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The impact of aromatic amines on the environment: risks and damages

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1. ABSTRACT

Aromatic amines are a group of chemicals whose ubiquitous presence in the environment is a result of the multitude of sources from which they originate. These compounds are widely used as raw materials or at intermediate stages in the manufacturing of industrial chemicals such as pesticides, medicines, dyestuffs, polymers, surfactants, cosmetics and corrosion inhibitors, especially in dyestuff factories. As with most chemical carcinogens, aromatic amines need to be metabolized into reactive electrophiles in order to exert their carcinogenic effects. This activation typically involves *N*-oxidation of arylamines to yield *N*-hydroxyarylamines. Since these amines are potential carcinogenic agents and are discharged into the atmosphere, water and soil, they constitute an important class of environmental pollutants of enormous concern due to the potential for human exposure.

2. INTRODUCTION

Aromatic amines or arylamines represent one of the most important classes of industrial and environmental chemicals. Many aromatic amines have been reported to be powerful carcinogens and mutagens, and/or hemotoxicants, which makes them the most studied single chemical class (1-2).

They have a wide variety of uses in many industries, i.e. in the manufacture of polymers, rubber, agricultural chemicals, dyes and pigments, pharmaceuticals and photographic chemicals (2). As a result of such applications, they are also dispersed into the environment, thereby creating a potential for human exposure (3). It is important to say that the toxicological properties of aromatic amines depend on chemical groups present in the molecule (4).

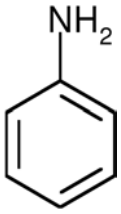


Figure 1. Aniline

Like most chemical carcinogens, aromatic amines require activation by drug-metabolizing enzymes to become reactive electrophiles, in order to exert their mutagenic and carcinogenic activity (2,5,6).

In order to evaluate the risks of these compounds, weight must be given to the extent to which they are distributed in the environment (7).

3. TYPES AND SOURCES OF AROMATIC AMINES IN THE ENVIRONMENT

Aromatic amines are generally identified as those chemical compounds having one or more aromatic rings bearing one or more amino substituents, in their molecular structure. They range from the simplest compound, known as aniline (Figure 1), to highly complex molecules with conjugated aromatic or heterocyclic structures and multiple substituents (8).

They are classified into the same chemical family but their toxicities differ from one compound to another (9). Amongst the aromatic amines the monocyclic ones are environmental pollutants and many of them are toxic, mutagenic and/or carcinogenic to humans and animals. Monocyclic aromatic amines contain a single aromatic ring and at least one amine function attached to the ring. This is the simplest form of aromatic amine and small modifications in the molecular structure greatly affect their mutagenicity (10).

Aromatic amines occur in a number of environments, such as the air, water, and soil (11). The major sources of amines in the environment include several chemical industrial sectors such as oil refining, synthetic polymers, dyes, adhesives and rubbers, pharmaceuticals, pesticides and explosives (6,8). An additional source of aromatic amines in the environment is the abiotic and biotic degradation of nitroaromatic compounds, azo dyes, several classes of pesticide and possibly polyurethanes (12).

Aromatic amines such as aniline, chloroanilines, naphthylamines, aminophenols, toluenediamines and 4-aminobiphenyl are biologically active compounds, well known as environmental pollutants because of their toxicity and carcinogenicity, and are widely used in industry to make dyes, cosmetics, medicines, rubber, textiles, agrochemicals and as reagent intermediates in many chemical syntheses (13,14).

Table 1 shows some examples of aromatic amines known to be potential hazards to human health and the environment.

Since these amines are discharged into the atmosphere, water and soil, they constitute an important class of environmental pollutants (15).

3.1. Aromatic amines in the air

Air pollution has adverse effects on respiratory and cardiovascular health, including acute reduction in lung function, aggravation of asthma, increased risk of pneumonia in the elderly, low birth weight in newborns and death (16). Aromatic amines in the air are associated with manufacturing processes, automobile exhaust fumes and the burning/pyrolysis of protein-rich vegetable matter such as in forest fires and tobacco smoking and in cooking. It has been reported that aniline and p-toluidine were found in outdoor air samples collected from six different points in Zonguldak province in Turkey, where pollution is very high due mainly to solid coal waste (17).

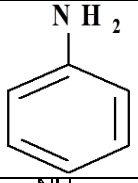
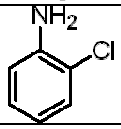
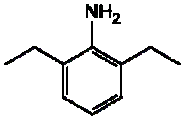
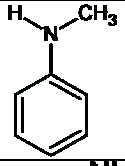
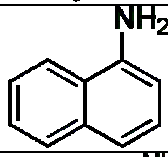
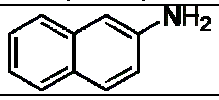
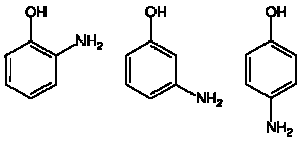
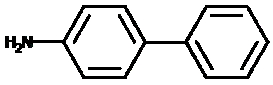
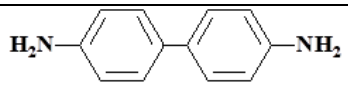
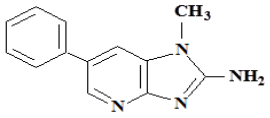
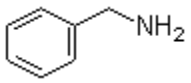
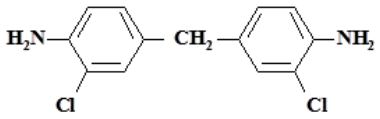
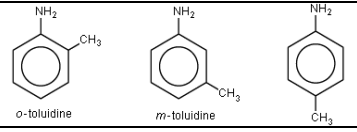
In addition, some aromatic amines were determined in ambient air samples collected from Brindisi town centre in Italy, where the aniline concentration was extremely variable (14). Another source of arylamines in the environment is cigarette smoke, although this kind of exposure is more expressive in the home environment, which is particularly important for the more vulnerable, sensitive groups of the population, such as children, the elderly and the chronically sick, who can spend most of their time indoors (13).

A number of different aromatic amines can be detected in mainstream and side stream cigarette smoke (11). Epidemiological studies have suggested that these compounds are a relevant risk factor for the induction of urinary bladder cancer in smokers. Since hemoglobin adducts of some aromatic amines have been detected in non-smokers, the possibility exists that indoor exposure to aromatic amines from passive smoking and outdoor aromatic amine contamination may have some toxicological effects. Arylamines originate in tobacco smoke from the pyrolysis of amino acids, and are more abundant in the side stream than in the mainstream smoke. Although some aromatic amines are present as contaminants in food and water, most of them are absorbed through the respiratory route and then activated to *N*-hydroxylamine intermediates. *N*-hydroxylamines, excreted by the kidney, generate arylnitrenium ions, powerful electrophilic compounds which bind the DNA of bladder epithelial cells and initiate carcinogenesis (14).

A study performed by Palmiotto *et al.* (2001) showed that aromatic amines contamination is a widespread phenomenon in indoor as well as in outdoor environments. According to them, the source of aniline in indoor environment is likely due to widespread presence of this amine in paints, cleaning fluids and house products; however, the authors consider harder explaining the presence of aniline in outdoor air since they found it at relatively high concentration also in “clean” rural sites (14).

Regarding air pollution by aromatic amines, it is important to point out that, as in the case of the majority of air contaminants, the concentrations of amines in the

Table 1. Chemical structure, source and risks of some types of aromatic amines

Aromatic amine	Chemical structure	Source	Risks and damage
Aniline		Naturally in some foods (e.g., corn, grains, beans, and tea). Manufacture of polymers, rubber, dyes, explosives, pesticides, pharmaceuticals. Oil refining; tobacco smoke; forest fires.	Toxicity to aquatic organisms and humans, possibly neurotoxic, carcinogenic and genotoxic
2-chloroaniline		Production of isocyanates, rubber. Processing chemicals, dyes and pigments, agricultural chemicals and pharmaceuticals.	Toxicity to humans and environment.
2,6-diethylaniline		Used as an intermediate mainly for the manufacture of herbicides (alachlor, butachlor and pretilachlor)	Toxicity to humans, including carcinogenicity, reproductive and developmental toxicity and neurotoxicity. Toxicity to aquatic organisms
N-methylaniline		Manufacture of dyes, agrochemicals and other organic products.	This substance irritates the eyes, the skin, the respiratory tract, may cause effects on the blood, resulting in formation of methaemoglobin
1-aminonaphthalene (alpha-naphthylamine);		Manufacture of dyes	Toxic to humans and carcinogenic
2-aminonaphthaleno (beta-naphthylamine)		Manufacture of dyes	Toxic to humans and carcinogenic
Aminophenols (2-aminophenol; 3-aminophenol; 4-aminophenol)		Used as a dye for textiles, hair, furs and feathers; also used as a photographic developer and chemical intermediate for pharmaceuticals and dyes.	Citotoxicity and nephrotoxicity
4-aminobiphenyl (ABP)		Used to manufacture azo dyes and present in cigarette smoke	Genotoxic and carcinogenic
Benzidine (4,4diaminobiphenyl)		Manufacture of dyes	Toxic and carcinogenic.
2-Amino-1-methyl-6-phenylimidazo (4,5-beta)pyridine (PhIP)		Cooking of meats	Genotoxic, mutagenic and carcinogenic.
Benzylamine		Used as a chemical intermediate in the manufacture of dyestuffs, pigments, optical brighteners, textile auxiliaries, agrochemicals, amino acids and other organic compounds.	Contact with skin and eyes may cause severe irritation including burns.
4,4'-Methylenebis (2-chloroaniline) (MOCA)		Manufacture of polyurethanes.	Toxic to humans and aquatic life. Possibly carcinogenic and genotoxic.
Toluidine (o-toluidine; m-toluidine; p-toluidine)		Manufacture of dyestuffs, production of rubber, chemicals and pesticides and as a curing agent for epoxy resin systems.	Carcinogenic

Reproduced with permission from (8)

environment depend mainly on the air temperature and rain frequency (17).

3.2. Aromatic amines in the water

Usually aromatic amines are released directly into the groundwater and river as industry effluents, or indirectly as breakdown products (i.e., metabolites) of herbicides and pesticides (18). The textile and several other industries generate a number of visible pollutants, which they discharge into the surrounding environment without any further treatment. These pollutants not only add color to the water but they also cause extensive toxicity to the aquatic and other forms of life (19). Especially in the dye producing process, a large amount of highly toxic wastewater containing aniline and its derivatives is discharged. Due to their relatively high solubility in water, aromatic amines can easily permeate through the soil and contaminate the groundwater (20), and they can enter into the body when people consume the food or water contaminated with them. As said previously, aromatic amines are highly toxic and may have a mutagenic impact on animals and humans, even at low concentrations. According to the Verhaar classification scheme, in aquatic toxicology aromatic amines have been classified as "polar narcotics" (21). This class is described as inert baseline toxicity chemicals in which the toxicity is evoked by non-specific mechanisms. For most species, the toxicity of these chemicals is hydrophobicity dependent. Moreover, "polar narcotics" are slightly more toxic (5–10 times lower effect concentrations) than predicted from their Quantitative Structure-Activity Relationship (QSAR) baseline toxicity, which is based on the n-octanol–water partition coefficient (22).

Akyuz and Ata (2006) analyzed river water samples collected from the Zonguldak province in Turkey, where the river is highly polluted by sewage effluent, industrial and coal wastes. They also analyzed river water seawater and sediments collected from the same spots. They found 2,6-diethylaniline, 2-chloroaniline, alpha-naphthylamine and beta-naphthylamine at relatively higher concentrations in the river sediment samples and in the river water. In addition, 4-ethylaniline was found in the river sediment samples, whereas it was not detected in most of the river water samples. The determination of aniline and aminophenols in both the river water and river sediment samples at relatively high concentrations may reflect the degradation of aniline under aerobic conditions to give 4-aminophenol. *N*-methylaniline, alpha-naphthylamine, beta-naphthylamine and benzylamine were found at relatively higher concentrations in the sea sediment and seawater samples. 4-aminobiphenyl was found in the sea sediment samples whereas it was not detected in most of the seawater samples, especially in the summer. Most of the compounds were found in relatively higher concentrations in the winter than in the summer (23).

Sun *et al.* (2009) studied the wastewaters from dyestuff manufacture and from the coking and tannery procedures, and found aniline in all the wastewaters and o-phenylenediamine in those from dyestuff manufacture and the tannery operation (24). Recently a textile azo dye processing plant effluent was identified as one of the sources of mutagenic activity detected in the Cristais River, a drinking water source in Brazil (25). Besides presenting high mutagenic activity in the Salmonella/microsome assay, mutagenic nitro-aminoazobenzenes dyes (26) and also benzidine, a known carcinogenic aromatic amine (27) were found in this effluent. Moreover a drinking water treatment plant, located approximately 6 Km from the point of discharge of this effluent failed to completely remove the contaminants present in the wastewater, and the water used for human consumption presented mutagenic activity related to nitro-aromatic and aromatic amine compounds, probably derived from the above-mentioned textile processing plant effluent discharge (25,26).

3.3. Aromatic amines in the soil

The occurrence of aromatic amines in the soil is associated with a variety of industrial effluents and solid wastes, fossil fuels and aerosols from combustion processes. A significant class of polar aromatic amines is related to microbial degradation processes in soils and waters contaminated with explosives (28).

Aromatic amines can interact with soils and sediments by numerous pathways. The three dominant modes of sorption include hydrophobic partitioning of the neutral amine into the solid matrix, covalent binding with solid phase surface reactive groups and cation exchange of the positively charged amine with negatively charged sites on soil particles. Each of these mechanisms is constrained by time, with hydrophobic partitioning and cation exchange dominating the early stages of sorption, followed by more slowly occurring covalent bonding processes (29,30). The individual contribution to sorption of each mechanism is dependent on the speciation of the amine as controlled by the pH-pK_a relationship, and the available soil domains (e.g., cation-exchange sites and soil organic matter). During characteristically short time periods (e.g. within 24 h), the sorption of organic bases is primarily reversible, with cation exchange being the predominant sorption mechanism (31).

Sorption mechanisms are in turn influenced by a number of properties, such as soil-solution pH, chemical solubility, soil organic carbon content, cation exchange capacity and the particle size distribution (30), but the soil-solution pH is the most significant factor controlling the magnitude of their sorption in soil systems. It has also been suggested that rather than a sequential process, these mechanisms are actually taking place simultaneously, with the rapid step influencing the slower step by limiting the amount of aromatic amine available for the reaction (31).

The competitive sorption of organic compounds on natural sorbents has been employed as a way to probe sorption mechanisms. For nonpolar and slightly polar organic chemicals primarily sorbed by partitioning to soil organic matter, little or no competition between solutes is observed. For moderately polar chemicals such as herbicides and pesticides, competition appears to occur only at high co-solute concentrations, with this competition being attributed to site-specific interactions. However,

these specific interactions must be relatively weak or less selective, since little competition has been observed at low co-solute concentrations (32).

Kosson and Byrne (1995) investigated an aniline-contaminated soil from an anonymous chemical manufacturer in order to describe the mechanisms of interaction between the aniline and the soil. Approximately five hectares of land area were contaminated to an unknown depth. The aniline was used as a raw material for a variety of chemical processes and the contamination had occurred by two methods. Firstly, aniline had been spilled onto the ground during the manufacturing process for several decades, and secondly, at least 40,000 pounds of aniline were spilled at the site in 1979. According to this work, sorption of the aniline by the soil was observed to occur through a two step mechanism. The first step was an ion exchange process with the protonated amine serving as an organic cation, this step being influenced by the solution pH and ionic composition. The second step was covalent bonding, most likely with quinone moieties, and oxidation with polymerization of the aniline. The extent of covalent bonding was influenced by the presence of oxygen and the redox potential. The majority of the aniline bound to the soil did not readily desorb under a variety of abiotic conditions. However, aniline was released to a significant extent in the presence of denitrifying and methanogenic microbial activity (33).

Alzaga *et al.* (1999) analyzed the sludge and contaminated soils affected by a toxic waste spill that occurred in the Los Frailes pyrite mine (Spain). A variety of mono- and di-chlorinated and brominated triphenylamines were identified in these samples. Of the halogenated amines (2-chlorotriphenylamine, 4-chlorotriphenylamine, 2-bromotriphenylamine, 2,29-dichlorotriphenylamine and 2,49-dichlorotriphenylamine) were identified, the chloro-substituted triphenylamines being the most abundant, particularly those substituted at position 4. The dichloro-substituted triphenylamine was also abundant, and its concentration exceeded the concentration of the two mono-substituted isomers (28).

4. METABOLISM AND BIOACTIVATION OF AROMATIC AMINES

It is well known that the aromatic amine carcinogens were thought to induce some tumors in humans, but most require to be metabolized to exert their genotoxicity in specific organs or tissues (34).

The wide variation in carcinogen metabolism in humans has long been regarded as an important determinant of individual susceptibility to chemical carcinogenesis. In the case of aromatic amine carcinogens, it has become apparent that the biochemical basis for these differences may be the polymorphic distribution of specific carcinogen-metabolizing enzymes involved in their activation and/or detoxification. These polymorphisms can arise from both heritable and environmental factors, which can be assessed by epidemiological studies. With the recent development of methods for metabolic phenotyping and genotyping, together with current techniques to detect carcinogen-protein and carcinogen-DNA adducts in human tissues, it should now be possible to assess individual cancer risk with much greater predictive capacity (35).

The role of aromatic amines has been well established in cancer of the human urinary bladder. In the early part of this century, industrial exposure to 4-aminobiphenyl (ABP), alpha-naphthylamine, and benzidine was clearly associated with a high incidence of transitional urothelial-cell carcinomas, and more recently, occupational exposure to 4,4'-methylenebis (2-chloroaniline) (MOCA) and o-toluidine was also correlated with increased bladder cancer risk. Likewise, cigarette smoking has often been implicated as a causative factor in urinary bladder carcinogenesis, and this association has been supported by findings that aromatic amines such as ABP are present in nanogram quantities in cigarette smoke, and that smokers have much higher levels of aromatic amine-hemoglobin adducts than nonsmokers (35).

Arylamines are mainly metabolized to hydroxylamines by oxidation mediated primarily by cytochrome P450 (CYP450) enzymes but also by flavin-containing monooxygenases (FMOs) and peroxidases. The resulting *N*-hydroxylamine products can be further activated to produce highly reactive ester derivatives that bind covalently to DNA (2,6,36).

At least four enzyme systems are known to be involved in the secondary activation step in mammals: *N*-acetyltransferase (NAT), sulfotransferase, prolyl tRNA synthetase and kinases, yielding reactive *N*-acetoxy, *N*-sulfonyloxy, *N*-prolyloxy and *N*-phosphatyl esters, respectively (36). This metabolic activation to electrophilic intermediates is initiated by the *N*-acetyltransferases 1 (NAT1) and 2 (NAT2), which are important enzymes in the biotransformation of these carcinogens and exhibit genetic polymorphism (5).

The NAT-catalyzed acetylation of *N*-hydroxy heterocyclic aromatic amines (HAAs) and arylamines enhances genotoxic activity and the levels of DNA adducts via the formation of reactive *N*-acetoxyl esters. In a similar way, the sulfur esters formed by the action of sulfotransferases are unstable and react. The role of the sulfotransferases has been given less attention than NAT in human epidemiology studies and even less information is available on the *in vivo* roles of the prolyloxy and phosphatyl esters (36).

According to Hein (2002), the high frequency of the NAT1 and NAT2 acetylation polymorphisms in human populations, together with the ubiquitous exposure to aromatic and heterocyclic amines, suggest that the NAT1 and NAT2 acetylator genotypes are important modifiers of human cancer susceptibility (5). Since human populations encounter many different chemicals and it is very difficult to quantify exposure to aromatic and heterocyclic amines over long periods of time, animal models are used to test the effects of acetylator polymorphisms on the carcinogenic risk of these compounds. Cats only have NAT1 and dogs

have no NAT enzymes, making them deficient in or unable to produce *N*-acetylate arylamine-containing compounds, respectively (37).

Moreover, the hydroxylamino, nitro- and nitroso-groups are able to generate amine groups due to metabolic interconversion. In addition to the nitrogen oxidation and reduction reactions (main activation pathways), certain aromatic amines and nitroaromatic hydrocarbons are converted into electrophilic derivatives via ring-oxidation pathways (2).

Arylamines and HAAs primarily yield adducts with guanine, reacting at the N2 and C8 atoms. The *N*-hydroxy HAAs can react directly with DNA, but the reaction is facilitated when reactive ester derivatives undergo heterocyclic cleavage to yield reactive arylnitrenium ion species, which preferentially react to form DNA adducts. Mechanisms for the reaction at the C8 atom are less clear. A direct reaction is possible, and a stepwise mechanism via an *N7*-guanyl intermediate has also been proposed (36).

Therefore, since aromatic amines must be metabolized to exert their carcinogenic effects, genetic polymorphisms in a number of important phase 1 (e.g. CYP450s) and 2 (e.g. glutathione-*S*-transferases) metabolizing enzymes may modify the cancer risk (5).

5. RISKS AND DAMAGE

Risk assessment is a key issue in environmental and medicinal chemistry. Among other biological endpoints, carcinogenicity is one of the most important toxicological issues when characterizing chemical compounds. Since it is impossible to experimentally investigate all chemicals in the human environment, prediction of the carcinogenic activity of chemicals is of great practical importance (4).

Epidemiological observations of the toxicity of aromatic amines were first reported in aniline dye factories by Rehn in 1895, with the report that German and Swiss workers suffered urinary bladder tumors. A major toxicological issue is the reaction with DNA and induction of carcinomas, primarily in the urinary bladder, liver or other tissues in humans and experimental animals (36).

These aromatic amines pose health hazards to human beings in two ways, i.e. by direct contact and via the environment. Some studies have shown the release of amines from consumer products such as clothing and footwear, and the absorption of aromatic amines via the skin by skin bacteria (38).

5.1. Toxicity, mutagenicity and carcinogenicity of aromatic amines

Aromatic amines are highly toxic, and are also suspected of being carcinogenic, which may have a mutagenic impact on animals and humans even at low concentrations (24).

Flückiger-Isler *et al.* (2004) tested several chemical substances including alpha-naphthylamine, 4,4'-methylene-bis (2-chloroaniline) (MOCA), benzidine and 3-amino-1,2,4-triazole, employing the Ames II mutagenicity assay in the presence and absence of Aroclor 1254-induced rat liver S9, using the strains TA98 (frameshift mutation) and TAMix (TA7001–7006) (base-pair substitutions). The authors observed that all the compounds induced mutagenicity in the presence of metabolic activation for both strains (TA98 and TAMix), except for 3-aminotriazole, which is a carcinogen not found to be mutagenic in the Ames II assay (39).

Ohe (1997) quantified and evaluated the genotoxicity of the following four carcinogenic heterocyclic amines, 3-amino-1,4-dimethyl-5*H*-pyrido (4,3-beta)indole (Trp-P-1), 3-amino-1- methyl-5*H*-pyrido (4,3-beta)indole (Trp-P-2), 2-amino-1-methyl-6-phenylimidazo (4,5-beta)pyridine (PhIP) and 2-amino-3,8-dimethylimidazo (4,5-f)quinoxaline (MeIQx), all present in cooked and charred meat and often excreted in human waste in genotoxic fractions. The author analyzed organic extracts obtained by the blue rayon hanging method, which is selective for the collection of heterocyclic amines, in water from the Yodo River (Japan), into which effluents from wastewater treatment plants or nightsoil treatment plants were discharged. This report showed the existence of mutagenic/carcinogenic MeIQx, Trp-P-1, Trp-P-2 and PhIP in the river water, and the heterocyclic amines quantified accounted for a mean of 24% of the total genotoxic activity of the blue rayon extracts recovered from the water of the Yodo River system (40).

Ono *et al.* (2000) also investigated the existence of carcinogenic heterocyclic amines in water samples from a river near Kyoto (Japan), where several sewage-treatment plants discharge their effluents and where water purification plants downstream use this polluted water as their source, and concluded that the carcinogens derived from the effluent of the sewage treatment plants, including human waste (41).

Tsukatani *et al.* (2002) examined the mutagenicity of soils sampled from hard shoulder, roadsides and a park bordering a roadside in Kurume City, Japan by Ames test. The authors observed that extracts from the soils showed much higher mutagenicity with respect to YG strains than to TA strains, indicating that these soils could be polluted with nitroarenes and aromatic amines (42).

The first QSAR analysis on the determinants of the mutagenic potency of the aromatic amines was carried out by studying a large database of chemicals using the TA98 and TA100 strains of *Salmonella typhimurium*, with S9 metabolic activation. It appears that the potency gradation of the mutagenic aromatic amines depends first on hydrophobicity and second on electronic and steric properties. Hydrophobicity, which controls the absorption and transportation of the chemicals into the living organisms and cells, as well as their interaction with the

metabolism enzymes, is known to be a major determinant in many biological activities (2).

A better understanding of the mechanisms of aromatic amine genotoxicity is required to provide important insights into the environmental and genetic origins of one or more human cancers and may reveal a substantial role for this group of compounds as human chemical carcinogens (7).

5.1.1 Azo pigments

Azo dyes are widely used in the textile, printing, paper manufacturing, pharmaceutical and food industries and also in research laboratories. These dyes are of great environmental concern due to their potential to form carcinogenic aromatic amines under reducing conditions (38,43,44).

Oliveira *et al.* (2010) demonstrated that the textile azo dyes Disperse Red 1, Disperse Orange 1 and Disperse Red 13 induced genotoxicity in the HepG2 cells and mutagenicity in the *Salmonella* strains TA98 and YG1041, and thus corresponded to an important source of environmental contamination, considering that a significant part of synthetic textile dyes are lost into waste waters during the manufacturing or processing operations (45).

The discharge of azo pigments into the environment is undesirable not only for aesthetic reasons but also because many azo dyes and their breakdown products are toxic to aquatic life and mutagenic to humans (45-47). Although the dye molecule is biologically inactive, when these compounds, either inadvertently or by design, enter the body via ingestion, they are metabolized to aromatic amines by intestinal microorganisms (38,44). Reductive enzymes in the liver can also catalyze the reductive cleavage of the azo linkage to produce aromatic amines. However, evidence indicates that the intestinal microbial azoreductase may be more important than the liver enzymes in azo reduction (44). Microorganisms on the skin or in the environment are also able to cleave the azo bonds and release the respective amines (38).

6. DETERMINATION OF AROMATIC AMINES IN THE ENVIRONMENT

To protect human health and the environment, it is imperative to develop a reliable, rapid, sensitive and environmentally friendly method to monitor aromatic amines in environmental samples (15). Several analytical methods have been reported, the most commonly employed techniques being gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) (15,24).

These methods show high sensitivity and excellent selectivity. However, some of the above techniques require large volumes of harmful organic solvents while others need complex derivatization procedures (GC) due to their polar nature, or involve the time-consuming process of sample clean-up to prevent deteriorating the chromatographic columns (15,24).

To overcome these difficulties, it is usually necessary to derivatize them before GC and flow injection coupled with voltammetry has been employed with diazotization or bromination reactions. These methods involve tedious and time-consuming sample preparation (15). Due to the low concentration of these amines in environmental samples, pretreatment and a pre-concentration step is generally required for determination of trace aromatic amine pollutants. Sample preparation is traditionally carried out by liquid–liquid extraction (LLE) or by solid-phase extraction (SPE), while in most cases, the final analysis is accomplished by either HPLC or capillary gas chromatography (GC). Since both the LLE and SPE techniques require the use of substantial amounts of organic solvents, much effort has been reported aimed at using solid phase microextraction (SPME) (34).

In recent years, capillary electrophoresis (CE) has also emerged as a fast, efficient tool for chemical analyses, due to its high separation speed and efficiency, low sample consumption and relatively simple instrumentation (15,24). A wide variety of detectors can be coupled with CE for the separation and detection of aromatic amines (24). Nevertheless, liquid chromatography (LC) is known as the most convenient technique for aromatic amines (15).

Aromatic amines are well known to show good electrochemical behavior due to the presence of the amino group, which permits their electrochemical detection (ECD) without making derivatization a prerequisite (15). ECD can offer high sensitivity and good selectivity for electroactive substances, and amperometric detection can avoid the interference caused by electro-inactive substances (24). The analysis of aromatic amines by LC– ECD has been reported, but this involves tedious procedures leading to an analysis taking more than 1 h (15). CE coupled with ECD has attracted considerable attention in environmental monitoring, and a few papers have been published for this purpose (24).

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Abbreviations: ABP: 4-aminobiphenyl; PhIP: 2-Amino-1-methyl-6-phenylimidazo (4,5-b)pyridine; MOCA: 4,4'-Methylenebis (2-chloroaniline); QSAR: Quantitative Structure-Activity Relationship; CYP450: cytochrome P450; FMOs: flavin-containing monooxygenases; NAT: *N*-acetyltransferase; NAT1: *N*-acetyltransferase 1; NAT2: *N*-acetyltransferase 2; HAAs: heterocyclic aromatic amines; TAMix: TA7001–7006; Trp-P-1: 3-amino-1,4-dimethyl-5*H*-pyrido (4,3-*b*)indole; Trp-P-2: 3-amino-1- methyl-5*H*-

pyrido (4,3-*b*)indole; PhIP: 2-amino-1-methyl-6-phenylimidazo (4,5-*b*)pyridine; MeIQx: 2-amino-3,8-dimethylimidazo (4,5-*f*)quinoxaline; GC-MS: gas chromatography–mass spectrometry; HPLC: high-performance liquid chromatography; LLE: liquid–liquid extraction; SPE: solid- phase extraction; GC: gas chromatography; SPME: solid phase microextraction; CE: capillary electrophoresis; LC: liquid chromatography; ECD: electrochemical detection

Key Words: Aromatic Amines, Air, Soil, Water, Risks, Review

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