue Pian Tablets	Merchant America	[NA]	[Retailer no longer found on the Internet.]
Aristolochia manschuriensis [*manshuriensis*]	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: *Akebia* 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.guangcitang.com/]	[http://maxnature.stores.yahoo.net/lodanxieganw.html] [Long Dan Xie Gan Wan with *Aristolochia manschuriensis* still available for sale.] [Three other products containing *Akebia* 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 *Aristolochia* plant parts were identified, and are listed below as *Aristolochia sp.*]
		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 *Aristolochia* 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 *Akebia* root rather than *Aristolochia manschuriensis*- see new listing below in Table 2.]
[*Aristolochia sp.*]	[Ma dou ling]	[Eastern Chinese Medicine Export Company]	[Eastern Chinese Medicine Export Company]	[http://www.tcmtreatment.com/images/wholesale/herb-price/6.htm) and http://www.tcmtreatment.com/herbs/0-madouling.htm] [*Aristolochia* fruit: *Aristolochiae fructus*]
	[Qing mu xiang]	[Eastern Chinese Medicine Export Company]	[Eastern Chinese Medicine Export Company]	[http://www.tcmtreatment.com/images/wholesale/herb-price/7.htm] [*Aristolochia* root: *Aristolochiae radix*]
[*Aristolochiae Mollissimae*]	[Xun gu feng]	[Eastern Chinese Medicine Export Company]	[Eastern Chinese Medicine Export Company]	[http://www.tcmtreatment.com/images/wholesale/herb-price/9.htm] [*Mollissima: Aristolochiae mollissimae*]
Aristolochia manschuriensis [*manshuriensis*]	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]
[*Aristolochia manschuriensis* (*manshuriensis*)]	[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 *aristolochia* stem.]

Species	Medicinal name	Retailer	Manufacturer	2007 update
[*Aristolochia manschuriensis* (*manshuriensis*)]	[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 *aristolochia* stem.]
Aristolochia 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/cougchikuany.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.
Aristolochia 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.]
Aristolochia sp.	Gui Pi Wan	Doc4Pain.com	[NA]	[Retailer no longer found on the Internet.]
[*Aristolochia* 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 (*Aristolochia serpentaria*).]
[*Aristolochia* sp.]	[Ma Dou Ling Aristolochia fruit (Aristolochiae Fructus)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/6.htm] [Product available in wholesale price list of Chinese herbs.]
[*Aristolochia* sp.]	[Qing Mu Xiang Aristolochia root (Aristolochiae Radix); Vladimiria root (Vladimiriae Radix)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/7.htm] [Product available in wholesale price list of Chinese herbs.]

Species	Medicinal name	Retailer	Manufacturer	2007 update
Aristolochia sp. (+ Coltsfoot)	Chuan Ke Wan	[Herbs West, LLC]	[Herbal Times brand]	http://herbswest.net/items/BL1355.shtml †
Aristolochia sp. + Clematis sp.	Circula	Opane.com PlazaQ.com	[NA] [NA]	[*Aristolochia* and *clematis* could not be confirmed on either website. Another product was found with an ingredients list including *Aristolochia* and *clematis* (see below) and several products containing *clematis* sold through these websites are listed in Table 2, below.]
[*Aristolochia* sp. + Clematis sp.]	[Eucommiae Musculoskeletal Support Pills: Du Zhong Zhuang Gu Wan]	[Opane.com] [PlazaQ.com]	[NA] [NA]	[http://opane.stores.yahoo.net/eucmussup100.html] [The ingredients listed include *clematis* root and Wooly Dutchmanspipe (i.e., *Aristolochia tomentosa* - http://plants.usda.gov/java/profile?symbol=ARTO3 - and wild ginger.] [http://plusq.stores.yahoo.net/eucmussup100.html] [Same ingredients list as on Opane.com website.]
Asarum canadense	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 *Zingiber officinale*, which is the botanical name for ginger. The listing below is for a product identified on the website as *Asarum canadense* (wild ginger).]
[*Asarum canadense*]	[Canada snake]	[Taylor's Organic Gardens]	NA	[http://www.taylorgarden.com/Products/BulkHerbList.asp] [Listing is for Canada snake (*Asarum canadense*).]
Asarum (canadense)	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 *Asarum* herb as an ingredient (see listing in Table 2, below).]

Species	Medicinal name	Retailer	Manufacturer	2007 update
Asarum canadense	Cold Away [Now called Winter Coat]	Sunrise Herbal Remedies	[Sunrise Herbal Remedies]	http://www.sunriseherbfarm.com/coldaway.html †
Asarum canadense	Cramp Relief	Sunrise Herbal Remedies	Sunrise Herbal Remedies	http://www.sunriseherbfarm.com/camprelief.html †
Asarum canadense	Formula 208	Web Vitamins	Heritage Products	[Product no longer available from retailer.]
Asarum canadense	Mother Earth's Cough Syrup / Mother Earth's Respiratory System Tonic	InterNatural	Heritage Products [Store]	http://www.internatural-alternative-health.com/ingr/ingr179190.cfm †
		Kalyx	Heritage Products [Store]	http://www.kalyx.com/store/proddetail.cfm/ItemID/569659.0/CategoryID/6000.0/SubCatID/985.0/file.htm] †
		DiscountBlvd.com NutrtionBlvd.com	[NA]	[Retailers DiscountBlvd.com and NutritionBlvd.com were not found on the web.]
Asarum canadense	Viral Resolve [called "Viral Vanish" in 2007]	[Sunrise Herbal Remedies]	[Sunrise Herbal Remedies]	http://www.sunriseherbfarm.com/viralresolve.html †
Asarum canadense	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
Asarum canadense	Wild Ginger tincture	Spring Valley Herbs and Natural Foods	Teeter Creek	http://www.springvalleyherbs.com/catalog.php?itemID=2025 [Wild Ginger tincture containing *Asarum canadense* 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]	[Spring Valley Herbs and Natural Foods]	[Teeter Creek]	[Teeter Creek Herbs Asthmaid Tincture containing wild ginger is available at http://www.springvalleyherbs.com/catalog.php?itemID=2045.]
Asarum canadense + *Akebia trifoliata*	Aller Relief	Spanda	Neo Concept	[*Asarum* 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 *Asarum*, 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
Akebia sp.	Akebia	Botanicum	[NA]	[Retailer no longer found on the Internet.]
Akebia sp.	Alive Energy: Mental and Emotional Strength Women's Courage 60's	InterNatural	[NA]	[Product not found on the Internatural.com website.]
Akebia sp.	Circulation: Specific Rubrella Care [Feng Zhen hwan]	Opane.com	[NA]	http://www.opane.com/cirspecrubca.html †
Akebia sp.	Eye Relief Capsules	diabetes-alternativemedicine.com	[NA]	[Retailer no longer found on the Internet.]
Akebia sp.	Genpriv	Mandarin Herbs	[NA]	[Product not found on the Mandarinherbs.com website]
Akebia sp.	K-C	The Herb Nook Virtualherbs.com	Nature's Sunshine	[Retailers no longer found on the Internet]
Akebia sp.	Lung Tan Xie Gan Wan Combination	Wing Hop Fung	[NA]	[Product not found on the Winghopfung.com website.]
Akebia sp.	Shi Chuan Xiu Xue Tang (General Purpose Stop Blood Formula)	Ancient Way Accupuncture & Herbs	[NA]	http://www.ancientway.com/Pages/MartialArtsFormulas.html †
Akebia sp.	Wind-Dispelling Powder (Xiao Feng San)	Nature's Health	[NA]	http://www.nature-s-health.com/products/theproduct1.asp?pid=287 †
[*Akebia* sp.]	[Yu Zhi Zi Foreknowledge Akebiae Fructus]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/10.htm] [Product available in wholesale price list of Chinese herbs.]
Akebia sp. + *Asarum* sp.	Nasixx	MyHerbalRx.com	[NA]	[http://myherbalrx.net/products/nasixx2.htm] †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
Akebia sp. + *Asarum* sp.	Sinus Clear Ephedra Free	Vitanet	Ridge Crest Herbals	http://store.yahoo.com/vitanet/sinclearnoep1.html †
Akebia sp. + *Stephania* sp.	Chinese Kidney Activator (formerly K-C)	Blessed Nutrition, Inc	[NA]	[Product not found on the Blessednutrition.net website]
		Herbshop.com	[NA]	[http://www.herbshop.com/urinary.htm#kc The website notes that the Chinese Kidney Activator product, which lists *Akebia* stem and *Stephania* root, is unavailable while it is reformulated to meet new FDA regulations.]
Akebia sp. + *Stephania* 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 *Akebia* and *Stephania* not present in ingredients list.]
		The Reynolds Office of Health and Nutrition	[NA]	http://www.reynoldsoffice.com/1872-5.htm [Product confirmed, but *Akebia* and *Stephania* 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 *Akebia* and *Stephania* 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 *Akebia* and *Stephania* not present in ingredients list.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
Akebia sp. + *Stephania* sp.	K-C (Eliminate Moisture/Qu Shi) - Kidney Support	Superlative Soundness	[NA]	[Retailer no longer found on the Internet.]
[*Akebia trifoliata*]	[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 *Akebia trifoliata* in the ingredients. An additional product contained both *Akebia trifoliata* stem and *Stephania tetrandra* root (see listing below), and 3 products containing *Asarum sieboldii* (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 *Akebia* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* 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 *Akebia trifoliata* stem.]
Akebia trifoliata	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) *Tribulus terrestris* rather than *Asarum*. See listing below.] [*Akebia* fruit was found on the Kalyx.com website (see entry above for Ba Yue Zha).]
Akebia trifoliata	Eight Righteous / Ba Zheng San Wan	Herbswest, LLC	[NA] [Same ingredients as in Plum Flower brand sold on the Kalyx website.]	http://www.herbswest.net/items/13325.shtml †
		Jade Chinese Herbs & Extracts	[NA]	[Retailer no longer found on the Internet.]
Akebia trifoliata	Hepataplex	2000 + Nutrition Center	[NA]	[Retailer no longer found on the Internet.]
Akebia trifoliata	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 †
Akebia trifoliata	Prostate: Kai Kit Pills	Opane.com	Hanyang pharmaceutical	http://www.opane.com/proskaikitpi.html †
Akebia trifoliata	Prostate: Kai Kit Wan	Healing Herbs of China	Plum Flower	http://store.yahoo.com/healingherbsofchina/prosenkaikit.html †

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
Akebia trifoliata	Prostate: Prostate Gland Care	Opane.com	[NA]	http://www.opane.com/prosprosglan.html [*Akebia* not found in ingredients list.]
[*Akebia trifoliata*]	[Snake & The Dragon Teapills]	[MaxNature]	[Plum Flower Brand]	[http://maxnature.stores.yahoo.net/sndrteldanxi.html] [Product ingredients list includes *Akebia trifoliata* stem.]
[*Akebia trifoliata*]	[Snake & The Dragon Teapills]	[MaxNature]	[Min Shan Brand (Lanzhou Foci herb factory)]	[http://maxnature.stores.yahoo.net/lodanxieganw1.html] [Product ingredients list includes *Akebia trifoliata* stem.]
[*Akebia trifoliata*]	[Coptis Purge Fire Formula]	[MaxNature]	[Health Concerns]	[http://maxnature.stores.yahoo.net/copufifoloda.html] [Product ingredients list includes *Akebia trifoliata* caulis (Mu Tong).]
[*Akebia trifoliata* + *Stephania tetrandra*]	[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 *Akebia trifoliata* stem and *Stephania tetrandra* root.]
Asarum heterotropoides	Bio-Antihist	Natural Health Consultants	Ameriden	http://www.naturalhealthconsult.com/Monographs/BioAntihist.html †
Asarum heterotropoides	100% Herbal Treatment for Tinnitis	Young Again Nutrients [Supplement Spot Nutrients (2007)]	[NA]	[http://www.supplementspot.com/tinnitus.html]
Asarum heterotropoides	Asarum	Botanicum	[NA]	[Retailer no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[*Asarum sieboldii*]	[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/120950/file.htm] [Product ingredients list includes *Asarum sieboldii* herb.]
Asarum sp.	AsthmaClear	LifeHealthEnergy.com	[NA]	[Retailer no longer found on the Internet.]
Asarum sp.	Azarina	Qlife	[NA]	http://www.qlife.com/azarina.html †
		Batory Asset Management	[NA]	http://www.merchantamerica.com/qlife/index.php?ba=product_enlarge&category=1843&product_id=6747 †
Asarum 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 *Asarum* herb.]
Asarum sp.	Breath Easy	NutraCompute	[NA]	[Product not found on Nutracompute.com website.]
Asarum 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
Asarum 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.chronicfatiguesyndromesupport.com/shop/product.cfm?product__code=N0161 †
		LifesVigor and many others	[NA]	[http://www.lifesvigor.com/17668.html] †
Asarum 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] †
Asarum sp.	Migrex	MyHerbalRx.com	[NA]	[http://www.figuerola.net/store/product_info.php?cPath=22&products_id=95&osCsid=7f251fd22c3df99ca2a8d77706c3b4b0] HTML [MigreX confirmed, but *Asarum* not found in list of ingredients.]
Asarum 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] †
Asarum 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
[*Asarum* 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 *Asarum* herb.]
[*Asarum* sp.]	[Bei Xi Xin Northern asarum Asiasari Herba cum Radice Septentionalis]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/herb.index.htm] [Product available in wholesale price list of Chinese herbs.]
[*Asarum* sp.]	[Bei Dou Gen Northern asarum Menispermi Daurici Radix]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/herb.index.htm] [Product available in wholesale price list of Chinese herbs.]
[*Asarum* sp.]	[Xi Xin Asarum Asiasari Herba cum Radice]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/9.htm] [Product available in wholesale price list of Chinese herbs.]
Clematis chinensis	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 *Clematis* 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 *Clematis* 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 *clematis* as an ingredient.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
Clematis chinensis	Flex N Spring	Health Products Distributors, Inc.	[NA]	[Product not found on the Health Products Distributors, Inc. (Integratedhealth.com) website.]
Clematis chinensis	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.]
Clematis chinensis	Kam Wo Herbal Tea	PlazaQ.com	Sing-lin	http://store.yahoo.com/plusq/kamwoherteak.html [Product confirmed, but *Clematis* not found in list of ingredients.]
[*Clematis chinensis*]	[Gam Wo Herbal Tea]	[MaxNature Health Products Co.]	[Sing-lin]	[http://maxnature.stores.yahoo.net/gamwoheteagh.html] [Product containing *Clematis chinensis* was found on this website by searching for Sing-Lin brand.]
Clematis chinensis	Tien Hsien Natural Nutritious Liquid	Cancerth.com	[NA]	[Retailer no longer found on the Internet]
Clematis chinensis	Yu Xiao San 8805	MaxNature Health Products Co	[Chong's Health Care, Inc.]	http://www.maxnature.com/yuxbasontrad.html] †
Clematis chinensis	40+ Nutritional System Joint Health 90's	InterNatural	Rainbow Light	[Product not found on the Internatural.com website.]
Clematis chinensis	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
[*Clematis chinensis*]	[Sciatica Pills]	[Opane. Com]	[NA]	[http://opane.stores.yahoo.net/arsciatpil12.html] [Product ingredients list includes *Clematis rhinensis* [*chinensis*] Osbeck.]
		[PlazaQ]	[NA]	[http://www.plazaq.com/arscpi1zh.html] [The same product containing *Clematis rhinensis* [*chinensis*] Osbeck is available at this website.]
Clematis sp.	Eucommia Extract	Opane.com	[NA]	http://store.yahoo.com/opane/eucex20cap.html †
	[Eucommia Extract (Du Jhong Waji Hwan)]	[PlazaQ.com]	[NA]	[http://plusq.stores.yahoo.net/euex20cadujh.html] [Product ingredients list includes *Clematis* root.]
Clematis sp.	Eucommiae Musculosketletal [sic] Support	PlazaQ.com	[NA]	http://store.yahoo.com/plusq/eucmussup100.html † [Product ingredients list includes *Clematis* and Wooly Dutchmanspipe (*Aristolochia tomentosa*) and wild ginger.]
Clematis sp.	Head Rescue Extract	[Afterglow of Sedon]	NOW brand	http://www.sedonalive.com/nowforms.html [Product confirmed, but *Clematis* not found in ingredients list.]
Clematis sp.	Joint Health	NutritionBlvd.com DiscountBlvd.com	[NA]	[Retailers no longer found on the Internet.]
Clematis 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
[*Clematis* sp.]	[Neck and Shoulders Support]	[VitaNet, LLC]	[Planetary Formulas]	[http://vitanetonline.com/description/PF0416/vitamins/Neck-and-Shoulder-Support/] [Product ingredients list includes Chinese *clematis* root extract.]
[*Clematis* sp.]	[Touku Rheumatic Pills]	[Opane. Com]	[NA]	[http://opane.stores.yahoo.net/rheumtoukrhe.html] [Product ingredients list includes *Clematis* root.]
[*Clematis* sp.]	[Mobility 2 (Clematis Combination Herbal Supplement)]	[MaxNature]	[Health Concerns]	[http://maxnature.stores.yahoo.net/mo2cohesuta.html] [Product ingredients list includes *Clematis* root (Wei Ling Xian).]
[*Clematis* sp.]	[AC-W Tabs (Da Huo Luo Dan Herbal Supplement)]	[MaxNature]	[Health Concerns]	[http://maxnature.stores.yahoo.net/acq.html] [Product ingredients list includes *Clematis* root (Wei Ling Xian).]
[*Clematis* sp.]	[Dao Chi San (Rehmannia & Armand's clematis Formula)]	[MaxNature]	[NA]	[http://maxnature.stores.yahoo.net/daochisanrar.html] [Clematis listed as part of product name; other ingredient information provided on website in Chinese only.]
[*Clematis* sp.]	[Shan Mu Tong Finet's clematis (Clematidis Finetianae Radix, Caulis et Folium)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/7.htm] [Product available in wholesale price list of Chinese herbs.]
[*Clematis* sp.]	[Wei Ling Xian Clematis root (Clematidis Radix])]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/9.htm] [Product available in wholesale price list of Chinese herbs.]
Clematis sp. + *Stephania* sp.	Clematis & Stephania	TCMM Formulas	[NA]	http://www.tcmformulas.com/studentliquidself.htm [*Clematis* & *Stephania* confirmed in product list, but no other details found.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[*Clematis* sp. + *Stephania* sp.]	Circula (Shu Jing Juo Zue Tang) (Clematis & Stephania Combination)]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/cisjihuoxuet.html] [Product identified as *Clematis* & *Stephania* combination.]
[*Clematis* sp. + *Stephania* sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) capsules]	[MaxNature]	[KPCformulas]	[http://maxnature.stores.yahoo.net/shujihuoxuet.html] [*Clematis* and *Stephania* confirmed as part of product name.]
[*Clematis* sp. + *Stephania* sp.]	[Shu Jing Huo Xue Tang (no Aristolochic Acid) (Clematis and Stephania Combination) tablets]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/shujihuoxuet1.html] [*Clematis* and *Stephania* confirmed as part of product name; however, it specifies "No Aristolochic Acid."]
[*Clematis* sp. + *Stephania* sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) tablets]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/shujihuoxuet2.html] [*Clematis* and *Stephania* confirmed as part of product name.]
[*Clematis* sp. + *Stephania* sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) herbal powder]	[MaxNature]	[Min Tong Herbs]	[http://maxnature.stores.yahoo.net/shujihuoxuet3.html] [*Clematis* and *Stephania* confirmed as part of product name.]
[*Clematis* sp. + *Stephania* sp.]	[Shu Jing Huo Xue Tang (Clematis and Stephania Combination) tablets]	[MaxNature]	[KPCformulas]	[http://maxnature.stores.yahoo.net/shujihuoxuet4.html] [*Clematis* and *Stephania* confirmed as part of product name.]

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

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
[*Saussurea sp.*]	[Spleen Activator (UC-C)]	[Klies Herbal Wellness and Colon Care]	[Nature's Sunshine]	[http://www.kliescolon.com/1880-8.htm] [Product ingredients list includes *Saussurea* 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 *Saussurea* 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 *Saussurea* root.] [NB: The Nature's Sunshine website does not list *Saussurea* in the ingredients for their "Spleen Activator, Chinese" product.] [http://www.naturessunshine.com/us/products/catalog/product/default.aspx?stocknum=1880]
Saussurea lappa	Chinese Stress Relief (STR-C)	Goherbal, Inc.	Nature's Sunshine	http://goherbal.stores.yahoo.net/1863-5.html †
		Greatest Herbs on Earth	Nature's Sunshine Product confirmed, but	http://www.greatestherbsonearth.com/nsp/chinese_stress_relief.htm [Product confirmed, but *Saussurea lappa* not found in ingredients list.]
		HerbNook.com	[NA]	[Retailer no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
Saussurea lappa	Complete Antioxidant Support	Betterlife.com, LLC N101, Inc.	Rainbow Light	[Product not found on the Betterlife.com, N101, or Rainbow Light website.]
Saussurea lappa	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. *Saussurea lappa* is listed in the ingredients.]
		[Healthy Store]	[Metagenics]	[The same product containing *Saussurea lappa* in the ingredients was also found at- http://www.healthstores.com/store/stores/HealthyStore/Browse_Item_Details.asp?Shopper_id=427632635234276&Item_ID=1107]
Saussurea lappa	Liver/Gallbladder Support	Health Designs International	Botanigest	[Link to "Liver/Gallbladder Support" product not found.]
Saussurea lappa	UC-C [Enhance Earth] Wen Zhong	HerbNook.com	Nature's Sunshine	[Retailer no longer found on the Internet.]
Saussurea lappa	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
Saussurea lappa	Ultra Energy Plus	Internatural.com	Rainbow Light	[http://www.internatural.com/ingr/ingr845210.cfm] [Product confirmed, but *Saussurea lappa* not listed in the ingredients.]
		Eng Natural [now called Enk Store]	[NA]	[http://www.enkueros.net/301086.html] [Product confirmed, but *Saussurea lappa* not listed in the ingredients.]
		Thymely Solutions	[NA]	http://www.absolutelythepurest.com/realestatesurvivalkit/ultraenergy.html [Product confirmed, but *Saussurea lappa* not listed in the ingredients.]
		Life's Vigor	[NA]	[http://www.lifesvigor.com/10087.html] [Product confirmed, but *Saussurea lappa* not listed in the ingredients.]
		Herbal Advisor and many others	[NA]	[Retailer no longer found on the Internet]
[*Saussurea* sp.]	[Yun Mu Xiang Yunnan saussurea root (Saussureae Radix Sichuanensis)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.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
[*Saussurea* sp.]	[Chuan Mu Xiang sichuan saussurea root (Vladiniriae Souliei Radix)	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/2.htm http://www.tcmtreatment.com/images/wholesale/herb-price/9.htm] [Product is listed in 2 places in the wholesale price list of Chinese herbs.]
Sinomenium acutum	Vine Essence Pills	Solstice Medicine Company	Vine Essence	http://www.sosusaco.com/product/productDetail.asp?iProductID=227 †
[*Sinomenium* sp.]	[Qing Feng Teng Orient Vine (Sinomenii seu sabiae Caulis et Rhizoma)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/7.htm] [Product available in wholesale price list of Chinese herbs.]
Stepania 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.]
Stephania clematis	OrthoFlex Plus	Betterlife.com, LLC	Pacific Biologics	[Product not found on the Betterlife.com website.]
Stephania delavaya + *Stephania sinica*	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).
Stephania pierrei	Boh Ra Phet Pung Chang Capsule (Saboo Luerd)	Phuketherb Ltd.	[NA]	http://phuketherbs.velocall.com/pd1086802810.htm †
[*Stephania* sp.]	[Chronofort]	[the Life Extension Vitamins]	[NA]	[http://lifeextensionvitamins.stores.yahoo.net/chnowwilu.html

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
Stephania 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/frmTemplateM.asp?CatalogID=2949&SubfolderID=31 †
Stephania 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.]
Stephania sp.	Ignite Your Life	NutritionStreet.com	[NA]	http://www.nutritionstreet.com/360facts.php †
		Healthynutritionaldiet.com	[NA]	[Retailer not found on the Internet.]
Stephania sp.	Ohco-Motion	NutritionBlvd.com DiscountBlvd.com	OHCO/Orient Herb Company	[Retailers not found on the Internet.]
Stephania sp.	Over-Eater's Diet	HerbsMD	Alive Energy	http://www.herbsmd.com/shop/xq/asp/pid.7716/qx/productdetail.htm †
Stephania sp.	Physical Transformation Formulas Over Eater's	InterNatural	Alive Energy	[Product not found on the Internatural.com website.]
Stephania sp.	Stephania & Astragalus Tea Pills	Morningstar Health	Plum Flower	http://www.morningstarhealth.com/store/product172.html †
Stephania sp.	Stephania Astragalus	Kang Le So	[NA]	[Retailer no longer found on the Internet.]

Species	Medicinal name	Retailer	Manufacturer	Web page with AA product
Stephania 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.]
[*Stephania* sp.]	[Bai Yao Zi Cepharantha Tuber (Stephaniae Cepharanthae Tuber); Dioscorea Root (Dioscoreae Rhizoma)]	[Eastern Chinese Medicine Export Company]	[NA]	[http://www.tcmtreatment.com/images/wholesale/herb-price/herb.index.htm] [Product available in wholesale price list of Chinese herbs.]
Stephania sp. + *Clematis* 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 *Stephaniae Tetrandrae* root (Han Fang Ji).
Stephania tetandra [*tetrandra*]	Stephania and Astragalus / Fang Ji Huang Qi Wan	Herbswest, LLC	[NA]	http://www.herbswest.net/items/13341.shtml †
Stephania tetandra [*tetrandra*]	Weight Loss	Alterna-Med, Inc.	Samra	[The link automatically relocates to - http://www.vitaminproshop.com/, but no product with *Stephania* 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 *Zingiber officinale* and not to Chinese wild ginger.]
[Chinese wild ginger]	[999 Zheng Tian Wan]	[Opane.com]	NA	[http://opane.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]	[Opane.com]	NA	[http://opane.stores.yahoo.net/heaidteamahe.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)]	[Opane.com]	[NA]	[http://opane.stores.yahoo.net/painbaozheng.html] [Product ingredients list includes Chinese wild ginger.]
		[PlazaQ.com]	[NA]	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	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.]
		Opane.com	Lanzhou Traditional Herbs	[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]	[Lanzhou Traditional Herbs]	[http://www.cgcmall.com/chuanxiong_mixture_p/hr00cxc1.htm] [Product ingredients list includes wild ginger.]
		[China-Herbs]	[Lanzhou Traditional Herbs]	[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.]
		[Wheatgrass for Your Health]	[Lanzhou Traditional Herbs]	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	[Product was not found in a search of the Internatural.com website.] [Other products with wild ginger as an ingredient were found on InterNatural website (see below).]
[Wild ginger]	[Four Elements Wild Ginger Flower Essence]	[InterNatural]	[Four Elements]	[http://www.internatural-alternative-health.com/ingr/ingr231722.cfm) [Product ingredients list includes wild ginger.]
Wild ginger	Mother Earth's Cough Syrup	Tao Herb Farm Vitanet	Heritage Products [Heritage Store brand]	[Product not found in a search of the Taoherbfarm.com website.] [http://www.myvitanet.com/motear4ozher.html [Product ingredients list includes wild ginger.] [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.] [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.]

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.taoofwellness.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 Wooly Dutchmanspipe (*Aristolochia tomentosa*)] [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
Aristolochia species	aristolochia guan mu tong guang mu tong
Aristolochia acuminata Lam. Syn. *Aristolochia tagala* Champ.	oval leaf Dutchman's pipe
Aristolochia argentina Griseb.	
Aristolochia baetica Linn. Syn. *Aristolochia bracteolata* Lam.	
Aristolochia bracteata Retz.	ukulwe
Aristolochia chilensis Bridges in Lindl.	
Aristolochia cinnabarina C.Y. Cheng & J.L. Wu	
Aristolochia clematitis L.	birthwort
Aristolochia contorta Bunge	ma dou ling tian xian teng
Aristolochia cymbifera Mart. & Zucc.	mil homens
Aristolochia debilis Siebold & Zucc. Syn. *Aristolochia longa* Thunb. Syn. *Aristolochia recurvilabra* Hance Syn. *Aristolochia sinarum* Lindl.	ma dou ling tian xian teng qing mu xiang sei-mokkou (Japanese) birthwort long birthwort
Aristolochia elegans Mast. Syn. *Aristolochia hassleriana* Chodat	
Aristolochia esperanzae Kuntze	
Aristolochia fangchi Y.C. Wu ex L.D. Chow & S.M. Hwang	guang fang ji fang ji mokuboi (Japanese) kwangbanggi (Korean) fang chi kou-boui (Japanese)
Aristolochia fimbriata Cham.	
Aristolochia indica L.	Indian birthwort
Aristolochia kaempferi Willd. Syn. *Aristolochia chrysops* (Stapf) E.H. Wilson ex Rehder Syn. *Aristolochia feddei* H. Lév Syn. *Aristolochia heterophylla* Hemsl Syn. *Aristolochia mollis* Dunn Syn. *Aristolochia setchuenensis* Franch. Syn. *Aristolochia shimadai* Hayata	yellowmouth Dutchman's pipe

Botanical name*	Common or other names
Syn. *Aristolochia thibetica* Franch. Syn. *Isotrema chrysops* Stapf Syn. *Isotrema heterophylla* (Hemsl.) Stapf Syn. *Isotrema lasiops* Stapf	
Aristolochia kwangsiensis Chun & F.C. How Syn. *Aristolochia austroszechuanica* C. B. Chien & C. Y. Cheng	
Aristolochia macrophylla Lam. Syn. *Aristolochia sipho* L'Hér.	Dutchman's-pipe
Aristolochia manschuriensis [*manshuriensis*] Kom. Syn. *Hocquartia manshuriensis* (Kom.) Nakai Syn. *Isotrema manchuriensis* (Kom.) H. Huber	manchurian birthwort manchurian Dutchman's pipe guang mu tong kan-mokutsu (Japanese) mokuboi (Japanese) kwangbanggi (Korean)
Aristolochia maurorum L.	
Aristolochia maxima Jacq. Syn. *Aristolochia maxima* var. *angustifolia Duchartre* in DC. Syn. *Howardia hoffmannii* Klotzsch	
Aristolochia mollissima Hance	
Aristolochia pistolochia L.	
Aristolochia rigida Duch.	
Aristolochia rotunda Linn.	
Aristolochia serpentaria L. Syn. *Aristolochia serpentaria* var. *hastata* (Nutt.) Duch.	Virginia snakeroot serpentaria Virginia serpentary
Aristolochia watsoni Wooton & Standley or *Aristolochia watsonii* Wooton & Standley Syn. *Aristolochia porphyrophylla* Pfeifer	
Aristolochia westlandii Hemsl. Or *Aristolochia westlandi* Hemsl.	
Aristolochia zollingeriana Miq. Syn. *Aristolochia kankauensis* Sasaki Syn. *Aristolochia roxburghiana* subsp. *kankauensis* (Sasaki) Kitam. Syn. *Hocquartia kankauensis* (Sasaki) Nakai ex Masam. Syn. *Aristolochia tagala* var. *kankauensis* (Sasaki) T. Yamaz.	
Asarum canadense Linn. Syn. *Asarum acuminatum* (Ashe) E.P. Bicknell Syn. *Asarum ambiguum* (E.P. Bicknell) Daniels Syn. *Asarum canadense* var. *ambiguum* (E.P. Bicknell) Farw. Syn. *Asarum canadense* var. *reflexum* (E.P. Bicknell) B.L. Rob. Syn. *Asarum furcatum* Raf. Syn. *Asarum medium* Raf.	wild ginger Indian ginger Canada snakeroot false coltsfoot colic root heart snakeroot Vermont snakeroot

Botanical name*	Common or other names
Syn. *Asarum parvifolium* Raf. Syn. *Asarum reflexum* E.P. Bicknell Syn. *Asarum rubrocinctum* Peattie	southern snakeroot
Asarum himalaicum Hook. f. & Thomson ex Klotzsch or *Asarum himalaycum* Hook. f. & Thomson ex Klotzsch	tanyou-saishin (Japanese)
Asarum splendens (F. Maek.) C.Y. Cheng & C.S. Yang	do-saishin (Japanese)
Bragantia wallichii R.Br. Specimen exists at New York Botanical Gardens. Tropicos does not list this species as a synonym for any *Thottea* species. Kew Gardens Herbarium does not recognize the genera *Bragantia*. Until additional information is obtained 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
Akebia species	akebia mu tong ku mu tong zi mutong bai mu tong mokutsu (Japanese) mokt'ong (Korean)
Akebia quinata (Houtt.) Decne. Syn. *Rajania quinata* Houtt.	chocolate vine fiveleaf akebia mu tong yu zhi zi mokutsu (japanese)
Akebia trifoliata (Thunb.) Koidz.	mu tong three leaf akebia yu zhi zi
Asarum forbesii Maxim.	batei-saishin (Japanese)
Asarum heterotropoides F. Schmidt Syn. *Asarum heterotropoides* F. Schmidt Syn. *Asiasarum heterotropoides* (F. Schmidt) F. Maek.	keirin-saishin (japanese) Chinese wild ginger Manchurian wild ginger bei xi xin xin xin
Asarum sieboldii Miq. Syn. *Asarum sieboldii* fo. *seoulense* (Nakai) C.Y. Cheng & C.S. Yang Syn. *Asarum sieboldii* var. *seoulensis* Nakai Syn. *Asiasarum heterotropoides* var. *seoulense* (Nakai) F. Maek. Syn. *Asiasarum sieboldii* (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
Clematis species	clematis mufangji clematidis ireisen (japanese) wojoksum (korean)
Clematis armandii Franch. Syn. *Clematis armandii* fo. *farquhariana* (W.T. Wang) Rehder & E.H. Wilson Syn. *Clematis armandii* var. *biondiana* (Pavol.) Rehder Syn. *Clematis biondiana* Pavol. Syn. *Clematis ornithopus* Ulbr.	armand's clematis chuan mu tong (stem) xiao mu tong armand's virgin bower
Clematis chinensis Osbeck.	chinese clematis wei ling xian (root)
Clematis hexapetala Pall.	
Clematis montana Buch.-Ham. ex DC. Syn. *Clematis insulari-alpina* Hayata	
Clematis uncinata Champ. ex Benth. Syn. *Clematis alsomitrifolia* Hayata Syn. *Clematis chinensis* var. *uncinata* (Champ. ex Benth.) Kuntze Syn. *Clematis drakeana* H. Lév. & Vaniot Syn. *Clematis floribunda* (Hayata) Yamam. Syn. *Clematis gagnepainiana* H. Lév. & Vaniot Syn. *Clematis leiocarpa* Oliv. Syn. *Clematis ovatifolia* T. Ito ex Maxim. Syn. *Clematis uncinata* var. *biternata* W.T. Wang Syn. *Clematis uncinata* var. *coriacea* Pamp. Syn. *Clematis uncinata* var. *floribunda* Hayata Syn. *Clematis uncinata* var. *ovatifolia* (T. Ito ex Maxim.) Ohwi ex Tamura Syn. *Clematis uncinata* var. *taitongensis* Y.C. Liu & C.H. Ou	
Cocculus species	cocculus
Cocculus carolinus (L.) DC. Syn. *Cebatha carolina* Britton Syn. *Epibaterium carolinum* (L.) Britton Syn. *Menispermum carolinum* L.	
Cocculus diversifolius DC. Syn. *Cocculus madagascariensis* Diels	
Cocculus hirsutus (L.) Diels Syn. *Cocculus villosus* DC. Syn. *Menispermum hirsutum* L.	
Cocculus indicus Royle Syn. *Anamirta paniculata* Colebr.	indian cockle
Cocculus laurifolius DC.	

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

Botanical name*	Common or other names
	tuteng zhuigufeng maofangji
Stephania species	stephania
Stephania tetrandra S. Moore	fen fang ji , fang ji fang ji (root) han fang ji kanboi (Japanese) hanbanggi (Korean) fun-boui (Japanese)
Vladimiria souliei (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 Quing 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 prdoucts 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 *Clematis armandi*	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 *Aristolochia serpentaria* (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 *Clematis armandi*	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

[a]Product 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	*Clematis chinensis* 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	*Single ingredient* Guan Mu Tong Ma Dou Ling *Herbal combinations including Guan Mu Tong as an ingredient* 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 [Tianjiang Pharmaceutical Co. Ltd., China]	Products contained arisotolochic acid	FDA 2001i (FDA News) http://www.fda.gov/bbs/topics/ENFORCE/2001/ENF00715.html
7/31/2001	Pacific Biologic Co., Clayton, CA	Herb- *Akebia Trifoliata Caulis* (Mu Tong) Herb- *Asarum Sieboldii Herba* cum Radix	Herbs contained aristolochic acid	FDA 2001j (Recalls)

Date	Company	Product(s) [manufacturers]	Reason for recall	Reference
		(Xi Xin) *Brands-* 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