GENERAL DISCUSSION OF COMMON MECHANISMS FOR AROMATIC AMINES

Ever since certain aromatic amines have been shown to be carcinogenic in humans the question has been raised how the chemical structure determines the biological effects, because a better understanding of this relationship could help assess the hazard and the risk associated with exposure to these chemicals. The common denominator is an amino-group bound to an aromatic system. The chemical reactivity of this amino group depends on the mesomeric interaction with the aromatic system, which is determined by further substituents and steric factors (Beland *et al.*, 1997; Marques *et al.*, 1997).

Recent progress in cancer research has revealed the complexity of the interaction between exogenous exposures and the physiology of an organism. The existing knowledge favours the idea that common principles combine the many aromatic amines and it might be concluded that most, if not all aromatic amines have a carcinogenic potential. The following considerations should help to answer the questions:

1. Do aromatic amines have generally a carcinogenic potential?
2. Is there a common mode of action that allows us to draw this conclusion?

1. Metabolic Activation

Metabolic activation was the leading concept to find out how aromatic amines cause biological effects. Both acute and chronic toxicity are held to depend on the metabolic activation of the amino group. The key reaction responsible for all the biological activities is the *N*-oxidation to aryl-*N*-hydroxylamines. Either the free amine or the acetamide can be *N*-oxidized. Thus an equilibrium exists between the two, which is determined by the competing activity of *N*-acetyltransferases and *N*-deacetylases. Frederick *et al.* (1985) described the equilibrium between benzidine, *N*-acetylbenzidine and *N,N′*-diacetylbenzidine in liver slices. The distribution in this eqilibrium is important, since acetylation of the amine to the acetamide is an inactivating reaction, as is the *C*-oxidation of the aromatic system. Dogs develop more readily bladder tumours with benzidine than several other species, because dogs as "non-acetylators" lack one of the inactivating metabolic steps (Lakshmi *et al.*, 1995).

Both the *N*-hydroxylamine and the *N*-hydroxyacetamide may be further activated by activating the leaving group through conjugation of the hydroxy group with sulfate or acetate, the sulfate being usually a better mutagen than the acetate. Eventually, the biological activity depends on the bioavailability of a nitrenium ion, an ultimate reactive metabolite that reacts with DNA, RNA, and proteins. The ultimate metabolites of most arylamines react *in vitro* and *in vivo* with C-8 of guanine and the respective adduct has been made responsible for point mutations.

The metabolic activation of benzidine is an interesting example that shows the complexity that follows from competing metabolic pathways and how these depend on the experimental system investigated. Assuming that *N*-hydroxy- *N*,*N*′-diacetylbenzidine (*N*-OH-DABZ) is a proximate carcinogen, it was used as the starting material in in-vitro experiments in which the esterification by cytosolic sulfotransferase to a reactive protein-binding metabolite could be demonstrated, and it was suggested that this pathway is involved in benzidine-induced carcinogenesis (Morton *et al.*, 1980).

Later on, the question was where the activating metabolism takes place. Acid-labile glucuronides are formed in the liver, which are transported to the bladder. In the acid urine they are hydrolysed either to *N*-acetylbenzidine, which could be activated by peroxidases, or to *N*′-hydroxy-*N*-acetylbenzidine, which could be further activated for instance by *O*-acetylation. The formation of the resulting guanine-C-8-adduct was explained by the reaction of the nitrenium ion with the intermediary formation of benzidine-diimine (Babu *et al.*, 1992; Zenser *et al.*, 1998). Already this summary outline of activating metabolism shows how many competing steps may be influenced by the circumstantial situation, the individual susceptibility, nutritional habits, voiding volume, dwelling time in the bladder, etc. It is clear that toxicokinetics will be influenced by species-, tissue- and cell-specific conditions.

The concept of metabolic activation was soon generally accepted as an essential prerequisite to explain the biological activity of an amine and it was expected that once the relationships between chemical structure of the amine and these conditions were understood, it would be possible to account for quantitative differences in the level of reactive metabolites and explain the diverse biological effects.

Other pathways have been proposed, such as the formation of reactive oxygen species which are made responsible for oxidative DNA damage and mutations induced by, e.g., 2-naphthylamine (Ohnishi *et al.*, 2002), 4-aminobiphenyl (ABP) and benzidine (Makena & Chung, 2007). But this is also a general property of aromatic amine metabolism. Human lung chromosomes contain high levels of arylamine peroxidase activity which readily activates ABP, benzidine, 4,4′-methylenebis(2-chloroaniline) (MOCA), 2-amino-fluorene (AF) and 2-naphthylamine as measured by DNA-adduct formation (Culp *et al.*, 1997). Prostaglandin H synthase activates *N*-acetylbenzidine leading to the typical guanine-C-8-acetylbenzidine-adduct (Lakshmi *et al.*, 1998). Peroxidase-mediated activation of aromatic amines can also be demonstrated by activating polymorphonuclear leukocytes with tumour promoters. Binding of metabolites to leukocyte DNA has been

found with benzidine, 2-aminofluorene and methylaminoazobenzene (Tsuruta *et al.*, 1985).

By far the most studies of aromatic amine metabolism were performed with 2-acetylaminofluorene (AAF) and to a lesser degree with 4-aminobiphenyl (ABP) and 2-aminonaphthalene (AN) and benzidine (BZ), which were among the first chemicals classified as human carcinogens. The ultimate goal of these studies – mostly performed in cell and tissue culture and less *in vivo* – was to find the critical metabolic pathway and the critical biological lesion, primarily in DNA. Many positive correlations were found and many species- and tissue-specific effects of individual arylamines could be explained by quantitative differences in toxicokinetics rather than by specific properties of the individual amine.

The role of metabolic activation for the individual susceptibility has particularly attracted interest with aromatic amines (Carreón *et al.*, 2006). One of the first examples for an enzymatic polymorphism was the observation that workers occupationally exposed to benzidine who were slow acetylators were at greater risk to develop bladder tumours than rapid acetylators (Golka *et al.*, 2002; Gu *et al.*, 2005. Sinués *et al.*, 1992). This was not confirmed in later studies, which incorporated phenotypic and genotypic analysis (Hayes *et al.*, 1993: Ma *et al.*, 2004). Similarly, there was no overall increase in bladder-cancer risk in the *GSTM1*-null genotype of benzidine-exposed workers (Shinka *et al.*, 1998), which is in contrast to its association with elevated bladder-cancer risk in the general population (Rothman *et al.*, 1996).

On the other hand, an elevated bladder-cancer risk for formerly benzidine-exposed workers in the Chinese dyestuff industry was associated with a homozygous mutant genotype of UDP-glucuronosyltransferase 2B7. This polymorphism is different from that in Caucasian populations (Lin *et al.*, 2005).

In summary, many enzymatic polymorphisms of enzymes involved in the metabolism of aromatic amines are now known and the various equilibria between activating and inactivating steps inevitably must be influenced by the individual set-up. One of the consequences is that epidemiological effects are likely to show up only in particularly exposed and rather homogeneous populations.

2. Mechanisms of Carcinogenesis

The concept of metabolic activation to an ultimate reactive metabolite was clearly supported by the finding that c-H-*ras*, the first oncogene that has been found in normal liver as well as in mouse-liver tumours, could be activated by a point mutation caused by exactly the guanine-C-8-AF adduct described above (Wiseman *et al.* 1986). This activation was considered an early effect in the development of liver tumours in the mouse (Anderson *et al.* 1992). In the meantime many proto-oncogenes have been identified that are activated and tumour-suppressor genes that are inactivated by genotoxic

effects. Most activated oncogenes found in human tumours are identical with those found in experimental animals.

Are there DNA-specific effects? Numerous carcinogens produce mutations in typical codons, such as 12, 13 and 61 of the H-*ras* gene, and the corresponding mutations were identified in different target tissues, i.e. in human lung and colon, but the pattern of mutations is too different to establish a clear cause-effect relationship. It is for instance not known why mutations of the H-*ras* gene are seen in mouse-liver tumours, but not in rat-liver tumours (Bitsch *et al.*, 1993). They seem to represent tumour-initiating lesions in mouse skin and liver and in rat mammary tissue (Stanley, 1995). Similar but distinguishable mutation profiles were seen with ABP, 2-aminoanthracene and PhIP in the *lac*Z-reversion assay (Garganta *et al.*, 1999). The selectivity of mutations in the *ras* oncogene in AAF-induced mouse lung and liver tumours was proposed to be tissue-specific as compared with those in spontaneously occurring mouse lung and liver tumours (Wang *et al.*, 1993).

Although both AAF and ABP produce the same type of DNA-adduct, i.e. dG-C8-AF and dG-C8-ABP, the pattern of mutations is different. AAF induces frame-shift and base-substitution mutations (G-T-transversions), ABP only base-substitution mutations (G-A-transversions). The mutagenic efficiency per adduct is greater with AAF than with ABP (Beland *et al.*, 1990). The dG-8-ABP adducts have been identified in human-bladder tumours (Zayas *et al.*, 2007), in bladder epithelial cells (Skipper & Tannenbaum, 1994), and in exfoliated bladder epithelial cells (Talaska *et al.*, 1993). The adduct levels correlate positively with cigarette smoking, type of tobacco and slow-acetylator phenotype. Do these results reflect bladder-specific or amine-specific effects?

From the very beginning interest focused on bladder tumours, because they were the first to be associated in humans with occupational exposure to aromatic amines. But as we know now, adduct formation and genotoxic effects are not target tissue-specific. The dG-C8-ABP adduct has been demonstrated in many human tissues, for instance in mammary tissue (Faraglia *et al.*, 2003).

The formation of dG-C8-ABP adducts correlates well with the formation of protein adducts such as that with haemoglobin (Talaska *et al.*, 1993; Kadlubar *et al.*, 1991). This protein adduct can be measured in blood samples and be used as a biomarker of exposure and as a biomarker of effect. It indicates that the *N*-hydroxylamine (or the nitroso-derivative) is distributed throughout the organism and in agreement with the general experience is available in most if not all tissues. Nukui *et al.* (2007) demonstrated the transplacental exposure during the pregnancy of smoking mothers.

The molecular basis by which 4-ABP mediates carcinogenic activity was believed to be its ability to produce mutations in the human genome. However, additional mechanisms have come up recently. Bladder cancer is now proposed to be the result of gross chromosome aberrations rather than point mutations (Saletta *et al.* 2007). Cells carrying chromosome instability and microsatellite instability have a selective advantage. Exposure to specific carcinogens can select for tumour cells with distinct forms of genetic instability (Bardelli *et al.*, 2002).

Despite many open questions the key steps outlined above are basically the same (Goodrow, 1996), and the similarities exceed the unexplained differences. The same metabolic scheme, the same kind of genotoxic lesions and in many cases the same or analogous tissue-specificity – like the generation of bladder tumours – support the view that the mode of action is the same for this whole group of chemicals.

Moreover, Benigni and Pino (1998) studied tumour profiles (target tissues) of 536 rodent carcinogens in the four experimental systems usually employed (rat/mouse, male/female). Aromatic amines and nitroarenes were among the classes most represented in the database. The authors come to the conclusion that no obvious association exists between chemical/mode of action class and tumour profile. It rather appears that each class produces tumours at a wide range of sites. It is suggested that the events surrounding the ultimate mechanism of reaction with DNA determine the differences in tumour profile.

Benigni and Passerini (2002) evaluated several QSAR-models and concluded that the gradation of potency of aromatic amines depends first on their hydrophobicity, and second on electronic properties (reactivity, propensity to be metabolically transformed) and steric characteristics. Although this regards some basic properties, it appears not to be possible to predict carcinogenicity and potency of an aromatic amine. However, the models help to verify the proposed mode of action and in fact support it.

In summary, a common mode of action is at the basis of the carcinogenic properties of aromatic amines and a carcinogenic potential seems to be associated with this whole group of chemicals. When and where a tumour will develop depends on the interaction of the chemical, with its specific properties, in a highly adaptable organism.

3. The Role of Monocyclic Aromatic Amines

It was believed for a long time that only the polycyclic aromatic amines, but not the monocyclic amines have carcinogenic potential. This conviction was abandoned when occupational exposure to 4-chloro-*ortho*-toluidine was shown to produce bladder tumours in workers. *ortho*-Toluidine had also to be classified as a carcinogen and the experimental results with aniline eventually put an end to this hypothesis. With each of a vast variety of monocyclic aromatic amines, *N*-hydroxylamines are metabolically formed under suitable conditions, and reactions with DNA and mutagenic activity can be demonstrated (Marques *et al.*, 1997). No criterium can be defined at present that would allow to seperate genotoxic from non-genotoxic, or carcinogenic from non-carcinogenic monocyclic arylamines. This is primarily due to results indicating that the role of genotoxicity was overestimated. It dominates potential and potency far less than hitherto believed.

This became particularly apparent with the recent developments concerning aniline and structurally related amines. The discussion focused for a long time on the question: is

aniline a genotoxic carcinogen and if not, should it be classified at all as a carcinogen. Tests for mutagenicity gave contradictory results and because of the low genotoxic potency these data were considered not to be sufficient to explain the spleen tumours observed in rats (Wilmer *et al.*, 1984; Bomhard and Herbold, 2005). It was concluded that these tumours must be caused by a non-genotoxic mechanism, with the possibility to establish a NOAEL (Bus & Popp, 1987 called it a threshold). It was hypothesized that with increasing doses more damaged erythrocytes are eliminated in the spleen, which causes vascular congestion, pericapsular inflammation, fibrosis and eventually sarcoma and angiosarcoma of the spleen. This would represent a typical high-dose phenomenon. In addition it was argued that spleen tumours in male rats are not relevant for the human situation.

The process starts with the *N*-oxidation of aniline to *N*-phenylhydroxylamine in the liver. In the erythrocytes, phenylhydroxylamine is then co-oxidized to nitrosobenzene, and Fe2+-haemoglobin is oxidized to Fe3+-methaemoglobin. Methaemoglobin has a reduced capacity to bind oxygen and causes a hypoxic situation. Both reactions are reversible, nitrosobenzene is reduced back to phenylhydroxylamine and Fe3+ to Fe2+. This regenerating process depends largely on the availability of reduced glutathione, which keeps methaemoglobin at a tolerable level. At the work-place only methaemoglobin levels of more than 5% are considered adverse. Khan *et al.*, (1997) expected that detrimental effects occur only when the degradation of erythrocytes in the spleen is overloaded. One of the hypotheses is that erythrocyte membranes become less plastic and like senescent erythrocytes are sequestered and degraded by the spleen. Iron is released in this process (Ciccoli *et al.*, 1999) which could activate oxygen, which in turn modifies cellular DNA. This would be an indirect genotoxic mechanism. At the same time lipids and proteins are oxidized and heme is excessively degraded. All these reactions contribute to cytotoxicity. Although iron is also released within the erythrocytes during methaemoglobin formation, the intravasal degradation of these cells is not thought to play a significant role (Pauluhn, 2004).

The example aniline shows how intimately genotoxic and non-genotoxic effects are connected and that genotoxicity alone will not answer the question.

Is it possible now to close the discussion and decide whether or not aniline has a carcinogenic potential, or more precisely, can a threshold be defined below which it does not contribute to carcinogenic risk? First of all, when metabolic activation and bioavailability of reactive metabolites are used as an end-point, an NEL was not reached at low doses in a 4-week study in male rats (Zwirner-Baier *et al.* 2003). It was, therefore, concluded that any exposure to aniline contributes to a background of methaemoglobin formation. A variety of endogenous and exogenous chemicals make up this background; other aromatic amines are particularly involved.

In addition to methaemoglobin formation, erythrocytes are damaged by reactive metabolites that react with proteins and membranes. Nitrosobenzene, for instance, reacts with the SH-groups of cysteine in the β-chain of haemoglobin. A stable sulfinamide-

adduct is formed, which has been used as a biomarker of effect (Albrecht and Neumann, 1985, Neumann, 2000, Sarkar *et al.* (2006).

The use of Hb-adducts as biomarkers clearly demonstrates that the general population is exposed to many monocyclic and polycyclic aromatic amines (Bryant *et al.*, 1987, Neumann *et al.*, 1995). The biomonitoring results make also clear that acute toxicity follows the same direction in humans as in experimental animals. Consequently, any quantitative considerations have to take into account additive or synergistic effects for most steps within this common mode of action.

4. The Role of Aromatic Nitrocompounds

At this point it is necessary to direct the attention to the fact that aromatic nitro-compounds have to be included into the group of chemicals whose correct collective name is *N*-substituted aryl compounds. The same *N*-hydroxylamine is formed by reducing the nitrogroup as by oxidizing the amine. These reactions take place at different locations. Nitrogroups are reduced to nitrosobenzene primarily in the reductive environment of the intestine, whereas amines are oxidized predominantly in liver. The ultimate metabolites may therefore be distributed differently. It is interesting to look at corresponding pairs of amino- and nitrocompounds, such as aniline and nitrobenzene (Neumann *et al*., 1995; Neumann, 2005). The reactive metabolites – phenylhydroxylamine and nitrosobenzene – are identical, the location of tumours in the rat, however, is different. Aniline causes sarcomas predominantly in the spleen, nitrobenzene produces liver adenoma and carcinoma in the rat. Both agents acutely produce methaemoglobin and chronic anaemia, and liver and kidney damage in rat and mouse. The biological tolerance values for aniline and nitrobenzene have therefore been set the same in Germany (DFG 2007; list of MAK and BAT values). The relationship between nitroarenes and amines becomes particularly important if it is realized that aromatic nitro-compounds are ubiquitously present in the environment as combustion products. Wherever organic material is combusted not only polycyclic aromatic hydrocarbons but – in the presence of nitrogen – also polycyclic aromatic nitro-compounds are formed (Neumann, 2001). Already in 1978, Johnson & Cornish (1978) studied in rats the conversion of 1- and 2-nitronaphthalene to 1- and 2-aminonaphthalene.

5. Genotoxicity Is Not the Only Mechanism

Soon it became clear that a single mutation was not sufficient to generate a tumour, but two or three such critical lesions in combination should be able to control the multistep process of tumour formation (Brandau & Böhle, 2001). In the case of large-bowel tumours up to eight irreversible alterations were postulated. The underlying

paradigm was that a genotoxic chemical, like an aromatic amine, is able to transform a normal cell into a tumour cell, which gains increasing growth advantage and ultimately grows to a tumour. Numerous types of genotoxic lesions may contribute, chromosome instability included. All the knowledge about the spectrum of DNA lesions formed upon administration of a single carcinogen as an initiator has not yet led to the identification of those critical lesions that predispose a cell to the development of neoplasia (Dragan and Pitot, 1992).

Despite many positive correlations between genotoxic lesions and species- and tissue-specific effects, genotoxic effects are necessary but not sufficient to explain the process of tumour formation. Early on, observations were reported indicating that pre-neoplastic lesions in rat liver were only seen when carcinogen treatment was coupled with a proliferative stimulus, partial hepatectomy being one of the possible triggers (Columbano *et al.*, 1981; Neumann, 1986, Nguyen-Ba & Vasseur, 1999).

A well known example for non-correlation of genotoxicity and tumour formation came from a dose–response study, the so-called megamouse experiment. Chronic administration of AAF to BALB/c mice produced liver as well as bladder tumours. The level of the typical guanine-C-8-AF-adduct as the relevant lesion increased linearly with dose in both tissues, more so in bladders than in livers. Tumor incidence however, increased linearly only in livers starting at the level of spontaneous liver tumours. Despite the higher adduct levels in bladder, the tumour incidence increased in this tissue steeply and nonlinearly only at some higher doses. This increase was associated with an increase in cell proliferation. This means that independent of the significantly higher DNA-damage in the bladder, tumours developed only when cell proliferation was stimulated by the carcinogenic agent.

In a corresponding experiment with 4-aminobiphenyl, bladder tumours were also obtained only with increased cell proliferation. Adduct levels were 2 to 3 times higher in bladder than in liver. In this case the yield of liver tumours was rather low, which was explained by an increased formation and the transport of *N*-hydroxy-4-aminobiphenyl-*N*-glucuronide from the liver, which led to lower exposures in the liver and higher exposures to the reactive metabolite in the bladder, where the glucuronide is hydrolized. This shows how pharmacokinetics can modify the genotoxic effect and how toxicity may determine tissue specificity (Poirier *et al.*, 1995).

In another example the carcinogenic effects of three polycyclic aromatic amines were compared: trans-4-acetylaminostilbene (AAS), 2-acetylaminophenanthrene (AAP), and 2-acetylaminofluorene (AAF). All three agents produce initiated, i.e. promotable cells in rat liver, but only one of them (AAF) produces liver tumours and, therefore, is a complete carcinogen for this tissue. A fundamental difference between the three agents is that only the complete carcinogen is hepatotoxic. In this case the adverse effect could be attributed at the molecular level as a non-genotoxic effect. AAF metabolites specifically uncouple the mitochondrial respiratory chain by detracting electrons, which opens the mitochondrial transition pore and interferes with the regulation of apoptosis (Bitsch *et al.*,

2000). Inhibition of apoptosis may help damaged cells to escape cell death and acquire a tumorigenic phenotype (Nguyen-Ba & Vasseur, 1999).

All three examples show that two different properties were required to make the aromatic amine a complete carcinogen: it must be mutagenic and cytotoxic. Other end-points, like progressive loss of histone H4 lysine 20 trimethylation, and increased histone H3 serine 10 phosphorylation, which were detected in rat liver, but not in kidney and spleen, indicate clearly the importance of epigenetic changes in carcinogenesis (Pogribny *et al.*, 2007).

Among the first authors who proposed a role for toxicity were Radomski *et al.* (1971). 1-Naphthylamine (1-NA) in contrast to the 2-isomer was considered to be non-carcinogenic, and the rat resistant to the formation of bladder tumours. In a study that compared the two isomers, the isomeric *N*-hydroxylamines (*N*-OH-NA) and their nitroso-derivatives (NO-N) were tested directly by i.p. injection in rats, and both oxidation products produced tumours (fibromas, fibrosarcoma and lymphosarcomas), but they also turned out to be hepatotoxic, such that the survival time was significantly reduced.. Both, 1-NOH-NA and 1-NO-N were more carcinogenic than the 2-isomers, and both gave the same type of tumours. When administered to newborne mice, it was the other way around: 2-NOH-NA was more carcinogenic than 1-NOH-NA, and 2-NOH-N more efficient than 2-NO-N. The original testing for carcinogenicity of the amines was evidently insufficient and both isomers have carcinogenic potential under suitable conditions. It also shows that the rat is not completely resistant to oral doses of 2-NA (Hicks *et al.* 1982). Toxicity has strongly influenced the outcome of the test results. The promoting effects of AAF have often been used in models of carcinogenicity testing and undefined toxicity was made responsible for this effect (Sparfel *et al.*, 2002).

6. Conclusions

The study of carcinogenic *N*-substituted aryl compounds, a large group of chemicals not only present at many workplaces but also in the general environment, teaches us an important lesson. If suitable conditions are chosen it is possible to demonstrate, with practically all of them, the formation of ultimate metabolites, their reaction with DNA, RNA and proteins, mutagenic activity, the formation of methaemoglobin and other acute toxic effects. Only in a few cases has it been possible so far to prove a causal relationship in humans, sufficient to classify the agent in IARC's Group 1.

What kind of information would be necessary to label an agent as hazardous to humans? It appears impossible to exclude the suspicion of a carcinogenic potential for this type of chemical. Together with the fact that many of these *N*-substituted chemicals are present in the environment and due to their common mode of action, additive or synergistic effects have to be expected. Tumour-promoting effects have been seen with

mixtures in which the level of most of the individual chemicals was below that expected to have such an effect (Crisp database, Benjamin, 2010).

7. References

Albrecht W, Neumann HG (1985). Biomonitoring of aniline and nitrobenzene. Hemoglobin binding in rats and analysis of adducts. *Arch Toxicol*, 57:1–5. doi:10.1007/BF00286566. PMID:4015392

Anderson MW, Reynolds SH, You M, Maronpot RM (1992). Role of proto-oncogene activation in carcinogenesis. *Environ Health Perspect*, 98:13–24. doi:10.2307/3431243. PMID:1486840

Babu SR, Lakshmi VM, Hsu FF *et al.* (1992). Role of N-glucuronidation in benzidine-induced bladder cancer in dog. *Carcinogenesis*, 13:1235–1240. doi:10.1093/carcin/13.7.1235. PMID:1638692

Bardelli A, Cahill DP, Lederer G *et al.* (2001). Carcinogen-specific induction of genetic instability. *Proc Natl Acad Sci USA*, 98:5770–5775. doi:10.1073/pnas.081082898. PMID:11296254

Beland FA, Fullerton NF, Kinouchi T *et al.* (1990) DNA adduct formation in relation to tumorigenesis in mice chronically fed 2-acetylaminofluorene. In: Mouse liver carcinogenesis: mechanisms and species comparison. Alan R Liss, Inc. pp 121–129.

Beland FA, Melchior WB Jr, Mourato LL *et al.* (1997). Arylamine-DNA adduct conformation in relation to mutagenesis. *Mutat Res*, 376:13–19. PMID:9202733

Benigni R, Passerini L (2002). Carcinogenicity of the aromatic amines: from structure-activity relationships to mechanisms of action and risk assessment. *Mutat Res*, 511:191–206. doi:10.1016/S1383-5742(02)00008-X. PMID:12088717

Benigni R, Pino A (1998). Profiles of chemically-induced tumors in rodents: quantitative relationships. *Mutat Res*, 421:93–107. PMID:9748520

Benjamin, SA (2010). Chemical mixtures as promoters of hepatocarcinogenesis. Crisp Database National Institute of Health. Available at: http://projectreporter.nih.gov/reporter.cfm

Bitsch A, Hadjiolov N, Klöhn PC *et al.* (2000). Dose response of early effects related to tumor promotion of 2-acetylaminofluorene. *Toxicol Sci*, 55:44–51. doi:10.1093/toxsci/55.1.44. PMID:10788558

Bitsch A, Röschlau H, Deubelbeiss C, Neumann HG (1993). The structure and function of the H-ras proto-oncogene are not altered in rat liver tumors initiated by 2-acetylaminofluorene, 2-acetylaminophenanthrene and trans-4-acetylaminostilbene. *Toxicol Lett*, 67:173–186. doi:10.1016/0378-4274(93)90054-2. PMID:8451759

Bomhard EM, Herbold BA (2005). Genotoxic activities of aniline and its metabolites and their relationship to the carcinogenicity of aniline in the spleen of rats. *Crit Rev Toxicol*, 35:783–835. doi:10.1080/10408440500442384. PMID:16468500

Brandau S, Böhle A (2001). Bladder cancer. I. Molecular and genetic basis of carcinogenesis. *Eur Urol*, 39:491–497. doi:10.1159/000052494..

Bryant MS, Skipper PL, Tannenbaum SR, Maclure M (1987). Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. *Cancer Res*, 47:602–608. PMID:3791245

Bus JS, Popp JA (1987). Perspectives on the mechanism of action of the splenic toxicity of aniline and structurally-related compounds. *Food Chem Toxicol*, 25:619–626. doi:10.1016/0278-6915(87)90024-X. PMID:3305247

Carreón T, LeMasters GK, Ruder AM, Schulte PA (2006). The genetic and environmental factors involved in benzidine metabolism and bladder carcinogenesis in exposed workers. *Front Biosci*, 11:2889–2902. doi:10.2741/2017. PMID:16720360

Ciccoli L, Ferrali M, Rossi V *et al.* (1999). Hemolytic drugs aniline and dapsone induce iron release in erythrocytes and increase the free iron pool in spleen and liver. *Toxicol Lett*, 110:57–66. doi:10.1016/S0378-4274(99)00138-1. PMID:10593595

Columbano A, Rajalakshmi S, Sarma DSR (1981). Requirement of cell proliferation for the initiation of liver carcinogenesis as assayed by three different procedures. *Cancer Res*, 41:2079–2083. PMID:6113048

Culp SJ, Roberts DW, Talaska G *et al.* (1997). Immunochemical, 32P-postlabeling, and GC/MS detection of 4-aminobiphenyl-DNA adducts in human peripheral lung in relation to metabolic activation pathways involving pulmonary N-oxidation, conjugation, and peroxidation. *Mutat Res*, 378:97–112. PMID:9288889

DFG Deutsche Forschungsgemeinschaft. (2007) List of MAK and BAT Values, Report No 42, Wiley-VCH-Verlag, Weinheim, pp 192–198.

Dragan YP, Pitot HC (1992). The role of the stages of initiation and promotion in phenotypic diversity during hepatocarcinogenesis in the rat. *Carcinogenesis*, 13:739–750. doi:10.1093/carcin/13.5.739. PMID:1586986

Faraglia B, Chen SY, Gammon MD *et al.* (2003). Evaluation of 4-aminobiphenyl-DNA adducts in human breast cancer: the influence of tobacco smoke. *Carcinogenesis*, 24:719–725. doi:10.1093/carcin/bgg013. PMID:12727801

Frederick CB, Weis CC, Flammang TJ *et al.* (1985). Hepatic N-oxidation, acetyl-transfer and DNA-binding of the acetylated metabolites of the carcinogen, benzidine. *Carcinogenesis*, 6:959–965. doi:10.1093/carcin/6.7.959. PMID:4017176

Garganta F, Krause G, Scherer G (1999). Base-substitution profiles of externally activated polycyclic aromatic hydrocarbons and aromatic amines determined in a lacZ reversion assay. *Environ Mol Mutagen*, 33:75–85. doi:10.1002/(SICI)1098-2280(1999)33:1<75::AID-EM9>3.0.CO;2-1. PMID:10037326

Golka K, Prior V, Blaszkewicz M, Bolt HM (2002). The enhanced bladder cancer susceptibility of NAT2 slow acetylators towards aromatic amines: a review considering ethnic differences. *Toxicol Lett*, 128:229–241. doi:10.1016/S0378-4274(01)00544-6. PMID:11869833

Goodrow TL (1996). One decade of comparative molecular carcinogenesis. *Prog Clin Biol Res*, 395:57–80. PMID:8895984

Gu J, Liang D, Wang Y *et al.* (2005). Effects of N-acetyl transferase 1 and 2 polymorphisms on bladder cancer risk in Caucasians. *Mutat Res*, 581:97–104. PMID:15725609

Hayes RB, Bi W, Rothman N *et al.* (1993). N-acetylation phenotype and genotype and risk of bladder cancer in benzidine-exposed workers. *Carcinogenesis*, 14:675–678. doi:10.1093/carcin/14.4.675. PMID:8472331

Hicks RM, Wright R, Wakefield JSJ (1982). The induction of rat bladder cancer by 2-naphthylamine. *Br J Cancer*, 46:646–661. PMID:7138770

Johnson DE, Cornish HH (1978). Metabolic conversion of 1- and 2-nitronaphthalene to 1- and 2-naphthylamine in the rat. *Toxicol Appl Pharmacol*, 46:549–573. doi:10.1016/0041-008X(78)90302-2. PMID:746547

Kadlubar FF, Dooley KL, Teitel CH *et al.* (1991). Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladder DNA adduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. *Cancer Res*, 51:4371–4377. PMID:1868460

Khan MF, Boor PJ, Gu Y *et al.* (1997). Oxidative stress in the splenotoxicity of aniline. *Fundam Appl Toxicol*, 35:22–30. doi:10.1006/faat.1996.2259. PMID:9024670

Lakshmi VM, Zenser TV, Davis BB (1998). N'-(3′-monophospho-deoxyguanosin-8-yl)-N-acetylbenzidine formation by peroxidative metabolism. *Carcinogenesis*, 19:911–917. doi:10.1093/carcin/19.5.911. PMID:9635882

Lakshmi VM, Zenser TV, Goldman HD *et al.* (1995). The role of acetylation in benzidine metabolism and DNA adduct formation in dog and rat liver. *Chem Res Toxicol*, 8:711–720. doi:10.1021/tx00047a011. PMID:7548754

Lin GF, Guo WC, Chen JG *et al.* (2005). An association of UDP-glucuronosyltransferase 2B7 C802T (His268Tyr) polymorphism with bladder cancer in benzidine-exposed workers in China. *Toxicol Sci*, 85:502–506. doi:10.1093/toxsci/kfi068. PMID:15615884

Ma QW, Lin GF, Chen JG *et al.* (2004). Polymorphism of N-acetyltransferase 2 (NAT2) gene polymorphism in shanghai population: occupational and non-occupational bladder cancer patient groups. *Biomed Environ Sci*, 17:291–298. PMID:15602826

Makena P, Chung KT (2007). Evidence that 4-aminobiphenyl, benzidine, and benzidine congeners produce genotoxicity through reactive oxygen species. *Environ Mol Mutagen*, 48:404–413. doi:10.1002/em.20288. PMID:17370336

Marques MM, Mourato LL, Amorim MT *et al.* (1997). Effect of substitution site upon the oxidation potentials of alkylanilines, the mutagenicities of N-hydroxyalkylanilines, and the conformations of alkylaniline-DNA adducts. *Chem Res Toxicol*, 10:1266–1274. doi:10.1021/tx970104w. PMID:9403181

Morton KC, Beland FA, Evans FE *et al.* (1980). Metabolic activation of N-hydroxy-N,N'-diacetylbenzidine by hepatic sulfotransferase. *Cancer Res*, 40:751–757. PMID:6937241

Neumann HG (1986). The role of DNA damage in chemical carcinogenesis of aromatic amines. *J Cancer Res Clin Oncol*, 112:100–106. doi:10.1007/BF00404390. PMID:3533949

Neumann HG (2000). Protein-Addukte als biochemische Effekt-Marker für ein biologisches Monitoring. *Arbeitsmed Sozialmed Umweltmed*, 35:351–356.

Neumann HG (2001). Health risk of combustion products: toxicological considerations. *Chemosphere*, 42:473–479. doi:10.1016/S0045-6535(00)00219-8. PMID:11219671

Neumann HG (2005) Monocyclic aromatic amino and nitro compounds: Toxicity, genotoxicity and carcinogenicity, classification in a carcinogen category. In: Greim H, ed, The MAK Collection for occupational health and safety, Part I: MAK-Value documentations Vol 21, DFG Deutsche Forschungsgemeinschaft, Wiley-VCH-Verlag, Weinheim, pp 3–45.

Neumann HG, van Dorp C, Zwirner-Baier I (1995). The implications for risk assessment of measuring the relative contribution to exposure from occupation, environment and lifestyle: hemoglobin adducts from amino- and nitro-arenes. *Toxicol Lett*, 82-83:771–778. doi:10.1016/0378-4274(95)03595-8. PMID:8597141

Nguyen-Ba G, Vasseur P (1999). Epigenetic events during the process of cell transformation induced by carcinogens (review). [review]. *Oncol Rep*, 6:925–932. PMID:10373683

Nukui T, Schreiber EM, Harger G *et al.* (2007) Association of maternal lifestyle factors and N-acetyltransferase 2 genotype to newborne 4-aminobiphenyl-Hb adduct levels. AACR Annual Meeting, Los Angeles, Abstract Number 4227.

Ohnishi S, Murata M, Kawanishi S (2002). Oxidative DNA damage induced by a metabolite of 2-naphthylamine, a smoking-related bladder carcinogen. *Jpn J Cancer Res*, 93:736–743. PMID:12149138

Pauluhn J (2004). Subacute inhalation toxicityof aniline in rats: analysis of time dependence and concentration-dependence fo hematotoxic and splenec effects. *Toxicol Sci*, 81:191–216. doi:10.1093/toxsci/kfh187.

Pogribny IP, Bagnyukova TV, Tryndyak VP *et al.* (2007) Early epigenetic dysregulation in rat liver during 2-acetylaminofluorene-induced hepatocarcinogenesis. AACR Annual Meeting, Los Angeles, Abstract No. 101.

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*, 16:2917–2921. doi:10.1093/carcin/16.12.2917. PMID:8603464

Radomski JL, Brill E, Deichmann WB, Glass EM (1971). Carcinogenicity testing of N-hydroxy and other oxidation and decomposition products of 1- and 2-naphthylamine. *Cancer Res*, 31:1461–1467. PMID:4328736

Rothman N, Hayes RB, Zenser TV *et al.* (1996). The glutathione S-transferase M1 (GSTM1) null genotype and benzidine-associated bladder cancer, urine mutagenicity, and exfoliated urothelial cell DNA adducts. *Cancer Epidemiol Biomarkers Prev*, 5:979–983. PMID:8959320

Saletta F, Matullo G, Manuguerra M *et al.* (2007). Exposure to the tobacco smoke constituent 4-aminobiphenyl induces chromosomal instability in human cancer cells. *Cancer Res*, 67:7088–7094. doi:10.1158/0008-5472.CAN-06-4420. PMID:17671175

Sarkar M, Stabbert R, Kinser RD *et al.* (2006). CYP1A2 and NAT2 phenotyping and 3-aminobiphenyl and 4-aminobiphenyl hemoglobin adduct levels in smokers and non-smokers. *Toxicol Appl Pharmacol*, 213:198–206. doi:10.1016/j.taap.2005.11.003. PMID:16405939

Shinka T, Ogura H, Morita T *et al.* (1998). Relationship between glutathione S-transferase M1 deficiency and urothelial cancer in dye workers exposed to aromatic amines. *J Urol*, 159:380–383. doi:10.1016/S0022-5347(01)63924-7. PMID:9649243

Sinués B, Pérez J, Bernal ML *et al.* (1992). Urinary mutagenicity and N-acetylation phenotype in textile industry workers exposed to arylamines. *Cancer Res*, 52:4885–4889. PMID:1516045

Skipper PL, Tannenbaum SR (1994). Molecular dosimetry of aromatic amines in human populations. *Environ Health Perspect*, 102 Suppl 6;17–21. doi:10.2307/3432145. PMID:7889842

Sparfel L, Loewert M, Huc L *et al.* (2002). Acute cytotoxicity of the chemical carcinogen 2-acetylaminofluorene in cultured rat liver epithelial cells. *Toxicol Lett*, 129:245–254. doi:10.1016/S0378-4274(02)00015-2. PMID:11888708

Stanley LA (1995). Molecular aspects of chemical carcinogenesis: the roles of oncogenes and tumour suppressor genes. *Toxicology*, 96:173–194. doi:10.1016/0300-483X(94)02991-3. PMID:7900159

Talaska G, Schamer M, Skipper P *et al.* (1993). Carcinogen-DNA adducts in exfoliated urothelial cells: techniques for noninvasive human monitoring. *Environ Health Perspect*, 99:289–291. doi:10.2307/3431503. PMID:8319646

Tsuruta Y, Subrahmanyam VV, Marshall W, O'Brien PJ (1985). Peroxidase-mediated irreversible binding of arylamine carcinogens to DNA in intact polymorphonuclear leukocytes activated by a tumor promoter. *Chem Biol Interact*, 53:25–35. doi:10.1016/S0009-2797(85)80081-8. PMID:2986860

Wang Y, Wang Y, Stoner G, You M (1993). ras mutations in 2-acetylaminofluorene-induced lung and liver tumors from C3H/HeJ and (C3H x A/J)F1 mice. *Cancer Res*, 53:1620–1624. PMID:8453632

Wilmer JL, Rosenkranz HS, Pet-Edwards J *et al.* (1984). On the carcinogenicity of aniline. *Environ Mutagen*, 6:629–632. doi:10.1002/em.2860060502. PMID:6479113

Wiseman RW, Stowers SJ, Miller EC *et al.* (1986). Activating mutations of the c-Ha-ras protooncogene in chemically induced hepatomas of the male B6C3 F1 mouse. *Proc Natl Acad Sci USA*, 83:5825–5829. doi:10.1073/pnas.83.16.5825. PMID:3016723

Zayas B, Stillwell SW, Wishnok JS *et al.* (2007). Detection and quantification of 4-ABP adducts in DNA from bladder cancer patients. *Carcinogenesis*, 28:342–349. doi:10.1093/carcin/bgl142. PMID:16926175

Zenser TV, Lakshmi VM, Davis BB (1998). N-glucuronidation of benzidine and its metabolites. Role in bladder cancer. *Drug Metab Dispos*, 26:856–859. PMID:9733663

Zwirner-Baier I, Deckart K, Jäckh R, Neumann HG (2003). Biomonitoring of aromatic amines VI: determination of hemoglobin adducts after feeding aniline hydrochloride in the diet of rats for 4 weeks. *Arch Toxicol*, 77:672–677. doi:10.1007/s00204-003-0473-8. PMID:14557919