PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 2.

Aromatic amines and aristolochic acids

Frederick A. Beland and M. Matilde Marques

Carcinogenicity in humans

Exposure to 4-aminobiphenyl, *o*-toluidine, 2-naphthylamine, and benzidine (Fig. 2.1) has been consistently associated with the induction of cancer of the urinary bladder in humans. This association is based upon occupational exposures, primarily of workers in the rubber and dye industries (IARC, 2010, 2012a). Similarly, occupational exposure to 4,4′-methylenebis(2-chloroaniline) (MOCA; Fig. 2.1), a curing agent for polyurethane pre-polymers, causes cancer of the bladder in humans, although the epidemiological data are not as strong as those for the other agents (IARC, 2010, 2012a). Certain azo dyes that are used in commercial products, for example, Direct Black 38, Direct Blue 6, and Direct Brown 95 (Fig. 2.2), are known to undergo azo reduction in vivo to yield the carcinogen benzidine. The overall evaluation for these dyes was raised to Group 1 based on this mechanistic information, although at present the corresponding epidemiological data are considered to provide *inadequate evidence* for the carcinogenicity of these dyes in humans (IARC, 2010, 2012a).

Cigarette smoke contains 4-aminobiphenyl, *o*-toluidine, and 2-naphthylamine, and tobacco smoking causes cancer of the bladder in humans (IARC, 1986, 2004, 2010, 2012a, b). The contribution of 4-aminobiphenyl, *o*-toluidine, and 2-naphthylamine to the induction of smoking-related cancer of the bladder is confounded by the presence of numerous other carcinogens, including carcinogenic aromatic amines, in tobacco smoke. Cigarette smoking also causes other cancers (e.g. cancer of the lung, oral cavity, and pancreas, and possibly breast cancer), but at present it is unclear whether these cancers can be attributed to 4-aminobiphenyl, *o*-toluidine, 2-naphthylamine, or other aromatic amines. Hair dyes are an additional source of exposure to 4-aminobiphenyl and *o*-toluidine (IARC, 2010, 2012a; Lizier and Boldrin Zanoni, 2012).

Exposure to phenacetin (Fig. 2.1), through its use as an analgesic, causes cancer of the kidney and ureter in humans (IARC, 2012c). Chlornaphazine (Fig. 2.1), a chemotherapeutic agent that has been used for the treatment of Hodgkin lymphoma and for the control of polycythaemia vera, causes cancer of the bladder in humans, presumably due to metabolism to 2-naphthylamine (IARC, 2012c). An additional source of human exposure to *o*-toluidine is from the anaesthetic prilocaine (Fig. 2.1) (IARC, 2010, 2012a).

Exposure to herbal remedies prepared from plant species of the genus *Aristolochia* has been causally

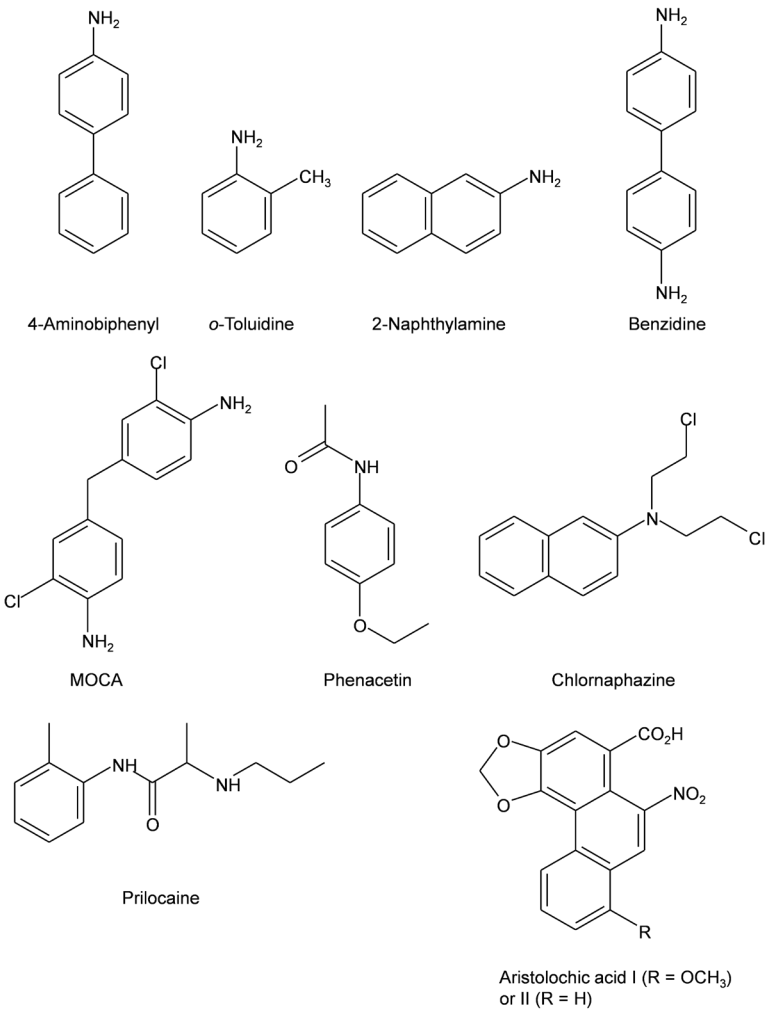
associated with the induction of urothelial cancer in humans (IARC, 2002, 2012c). The induction of urothelial tumours has been attributed to the presence of aristolochic acid I and aristolochic acid II (Fig. 2.1).

Metabolism and DNA adduct formation

4-Aminobiphenyl, *o*-toluidine, 2-naphthylamine, and MOCA are metabolized to electrophilic *N*-hydroxyarylamines by hepatic cytochrome P450 enzymes (IARC, 2010, 2012a, c). The *N*-hydroxyarylamines undergo acid-catalysed reactions with DNA to form a variety of DNA adducts. With the exception of MOCA, C8-substituted deoxyguanosine adducts are typically the major products, along with smaller amounts of N^2-substituted deoxyguanosine and N^6-substituted deoxyadenosine adducts (Fig. 2.3); with MOCA, only C8-substituted deoxyadenosine adducts have been detected (IARC, 2010, 2012a). These DNA adducts can also be formed from reactive esters of *N*-hydroxyarylamines (e.g. *N*-sulfoxyarylamines and *N*-acetoxyarylamines). Benzidine, which has two amino groups, also forms a C8-substituted deoxyguanosine adduct via a pathway involving an initial *N*-acetylation followed by *N*-hydroxylation of the remaining amino function (Fig. 2.3).

The carcinogenic activity of aromatic amines in the bladder in humans has been attributed to an initial *N*-hydroxylation, catalysed by hepatic cytochrome P450 enzymes, followed by transport of the *N*-hydroxyarylamines to the bladder as either aglycones or *N*-glucuronide conjugates (Bois et al., 1995). In the bladder lumen, the *N*-hydroxyarylamine *N*-glucuronides can undergo acid-catalysed hydrolysis to release the *N*-hydroxyarylamines, which can enter the bladder epithelium and react with DNA either directly or after esterification. DNA adducts derived from 4-aminobiphenyl, *o*-toluidine, benzidine, and MOCA have been detected in bladder tissue or exfoliated bladder cells from exposed individuals (IARC, 2010, 2012a; Böhm et al., 2011; Lee et al., 2014). With the exception of MOCA, which forms only C8-substituted deoxyadenosine adducts, the major – if not the only – adduct detected in each instance was a C8-substituted deoxyguanosine adduct. The importance of urinary acidity for the hydrolysis of the *N*-hydroxyarylamine *N*-glucuronides and perhaps for the reaction of the *N*-hydroxyarylamines with urothelial DNA has been demonstrated by the positive correlation between urinary acidity and the levels of benzidine DNA adducts in exfoliated bladder cells from exposed workers (Rothman et al., 1997). Additional support for this mechanism comes from the observation of a positive

Fig. 2.1. Structures of IARC Group 1 aromatic amines, drugs that are metabolized to Group 1 aromatic amines, and aristolochic acids. MOCA, 4,4′-methylenebis(2-chloroaniline).

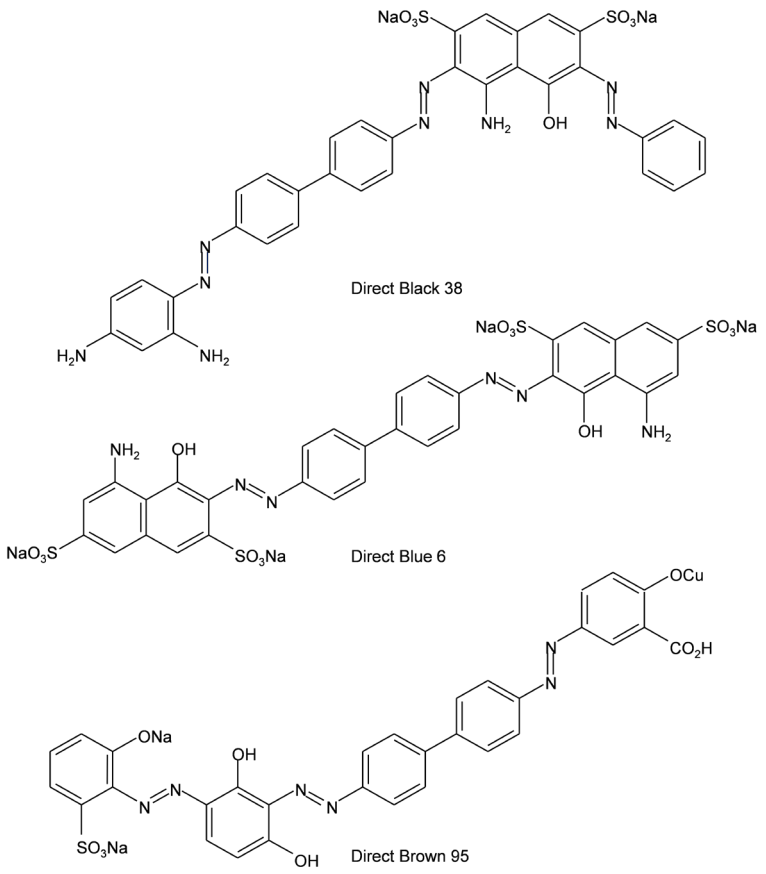


correlation between urinary acidity and the incidence of bladder cancer in smokers (Alguacil et al., 2011).

The major metabolic activation pathway for aristolochic acid I and aristolochic acid II involves nitro reduction, followed by cyclization to give *N*-hydroxyaristolactams, which – in contrast to other *N*-hydroxyarylamides – do not appear to require additional activation to react with DNA (Stiborová et al., 2011, 2013). Nonetheless, *N*-hydroxyaristolactams have been shown to serve as substrates for human sulfotransferases, particularly sulfotransferase family cytosolic 1B member 1 (SULT1B1), forming highly reactive *N*-sulfoxy derivatives (Sidorenko et al., 2014). The major adducts resulting from the *N*-hydroxyaristolactams are N^2-substituted deoxyguanosines and N^6-substituted deoxyadenosines (Fig. 2.4) (IARC, 2002, 2012c).

DNA adducts derived from aristolochic acids have been detected in renal tissue from patients who had been exposed to aristolochic acid-containing herbal products and from individuals who had consumed wheat grains contaminated with *Aristolochia* (IARC, 2002, 2012c; Chen et al., 2012; Jelaković et al., 2012; Schmeiser et al., 2012, 2014; Yun et al., 2013, 2014). Typically, the major lesion detected is an N^6-deoxyadenosine adduct derived from aristolochic acid I, accompanied by smaller amounts of a similar adduct derived from aristolochic acid II and an N^2-deoxyguanosine adduct derived from aristolochic acid I.

Fig. 2.2. Structures of benzidine-derived azo dyes.



Alterations in the *TP53* tumour suppressor gene in humans

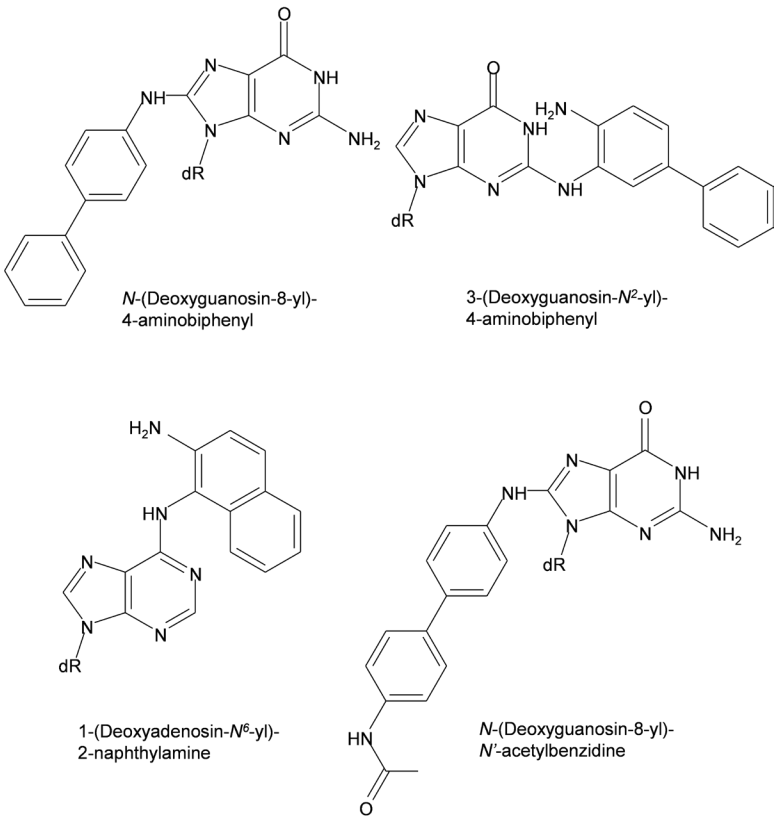
Mutations in the *TP53* tumour suppressor gene have been found in approximately 50% of all bladder cancers in humans (Petitjean et al., 2007), with G:C base substitution mutations occurring to a greater extent than A:T base substitution mutations.

TP53 gene mutations have been detected in bladder cancer patients exposed occupationally to 4-aminobiphenyl, 2-naphthylamine, and/or benzidine (Sørlie et al., 1998). The mutations occurred exclusively in higher-grade tumours (grades 2 or 3, i.e. moderately or poorly differentiated) and only at G:C base pairs.

Mutant p53 protein has also been detected in workers exposed occupationally to benzidine (Xiang et al., 2007). The occurrence and the amount of mutant protein were positively correlated with the level of benzidine exposure and the extent of neoplastic changes in exfoliated urothelial cells.

Urothelial tumours arising from exposure to aristolochic acids have been consistently shown to carry mutations in the *TP53* tumour suppressor gene, of which the most common mutation is an A → T transversion

Fig. 2.3. Structures of representative DNA adducts obtained from Group 1 aromatic amines. dR, deoxyribose.



mutation (reviewed in IARC, 2002, 2012c; Hollstein et al., 2013; see also Schmeiser et al., 2012; Chen et al., 2013; Hoang et al., 2013; Poon et al., 2013; Aydin et al., 2014). These mutations have been demonstrated in tumour tissue from patients who had consumed herbal preparations containing aristolochic acids and in urothelial cancer tissues of patients from regions with a high incidence of endemic (Balkan) nephropathy due to consumption of grains contaminated with *Aristolochia*. The presence of A → T transversion mutations is consistent with the observation that the major lesion detected in patients is an N^6-deoxyadenosine adduct derived from aristolochic acid I.

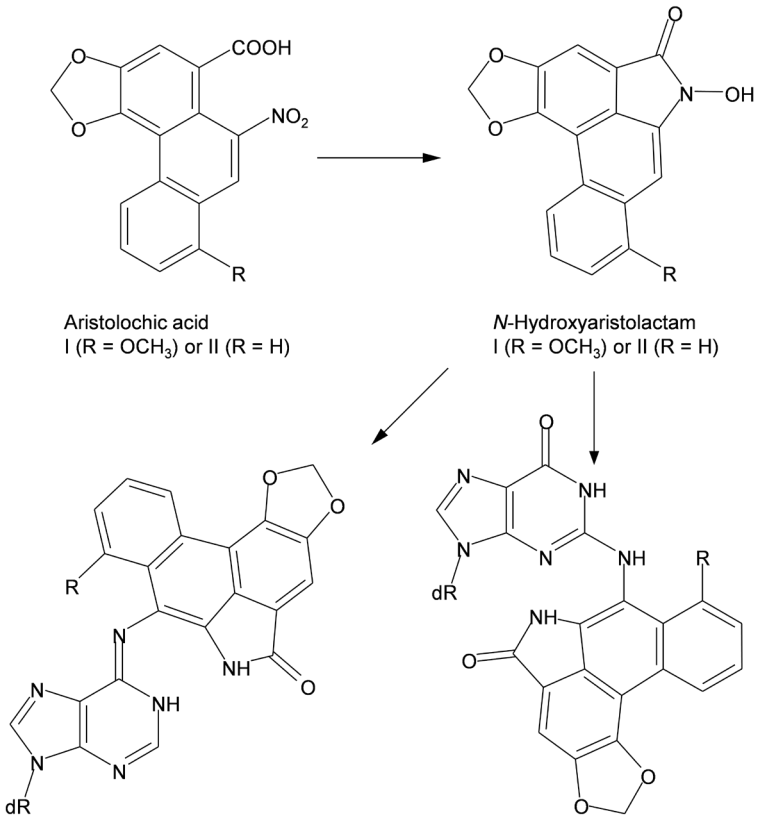
Tumour sites and the mechanism of tumour induction in experimental animals

4-Aminobiphenyl, 2-naphthylamine, and MOCA are bladder carcinogens in dogs (IARC, 2010, 2012a). Bladder tumours also occur in mice treated with 4-aminobiphenyl. As with humans, the induction of bladder tumours in dogs is thought to result from hepatic *N*-hydroxylation, transport of the *N*-hydroxyarylamines to the bladder as either aglycones or *N*-hydroxyarylamine *N*-glucuronides, and subsequent hydrolysis of the *N*-hydroxyarylamine *N*-glucuronides in the bladder lumen to release the *N*-hydroxyarylamines. Support for this mechanism comes from the observation that the DNA lesions detected in the bladders of dogs treated with 4-aminobiphenyl or MOCA appear to be C8-substituted deoxyguanosine and deoxyadenosine adducts that are identical to the DNA adducts detected in bladder tissues or exfoliated bladder cells from humans exposed to these carcinogens (IARC, 2010, 2012a). 2-Naphthylamine DNA adducts detected in the bladders of dogs exposed to 2-naphthylamine are entirely consistent with a mechanism involving the formation of *N*-hydroxy-2-naphthylamine (IARC, 2010).

In contrast to what is observed in humans, benzidine in not a bladder carcinogen in dogs. This lack of carcinogenicity has been attributed to the inability of dogs to *N*-acetylate aromatic amines (IARC, 2010). With most aromatic amines, *N*-acetylation is considered to be a detoxification event; however, with benzidine, *N*-acetylation appears to be required to give *N*-acetylbenzidine, which undergoes a subsequent *N*-hydroxylation of the second amino function. This metabolic pathway occurs in humans but not in dogs.

4-Aminobiphenyl, *o*-toluidine, 2-naphthylamine, benzidine, benzidine-based dyes, and MOCA induce hepatocellular tumours in mice (IARC, 2010, 2012a). DNA adducts derived from 4-aminobiphenyl and benzidine have been examined in liver tissue from exposed mice, and the major DNA lesions detected in each instance were C8-substituted deoxyguanosine adducts, consistent with formation of an *N*-hydroxyarylamine intermediate (IARC, 2010). In rats, DNA adducts derived from benzidine-based dyes and MOCA have been examined in the liver, which is also a target tissue for these

Fig. 2.4. Structures of DNA adducts derived from aristolochic acids through *N*-hydroxyaristolactam intermediates. dR, deoxyribose.



carcinogens, and again the major DNA adducts detected in each instance were consistent with formation of an *N*-hydroxyarylamine intermediate (IARC, 2010).

In mice, there appears to be a balance between hepatic *N*-acetylation, which is considered to be a detoxification step, and hepatic *N*-hydroxylation, which is considered to be an activation step. Should *N*-hydroxylation occur, the *N*-hydroxyarylamines can be further activated by hepatic *O*-acetylation to yield *O*-acetoxyarylamines, which can give rise to the DNA adducts detected in liver tissue (IARC, 2010). C8-substituted deoxyguanosine adducts have also been detected in the bladder DNA of mice treated with 4-aminobiphenyl (Poirier et al., 1995). These adducts presumably arise from hepatic *N*-hydroxylation and possibly *O*-acetylation of *N*-hydroxy-4-aminobiphenyl in the bladder epithelium.

The carcinogenicity of aristolochic acids has been assessed in rats and to a lesser extent in mice and rabbits, primarily by oral dosing (IARC, 2002, 2012c). Aristolochic acid I and mixtures of aristolochic acids I and II consistently induce tumours of the forestomach in rats. Tumours of the kidney have been reported to occur sporadically. Mice treated with mixtures of aristolochic acids I and II develop tumours of the forestomach, kidney, and lung. In rabbits, mixtures of aristolochic acids I and II administered intraperitoneally are associated with tumours of the kidney, ureter, and peritoneal cavity.

DNA adducts derived from aristolochic acid I and aristolochic acid II have been detected in target tissues in mice (forestomach, kidney, and lung), rats (forestomach and kidney), and rabbits (kidney) (IARC, 2002, 2012c; Debelle et al., 2003; Gillerot et al., 2003; Dong et al., 2006; Mei et al., 2006; Shibutani et al., 2007; Chan et al., 2008; Rosenquist et al., 2010; Shibutani et al., 2010; Baudoux et al., 2012; McDaniel et al., 2012; Wang et al., 2012a; Yun et al., 2013, 2014). Typically, three DNA adducts are detected: an N^6-deoxyadenosine adduct derived from aristolochic acid I, an N^6-deoxyadenosine adduct derived from aristolochic acid II, and an N^2-deoxyguanosine adduct derived from aristolochic acid I.

Oncogene alterations in experimental animals

Transversion mutations at codon 61 of the H-*Ras* oncogene (CAA → AAA) have been observed in the livers of mice treated with 4-aminobiphenyl (IARC, 2010, 2012a). G → T transversion mutations in the *cII* transgene have been detected in the livers and bladders of transgenic mice treated with 4-aminobiphenyl (Wang et al., 2012b; Yoon et al., 2012). The occurrence of these mutations at G:C base pairs is consistent with the observation that the major DNA adduct detected in target tissues

after exposure to 4-aminobiphenyl is a C8-substituted deoxyguanosine adduct.

Transversion mutations at codon 61 of the H-*Ras* oncogene (CAA → CTA) have been detected in tumours from rats and mice fed mixtures of aristolochic acids I and II and/or aristolochic acid I (IARC, 2002, 2012c; Wang et al., 2011, 2012a). A → T transversion mutations have also been detected in the *cII* transgene of rats and the *cII* and *lacZ* transgenes of mice treated with mixtures of aristolochic acids I and II, and in the *gpt* transgene of mice treated with aristolochic acid I or aristolochic acid II (IARC, 2012c; McDaniel et al., 2012; Xing et al., 2012). The occurrence of these mutations at A:T base pairs is consistent with the observation that the major DNA lesions detected in target tissues after exposure to aristolochic acids are N^6-substituted deoxyadenosine adducts.

Summary

In humans, exposure to aromatic amines and aristolochic acids that are IARC Group 1 carcinogens has been associated with induction of tumours of the urinary tract. With aromatic amines, the primary tumour site is the bladder; with aristolochic acids, the primary site for tumour formation is the kidney. Experimental animals treated with aromatic amines or aristolochic acids develop tumours of the urinary tract; tumours also arise in other tissues, primarily the liver.

Aromatic amines and aristolochic acids that are IARC Group 1 carcinogens are metabolized by amine oxidation (in the case of aromatic amines) or nitro reduction (in the case of aristolochic acids) to *N*-hydroxyarylamine metabolites in both humans and experimental animals. These *N*-hydroxyarylamine intermediates can react directly with DNA or be further activated by *O*-esterification to give rise to DNA adducts, predominantly at deoxyguanosine (primarily with aromatic amines) and deoxyadenosine (primarily with aristolochic acids), in tumour target tissues of humans and experimental animals.

Mutations of the *TP53* tumour suppressor gene consistent with the major DNA adducts derived from aromatic amines and aristolochic acids have been detected in tumours from exposed humans. Similarly, mutations of the H-*Ras* oncogene consistent with the major DNA adducts derived from aromatic amines and aristolochic acids have been found in target tissues of experimental animals.

Disclaimer

The views expressed in this chapter do not necessarily represent those of the United States Food and Drug Administration.

References

Alguacil J, Kogevinas M, Silverman DT, Malats N, Real FX, García-Closas M, et al. (2011). Urinary pH, cigarette smoking and bladder cancer risk. Carcinogenesis. 32(6):843–7. http://dx.doi.org/10.1093/carcin/bgr048 PMID:21402590

Aydin S, Dekairelle A-F, Ambroise J, Durant J-F, Heusterspreute M, Guiot Y, et al. (2014). Unambiguous detection of multiple *TP53* gene mutations in AAN-associated urothelial cancer in Belgium using laser capture microdissection. PLoS ONE. 9(9):e106301. http://dx.doi.org/10.1371/journal.pone.0106301 PMID:25184754

Baudoux TER, Pozdzik AA, Arlt VM, De Prez EG, Antoine M-H, Quellard N, et al. (2012). Probenecid prevents acute tubular necrosis in a mouse model of aristolochic acid nephropathy. Kidney Int. 82(10):1105–13. http://dx.doi.org/10.1038/ki.2012.264 PMID:22854641

Böhm F, Schmid D, Denzinger S, Wieland WF, Richter E (2011). DNA adducts of *ortho*-toluidine in human bladder. Biomarkers. 16(2):120–8. http://dx.doi.org/10.3109/1354750X.2010.534556 PMID:21117897

Bois FY, Krowech G, Zeise L (1995). Modeling human interindividual variability in metabolism and risk: the example of 4-aminobiphenyl. Risk Anal. 15(2):205–13. http://dx.doi.org/10.1111/j.1539-6924.1995.tb00314.x PMID:7597257

Chan W, Yue H, Poon WT, Chan Y-W, Schmitz OJ, Kwong DWJ, et al. (2008). Quantification of aristolochic acid-derived DNA adducts in rat kidney and liver by using liquid chromatography-electrospray ionization mass spectrometry. Mutat Res. 646(1–2):17–24. http://dx.doi.org/10.1016/j.mrfmmm.2008.08.012 PMID:18812181

Chen C-H, Dickman KG, Moriya M, Zavadil J, Sidorenko VS, Edwards KL, et al. (2012). Aristolochic acid-associated urothelial cancer in Taiwan. Proc Natl Acad Sci USA. 109(21):8241–6. http://dx.doi.org/10.1073/pnas.1119920109 PMID:22493262

Chen C-H, Dickman KG, Huang C-Y, Moriya M, Shun C-T, Tai H-C, et al. (2013). Aristolochic acid-induced upper tract urothelial carcinoma in Taiwan: clinical characteristics and outcomes. Int J Cancer. 133(1):14–20. http://dx.doi.org/10.1002/ijc.28013 PMID:23292929

Debelle F, Nortier J, Arlt VM, De Prez E, Vienne A, Salmon I, et al. (2003). Effects of dexfenfluramine on aristolochic acid nephrotoxicity in a rat model for Chinese-herb nephropathy. Arch Toxicol. 77(4):218–26. http://dx.doi.org/10.1007/s00204-003-0438-y PMID:12698237

Dong H, Suzuki N, Torres MC, Bonala RR, Johnson F, Grollman AP, et al. (2006). Quantitative determination of aristolochic acid-derived DNA adducts in rats using ^{32}P-postlabeling/polyacrylamide gel electrophoresis analysis. Drug Metab Dispos. 34(7):1122–7. http://dx.doi.org/10.1124/dmd.105.008706 PMID:16611860

Gillerot G, Goffin E, Moulin P, Arlt VM, Phillips DH, Cosyns J-P, et al. (2003). Aristolochic acid nephropathy and the peritoneum: functional, structural, and molecular studies. Kidney Int. 64(5):1883–92. http://dx.doi.org/10.1046/j.1523-1755.2003.00275.x PMID:14531824

Hoang ML, Chen C-H, Sidorenko VS, He J, Dickman KG, Yun BH, et al. (2013). Mutational signature of aristolochic acid exposure as revealed by whole-exome sequencing. Sci Transl Med. 5(197):197ra102. http://dx.doi.org/10.1126/scitranslmed.3006200 PMID:23926200

Hollstein M, Moriya M, Grollman AP, Olivier M (2013). Analysis of *TP53* mutation spectra reveals the fingerprint of the potent environmental carcinogen, aristolochic acid. Mutat Res. 753(1):41–9. http://dx.doi.org/10.1016/j.mrrev.2013.02.003 PMID:23422071

IARC (1986). Tobacco smoking. IARC Monogr Eval Carcinog Risks Hum. 38:1–421. PMID:3460963. Available from: http://publications.iarc.fr/56.

IARC (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monogr Eval Carcinog Risks Hum. 82:1–556. PMID:12687954. Available from: http://publications.iarc.fr/100.

IARC (2004). Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risks Hum. 83:1–1438. PMID:15285078. Available from: http://publications.iarc.fr/101.

IARC (2010). Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risks Hum. 99:1–658. PMID:21528837. Available from: http://publications.iarc.fr/117.

IARC (2012a). Chemical agents and related occupations. IARC Monogr Eval Carcinog Risks Hum. 100F:1–599. PMID:23189753. Available from: http://publications.iarc.fr/123.

IARC (2012b). Personal habits and indoor combustions. IARC Monogr Eval Carcinog Risks Hum. 100E:1–575. PMID:23193840. Available from: http://publications.iarc.fr/122.

IARC (2012c). Pharmaceuticals. IARC Monogr Eval Carcinog Risks Hum. 100A:1–437. PMID:23189749. Available from: http://publications.iarc.fr/118.

Jelaković B, Karanović S, Vuković-Lela I, Miller F, Edwards KL, Nikolić J, et al. (2012). Aristolactam-DNA adducts are a biomarker of environmental exposure to aristolochic acid. Kidney Int. 81(6):559–67. http://dx.doi.org/10.1038/ki.2011.371 PMID:22071594

Lee HW, Wang HT, Weng MW, Hu Y, Chen WS, Chou D, et al. (2014). Acrolein- and 4-aminobiphenyl-DNA adducts in human bladder mucosa and tumor tissue and their mutagenicity in human urothelial cells. Oncotarget. 5(11):3526–40. http://dx.doi.org/10.18632/oncotarget.1954 PMID:24939871

Lizier TM, Boldrin Zanoni MV (2012). Effect of ionic liquid on the determination of aromatic amines as contaminants in hair dyes by liquid chromatography coupled to electrochemical detection. Molecules. 17(7):7961–79. http://dx.doi.org/10.3390/molecules17077961 PMID:22751261

McDaniel LP, Elander ER, Guo X, Chen T, Arlt VM, Mei N (2012). Mutagenicity and DNA adduct formation by aristolochic acid in the spleen of Big Blue® rats. Environ Mol Mutagen. 53(5):358–68. http://dx.doi.org/10.1002/em.21696 PMID:22508110

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. http://dx.doi.org/10.1016/j.mrfmmm.2006.08.004 PMID:17010389

Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P, et al. (2007). Impact of mutant p53 functional properties on *TP53* mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat. 28(6):622–9. http://dx.doi.org/10.1002/humu.20495 PMID:17311302

Poirier MC, Fullerton NF, Smith BA, Beland FA (1995). DNA adduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. Carcinogenesis. 16(12):2917–21. http://dx.doi.org/10.1093/carcin/16.12.2917 PMID:8603464

Poon SL, Pang S-T, McPherson JR, Yu W, Huang KK, Guan P, et al. (2013). Genome-wide mutational signatures of aristolochic acid and its application as a screening tool. Sci Transl Med. 5(197):197ra101. http://dx.doi.org/10.1126/scitranslmed.3006086 PMID:23926199

Rosenquist TA, Einolf HJ, Dickman KG, Wang L, Smith A, Grollman AP (2010). Cytochrome P450 1A2 detoxicates aristolochic acid in the mouse. Drug Metab Dispos. 38(5):761–8. http://dx.doi.org/10.1124/dmd.110.032201 PMID:20164109

Rothman N, Talaska G, Hayes RB, Bhatnagar VK, Bell DA, Lakshmi VM, et al. (1997). Acidic urine pH is associated with elevated levels of free urinary benzidine and *N*-acetylbenzidine and urothelial cell DNA adducts in exposed workers. Cancer Epidemiol Biomarkers Prev. 6(12):1039–42. http://cebp.aacrjournals.org/content/6/12/1039.full.pdf PMID:9419400

Schmeiser HH, Kucab JE, Arlt VM, Phillips DH, Hollstein M, Gluhovschi G, et al. (2012). Evidence of exposure to aristolochic acid in patients with urothelial cancer from a Balkan endemic nephropathy region of Romania. Environ Mol Mutagen. 53(8):636–41. http://dx.doi.org/10.1002/em.21732 PMID:22987305

Schmeiser HH, Nortier JL, Singh R, Gamboa da Costa G, Sennesael J, Cassuto-Viguier E, et al. (2014). Exceptionally long-term persistence of DNA adducts formed by carcinogenic aristolochic acid I in renal tissue from patients with aristolochic acid nephropathy. Int J Cancer. 135(2):502–7. http://dx.doi.org/10.1002/ijc.28681 PMID:24921086

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. http://dx.doi.org/10.1124/dmd.107.014688 PMID:17392392

Shibutani S, Bonala RR, Rosenquist T, Rieger R, Suzuki N, Johnson F, et al. (2010). Detoxification of aristolochic acid I by O-demethylation: less nephrotoxicity and genotoxicity of aristolochic acid Ia in rodents. Int J Cancer. 127(5):1021–7. http://dx.doi.org/10.1002/ijc.25141 PMID:20039324

Sidorenko VS, Attaluri S, Zaitseva I, Iden CR, Dickman KG, Johnson F, et al. (2014). Bioactivation of the human carcinogen aristolochic acid. Carcinogenesis. 35(8):1814–22. http://dx.doi.org/10.1093/carcin/bgu095 PMID:24743514

Sørlie T, Martel-Planche G, Hainaut P, Lewalter J, Holm R, Børresen-Dale A-L, et al. (1998). Analysis of *p53*, *p16*^{MTS1}, *p21*^{WAF1} and H-*ras* in archived bladder tumours from workers exposed to aromatic amines. Br J Cancer. 77(10):1573–9. http://dx.doi.org/10.1038/bjc.1998.259 PMID:9635831

Stiborová M, Mareš J, Frei E, Arlt VM, Martínek V, Schmeiser HH (2011). The human carcinogen aristolochic acid I is activated to form DNA adducts by human NAD(P)H:quinone oxidoreductase without the contribution of acetyltransferases or sulfotransferases. Environ Mol Mutagen. 52(6):448–59. http://dx.doi.org/10.1002/em.20642 PMID:21370283

Stiborová M, Martínek V, Frei E, Arlt VM, Schmeiser HH (2013). Enzymes metabolizing aristolochic acid and their contribution to the development of aristolochic acid nephropathy and urothelial cancer. Curr Drug Metab. 14(6):695–705. http://dx.doi.org/10.2174/1389200211314060006 PMID:23701164

Wang S, Sugamori KS, Brenneman D, Hsu I, Calce A, Grant DM (2012b). Influence of arylamine *N*-acetyltransferase, sex, and age on 4-aminobiphenyl-induced in vivo mutant frequencies and spectra in mouse liver. Environ Mol Mutagen. 53(5):350–7. http://dx.doi.org/10.1002/em.21695 PMID:22508569

Wang Y, Arlt VM, Roufosse CA, McKim KL, Myers MB, Phillips DH, et al. (2012a). ACB-PCR measurement of H-*ras* codon 61 CAA→CTA mutation provides an early indication of aristolochic acid I carcinogenic effect in tumor target tissues. Environ Mol Mutagen. 53(7):495–504. http://dx.doi.org/10.1002/em.21710 PMID:22729866

Wang Y, Meng F, Arlt VM, Mei N, Chen T, Parsons BL (2011). Aristolochic acid-induced carcinogenesis examined by ACB-PCR quantification of H-*Ras* and K-*Ras* mutant fraction. Mutagenesis. 26(5):619–28. http://dx.doi.org/10.1093/mutage/ger023 PMID:21642617

Xiang C-Q, Shen C-L, Wu Z-R, Qin Y-Q, Zhang Y-Y, Liu C-Z, et al. (2007). Detection of mutant p53 protein in workers occupationally exposed to benzidine. J Occup Health. 49(4):279–84. http://dx.doi.org/10.1539/joh.49.279 PMID:17690521

Xing G, Qi X, Chen M, Wu Y, Yao J, Gong L, et al. (2012). Comparison of the mutagenicity of aristolochic acid I and aristolochic acid II in the *gpt* delta transgenic mouse kidney. Mutat Res. 743(1–2):52–8. http://dx.doi.org/10.1016/j.mrgentox.2011.12.021 PMID:22245565

Yoon J-I, Kim S-I, Tommasi S, Besaratinia A (2012). Organ specificity of the bladder carcinogen 4-aminobiphenyl in inducing DNA damage and mutation in mice. Cancer Prev Res (Phila). 5(2):299–308. http://dx.doi.org/10.1158/1940-6207.CAPR-11-0309 PMID:22086680

Yun BH, Rosenquist TA, Nikolić J, Dragičević D, Tomić K, Jelaković B, et al. (2013). Human formalin-fixed paraffin-embedded tissues: an untapped specimen for biomonitoring of carcinogen DNA adducts by mass spectrometry. Anal Chem. 85(9):4251–8. http://dx.doi.org/10.1021/ac400612x PMID:23550627

Yun BH, Yao L, Jelaković B, Nikolić J, Dickman KG, Grollman AP, et al. (2014). Formalin-fixed paraffin-embedded tissue as a source for quantitation of carcinogen DNA adducts: aristolochic acid as a prototype carcinogen. Carcinogenesis. 35(9):2055–61. http://dx.doi.org/10.1093/carcin/bgu101 PMID:24776219