NTP
National Toxicology Program

Report on Carcinogens
Draft Substance Profile for Aristolochic Acids

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Aristolochic Acids

Family of nitrophenanthrene carboxylic acids that occurs naturally in plants, primarily of the genera *Aristolochia* and *Asarum*

- Principal acids: AA1 and AA2

Use and Human Exposure

Use

- Traditional folk medicines
  - Chinese herbal medicine
  - Weight-loss regimen (inadvertently)
  - Name confusion

Exposure

- Ingestion of plants and botanical products
- FDA warning in 2001
- Still available legally in other countries
- Currently available via the Internet

Proposed Listing

Aristolochic acids are *known to be human carcinogens*

- Sufficient evidence from studies in humans
- Supporting mechanistic data

Sufficient Evidence in Humans

- Studies on individuals consuming botanical products containing aristolochic acids (AAs)
- Mechanistic data that aristolochic acids are the causative agent in the botanical products

Human Studies

- Aristolochic acid nephropathy (AAN): Belgian epidemic
- Urothelial cancer: Belgian epidemic
- Urothelial cancer: worldwide
- Summary of human data

AAN: Belgian Epidemic

- Chinese herbs containing AAs inadvertently consumed by 1,500 to 2,000 people as part of a weight loss regimen
  - ~ 5% developed nephropathy
  - > 100 cases at 2 major centers
- Association of nephropathy with AAs
  - Nephropathy developed immediately after ingestion of the herbs
  - No exposure to other agents known to be risk factors for nephropathy
  - AAs identified in the herbal products
  - AA-DNA adducts found in tissues (usually kidney or urothelial tissue) from some of the patients

Urothelial Cancer: Belgian Epidemic

- Prevalence of urothelial cancer in AAN patients undergoing renal transplant
  - Cosyns *et al.* 1999 (St. - Luc): 40% prevalence
  - Nortier *et al.* 1999, 2001 (Hospital Erasme): 46% prevalence
    - AA-DNA adducts detected in tissues from patients
    - Higher cumulative dose of AA in AAN patients with cancer compared to patients without cancer
    - No significant differences with other risk factors (e.g., smoking status, drug use) in AAN patients with and without urothelial cancer
  - Lemy *et al.* 2008 (update of Nortier) - similar findings
- Most tumors were in the upper urothelial tract; upper urothelial tract tumors are rare

Urothelial Cancer: Worldwide

- Balkan endemic nephropathy (BEN)
  - Similar pathology as AAN
  - Associated with upper urothelial cancer
  - Exposure is thought to be from contaminated wheat
  - AA proposed as risk factor based on mechanistic evidence
    - AA–DNA adducts
    - A:T → T:A transversions in the *p53* gene in tumor tissue (Grollman *et al.* 2007)

Urothelial Cancer: Worldwide

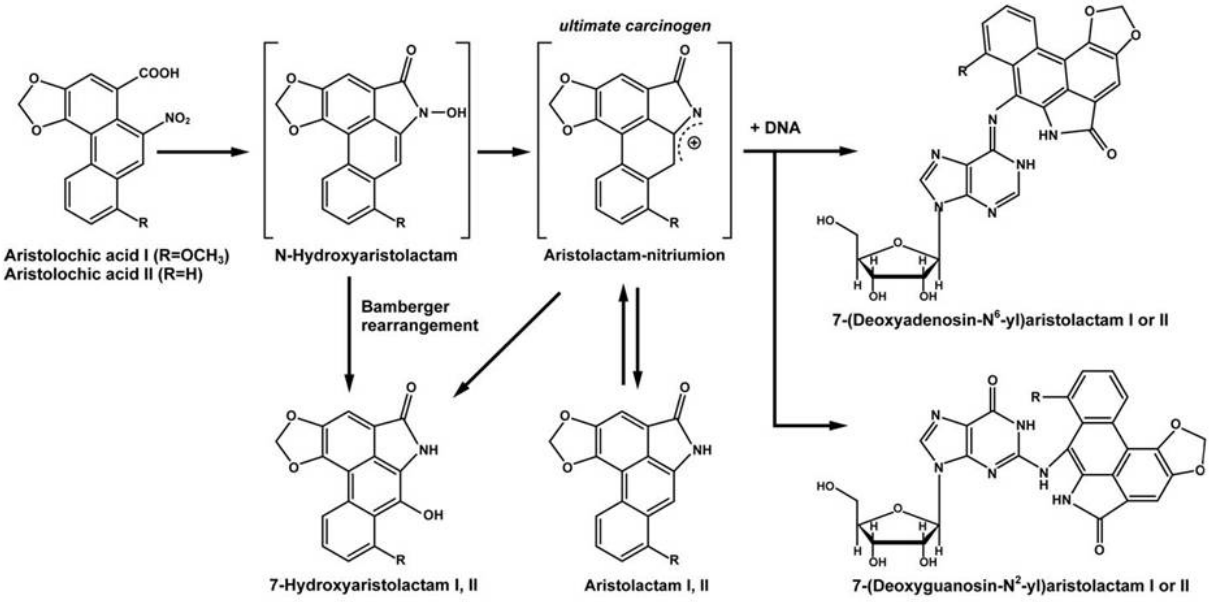
Clinical studies in China

- Transitional cell carcinoma among renal transplant or dialysis patients
  - Significant increased risks among patients consuming herbal remedies containing AA
    - 283 dialysis patients (Li *et al.* 2005)
      - OR = 37, 95% CI = 11 to 216
    - 1,429 renal transplant patients (Li *et al.* 2008)
      - RR = 5.85, 95% CI NR, $P < 0.0001$

Summary of Human Data

- Evidence supporting a causal association
  - High prevalence or risk estimates
  - Consistency
  - Dose/response relationship
  - AA-DNA adducts
  - A to T transversions in the p53 gene
- Agreement with IARC (2002, 2008)
  - Plants containing AA are carcinogenic to humans (IARC 2002)
  - Aristolochic acids are carcinogenic to humans (IARC 2008)

Metabolic Activation/Mechanism



Supporting Mechanistic Data

- Adducts associated with A to T transversions
  - Mammalian cells
  - Tissue (liver and kidney) from animals exposed to AA
- A to T transversions detected in oncogenes of AA-induced tumors
  - Animal studies: tumors of the forestomach, ear duct and lung
    - c-Ha-*ras*
  - Human: urothelial tumors
    - *p53* gene in AA-exposed patients

Studies in experimental animals

Study characteristics

- Substance
  - Individual or mixture of AA (AA1 or AA2)
  - Extracts from plants
  - Weight loss regimen
- Limitations
  - Small numbers
  - Short duration (most studies)
  - Few studies reported statistics

Sufficient Evidence from Studies in Experimental Animals

- Multiple tissues sites
- Multiple routes of exposure: oral, subcutaneous injection, intraperitoneal injection
- Multiple species: mice, rats and rabbits

Exposure to Extracts or Products Containing AAs

Substance	Species	Route	Tumor Sites
Extracts *Aristolochia* spp.	Rats	Oral	Forestomach, kidney
Extracts *Aristolochia* spp.	Rats	Injection	Sarcomas
Weight loss regimen	Rats (M)	Oral	Forestomach

Exposure to AAs

Species	Route	Tumor Sites (Primary)
Mice (F)	Oral	Forestomach, stomach, kidney, lung, uterine and malignant lymphoma
Rats	Oral	Forestomach, kidney, renal pelvis, urinary bladder, ear duct, thymus, small intestine, and pancreas
Rats (M)	Injection	Renal pelvis, sarcoma at injection site
Rabbits (F)	Injection	Kidney, urinary tract, mesothelioma of the peritoneal cavity

Aristolochic Acids

Proposed Listing: *Known to be Human Carcinogens*

- Sufficient evidence in humans
  - Excess urothelial cancer among AA-exposed patients
- Supporting mechanistic evidence
  - AA adducts cause A to T transversions
  - Transversions observed in oncogenes from AA-induced tumors
- AAs carcinogenic in experimental animals