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Biomonitoring of human exposure to arylamines

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1. ABSTRACT

Extensive industrial use of arylamines started in the middle of the 19th century in the dye industry. Because of the high incidence of bladder cancer, arylamines belong to the first and most intensively studied occupational and environmental carcinogens. In workers, biomonitoring of exposure to arylamines including *ortho*-toluidine started in the first half of the 20th century. This review highlights the many gaps in our knowledge on the human carcinogen *ortho*-toluidine.

2. INTRODUCTION

Commercial use of *o*-toluidine started after the important discovery by William Henry Perkin in 1856 of the first synthetic organic dye that could be made from coal tar (1-3). Perkin was a research assistant of the German scientist August Wilhelm Hofmann (4), director of the Royal Society of Chemistry in London. After potassium dichromate treatment of rather impure aniline, prepared by nitration of a fraction distilled from coal tar, Perkin obtained a striking colorant, which he called purple aniline, later to be known as mauve or mauveine. Perkin left academics and pursued commercial production of the purple dye. However, success of mauveine was short lived and was soon replaced by the second most famous aniline dye, fuchsine or magenta (Figure 1), which was obtained from coal tar fractions with different oxidation procedures. It was Hofmann who demonstrated that it was not pure aniline, but a mixture of aniline and toluidines that resulted in these colors (5). Nowadays, mauveine synthesis can be easily performed as a microscale organic chemistry experiment using a mixture of aniline, *o*-toluidine and *p*-toluidine (6).

Commercial success in producing fuchsine was first achieved in 1859 when the French chemist François-Emmanuel Verguin (1814-1864) joined forces with two dyers in Lyon, Messrs. Reynard Bros. They called the dye fuchsine either because of its brilliant blue-red color similar to the color of the fuchsia flower or because Fuchs is the German name for the French Reynard. Hofmann named this color aniline red or rosaniline/roseine (3). Later, the name magenta commemorating the devastating 1859 battle of Magenta, a town in northern Italy, became popular in Britain. Historically, the name magenta referred to four constituents from which all but magenta II are still commercially available (Figure 1) (7). In 1877 Fischer and Fischer (8) showed that the major part of commercial fuchsine is produced by the reaction of *o*-toluidine with aniline. It should be noted that *o*-toluidine was used for synthesis of many other dyes discovered in the last half of the 19th century (9). At that time the carcinogenic potential of *o*-toluidine was not known. In their historical review Dietrich and

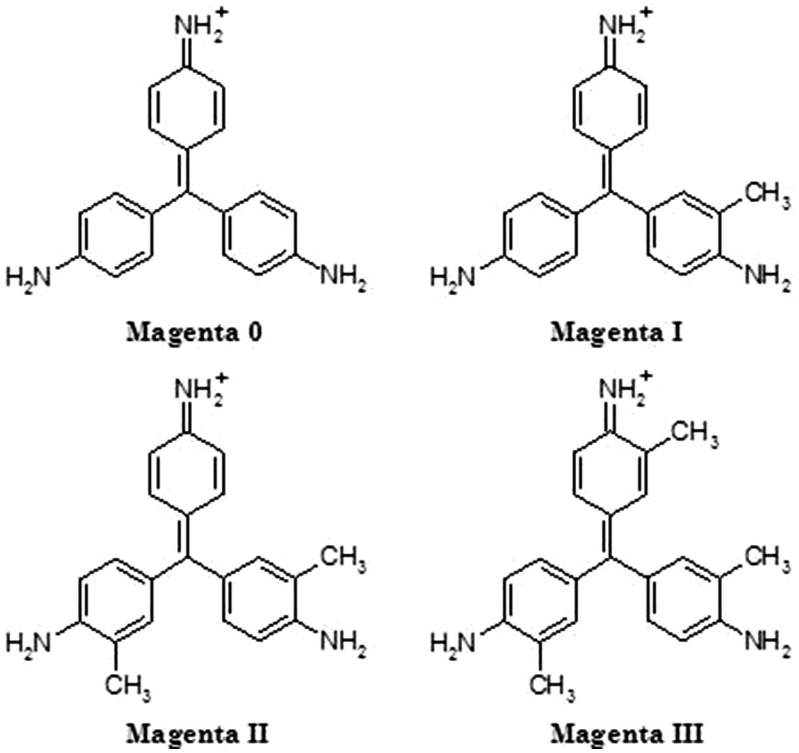

Figure 1. The four components of fuchsine (magenta).

Golka (10) mentioned that a student of Hofmann, W. H. Perkin, reported between 1856 and 1869 on the phenomenon of urinary bladder tumors among workers in the paint industry. Later, Ludwig Rehn in his seminal article (11) reported on the high incidence of occupational bladder tumors in fuchsine workers and blamed aniline as the responsible agent for this cancer. Although it is now well-known that exposure to aniline was not the cause of bladder cancer in these workers, nobody has considered *o*-toluidine as the most probable agent responsible for the high incidence of bladder cancer in industries workers producing fuchsine/magenta.

In this review the reasons for the underestimation of *o*-toluidine as a cause of bladder cancer will be discussed and the important role that biomonitoring has already played in shedding more light on this subject.

3. CARCINOGENIC POTENTIAL OF *O*-TOLUIDINE

Shortly after the publication of Rehn in 1895 (11), several observations of bladder cancer among workers in aniline factories were reported. In a report of the International Labour Office in Geneva published in 1921, toluidine still appeared on the list of the products that may cause bladder tumors (12). Based on animal experiments, monocyclic arylamines were not considered to have contributed to the extremely high incidence of bladder cancer observed in workers engaged in the production of fuchsine.

In 1972, Homburger *et al.* (13) in a meeting report referring to *o*-toluidine stated, "that even monocyclic aromatic amines may be carcinogenic". However, three decades earlier results from two rather imperfect studies by Japanese researchers published in 1940 and 1941 (14,15), suggested that *o*-toluidine gave rise to papilloma in the bladder of rats, guinea pigs and rabbits. These results were ignored by Case in his of review of bladder cancer in Britain (16), when he clearly showed that the manufacture of magenta (fuchsine) "appears to have a definite occupational hazard of causing tumour of the urinary bladder" but did not consider *o*-toluidine as the most probable causative agent. After 1970 several reports clearly demonstrating the carcinogenic activity of *o*-toluidine

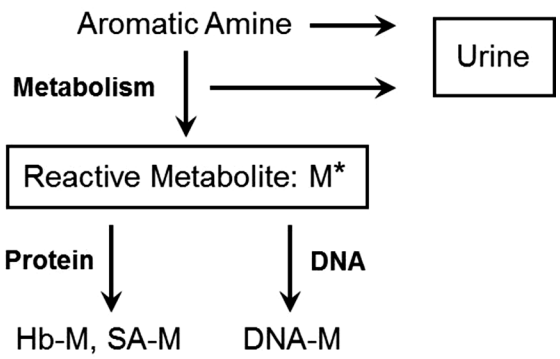

Figure 2. Human biomonitoring of arylamine exposure. Hb, hemoglobin; SA, serum albumin.

in rats and mice were published (7). Consumer use of azo dyes that, by reductive cleavage of one or more azo groups, may release detectable concentrations of carcinogenic arylamines including *o*-toluidine has been prohibited in Germany since 1998 (17). Fourteen years later this restriction was adopted by Directive 2002/61/EC of the European Union (18).

Based on animal experiments a "no significant risk level" of 4 µg *o*-toluidine/day was estimated to be associated with a lifetime cancer risk of 10^{-5} for an adult weighing 70 kg (19). This is about 10- and 100-fold lower than the estimate for 2-naphthylamine and 4-aminobiphenyl, respectively. Female beagle dogs are the most suitable animal species for testing the bladder carcinogens such as 2-naphthylamine, 4-aminobiphenyl as well as benzidine. In a comparison of the relative carcinogenic potency, 4-aminobiphenyl was 6- and 27-fold more potent than 2-naphthylamine and benzidine, respectively (20). However, *o*-toluidine was not on this list because three female dogs administered *o*-toluidine orally at a dose of 100 mg/day, 5 days/week for 6 years did not develop any abnormalities of the urinary bladder (21). However, in another study, two dogs administered an oral dose of 125 mg *o*-toluidine/kg/day developed bladder tumors after 9 and 10 years, respectively (22). In this study the cumulative dose of *o*-toluidine was about 10 times higher than in the former study and about 50 times higher than the cumulative dose of 4-aminobiphenyl giving bladder tumors in dogs after 3 years (23).

Ward *et al.* (24) reported an excess number of bladder cancers in workers exposed to *o*-toluidine at a chemical plant in western New York State, which stimulated discussion on the carcinogenicity of *o*-toluidine to humans. In 2006, the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area classified *o*-toluidine as a proven human bladder carcinogen (25). Two years later the International Agency for Research on Cancer (IARC) announced an upgrade of *o*-toluidine from a probable (group 2A) to a proven (group 1) carcinogen for humans (7). This upgrade remains to be acknowledged by the European Union which still classifies *o*-toluidine in Category 1B as a substance whose carcinogenic potential to humans is presumed but primarily based on animal data (26). In the USA *o*-toluidine has been assigned an A3 notation "Confirmed Animal Carcinogen with Unknown Relevance to Humans" by the American Conference of Governmental Industrial Hygienists (7). The National Toxicology Program has classified *o*-toluidine as "reasonably anticipated to be a human carcinogen" (7). However, according to a revised draft report this classification is expected to be changed to "*ortho*-toluidine is known to be a human carcinogen" (27). That there is still little acceptance in the scientific community as to the role of *o*-toluidine for human bladder cancer is best exemplified by a recent "platinum priority review on bladder cancer" in which *o*-toluidine was not even mentioned (28). Concern about the carcinogenic risk of *o*-toluidine also did not reduce its commercial use. The IARC has estimated the worldwide production volume in 2006 to be in the range between 10 and 50 million pounds not lower than in 1986 (7).

4. HUMAN BIOMONITORING OF ARYLAMINES

For human biomonitoring of exposure to arylamines the parent compound and its metabolites are noninvasively determined in urine (29). For biomonitoring of the metabolically activated moiety of arylamines, hemoglobin and serum albumin adducts can be determined as well as DNA adducts in leukocytes and target tissue (Figure 2). Excellent reviews are available on human biomonitoring of arylamines (29-35). After giving a short overview, available and missing results for biomonitoring of exposure to *o*-toluidine will be presented in detail.

Because of the implication of arylamines in occupational cancer, biomonitoring of exposure started first in workers of chemical plants. In 1908, the possibility of monitoring benzidine and some of its metabolites in urine was shown in animal experiments by Adler (36). First reports in the literature on biomonitoring of carcinogenic arylamines in urine date to the late 1940s when it

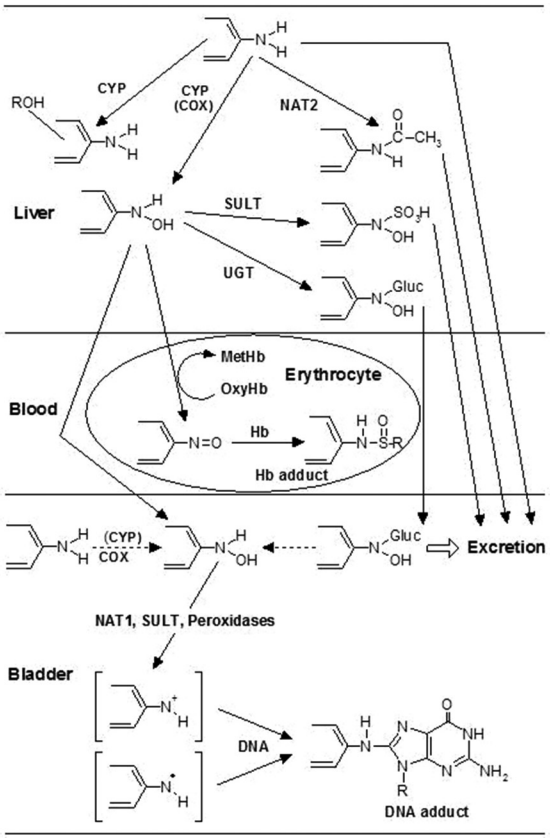

Figure 3. Major metabolic activation and detoxification pathways of aromatic amines. COX, cyclooxygenase 1/2; CYP, cytochrome P450; Hb, hemoglobin; NAT1/2, *N*-acetyltransferase 1/2; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase.

was realized that occupational health surveillance is best performed by determining human exposure in the workplace (37,38). The drawback of monitoring human urine is that arylamines undergo significant metabolism in the human body resulting in the excretion of only small amounts of the parent amines together with a large number of different metabolites. Furthermore, significant polymorphism of enzymes involved in the metabolism of arylamines, including cytochromes P450, *N*-acetyltransferases, UDP-glucuronosyltransferases, sulfotransferases, cyclooxygenases and peroxidases (Figure 3), can result in considerable variation in the percentage of dose excreted as the parent compound or certain individual metabolites and, therefore, provides only a crude estimate of an individual's exposure to arylamines. However, in the workplace, comparison of pre- and post-shift concentrations of arylamines (and/or their metabolites) is still the most valuable indicator of individual occupational workplace exposure.

Based on the work of Ehrenberg (39), biomonitoring of hemoglobin adducts have been established as biochemical markers of exposure and effect both in the workplace (40) and as markers of environmental exposure in smokers and nonsmokers exposed to environmental tobacco smoke (41-43). Hemoglobin adducts are formed after activation of arylamines through *N*-hydroxylation and do not undergo any significant repair (Figure 2). In man, hemoglobin adducts persist as long as the lifespan of hemoglobin, which is ~120 days. Therefore, adduct levels reflect a 4 month record of the time-weighted average exposure (44). However, only that part of the arylamine that has undergone *N*-hydroxylation is recorded. Studies on smokers indicate that the extent of 4-aminobiphenyl hemoglobin adduct formation depends on the balance between metabolic *N*-oxidation and *N*-acetylation (45).

Because of its shorter turnover (half-life of 20-25 days) serum albumin adducts are regarded as more suitable for tracking exposure in the workplace (44).

Fundamental studies of chemical carcinogenesis have revealed a central role for DNA adducts in the genesis of cancer (46). This has been especially well documented for arylamines (34). However, DNA adduct measurements are often precluded by the unavailability of target tissue samples in large scale human studies and by the need of extremely low analytical detection limits approaching the part-per-billion threshold (47). In contrast to hemoglobin adducts, determination of DNA adducts in human tissues have been restricted mostly to 4-aminobiphenyl (34,46). Besides unambiguous verification of 4-aminobiphenyl in human urinary bladder tissue and exfoliated buccal cells in saliva using mass spectrometric methods, arylamine adducts have also been tentatively detected in human leukocytes and mammary tissue by ^{32}P-postlabeling. In workers highly exposed to benzidine, a specific acetylated guanine adduct from benzidine has been be identified in both white blood cells and urothelial cells of workers (48).

4.1. Biomonitoring of *o*-toluidine

4.1.1. Urine and other body fluids

According to Ott and Langner (38) monitoring of *o*-toluidine in worker's urine date back to the mid-1940s. At that time unchanged

Table 1. Biomonitoring of o-toluidine in human urine

Occupational Exposure							
			Unexposed		Exposed		Ref
Origin of samples	Dimension, value	Smoking status	Pre-shift	Post-shift	Pre-shift	Post-shift	
New York chemical plant	µg/L, Median (N)	Yes	1.0 (12)	2.6 (12)	20.0 (19)	135.6 (20)	(55)
		No	1.2 (20)	2.8 (20)	17.5 (29)	83.9 (32)	
New York chemical plant	µg/L, Mean±SD (N)	-	1.1±1.0 (31)	2.7±1.4 (31)	18.0±27.0 (46)	104.0±111.0 (46)	(56)
New York chemical plant	µg/L, Mean±SD (N)	Yes	0.9±0.7 (10)	2.8±1.2 (9)	14.3±10.2 (15)	132.1±153.1 (15)	(57)
		No	1.3±1.3 (16)	2.8±1.6 (16)	16.1±33.0 (28)	80.1±94.0 (27)	
German chemical plants[a]	µg/L, Mean±SD (N)	Yes	1.7±1.6 (8)		0.6±0.1.1 (22)		(54)
		No	nd (8)		0.4±1.1 (21)		
French chemical plant	µg/L, Mean±SD (N)	-			1.7±1.5 (8)	523.0±312.6 (8)	(52)
German rubber industries	µg/L, Median, Range (N)	Yes				0.6, nd-242.9 (36)	(51)
		No				6.0, nd-292.4 (15)	
Swedish rubber industries	µg/L, Median, Range (N)	-				0.46, 0.03-0.11 (157)	(53)
Environmental Exposure							
Origin of samples	**Dimension, value**	**Smokers**	**Nonsmokers**	**Passive Smokers**	**Ratio[e]**		
New York area	ng/24 h, Mean±SD (N)	6357±4041 (10)	4222±3798 (9)		1.5		(58)
Munich, Germany	ng/24 h, Median, Range (N)		61.8, nd-401 (81)				(64)
North-Rhine Westphalia, Germany	ng/L, Median, Range (N)	206, nd-838 (45)	85, nd-1660 (115)	87, nd-209 (37)	2.4		(65)
Munich, Germany	ng/24 h, Mean±SD (N)	204±59 (10)	105±26 (9)[b]		1.9		(62)
Bavaria, Germany	ng/L, Mean, Range (N)	130, nd-173 (145)	100, nd-34		1.3		(60)
Lincoln, NE	ng/24 h, Mean±SD (N)	87.6±61 (20)	40.9±29.2 (20)[c]		2.1		(59)
Lincoln, NE	ng/24 h, Mean±SD (N)	278±223 (15)	54±16 (15)[c]		5.1		(63)
3 European countries[d]	ng/24 h, Mean±SD (N)	179±491 (1148)	64±128 (395)		2.8		(61)

[a]Workers processing aniline and 4-chloroaniline, time of sampling not specified; unexposed controls from general population; [b]one nonsmoker with 731 ng/24 h excluded; [c]smokers who have stopped smoking for 8 days; [d]Germany, Switzerland, United Kingdom; [e]smokers vs. nonsmokers.

arylamines in urine were solvent extracted and semiquantitatively estimated by specific color reactions either directly or after separation by paper chromatography (49). Further studies on occupational exposure to *o*-toluidine using more sensitive and specific analytical methods are summarized in Table 1 (50-57). Large increases of the concentration in post-shift urine samples clearly demonstrate the

effectiveness of monitoring occupational exposure to *o*-toluidine by determination of free *o*-toluidine in workers' urine. Korinth *et al.* (50,51) provided evidence that in rubber industry workers skin absorption may be more important than inhalation. Impaired skin leads to higher internal exposure and use of skin barrier creams further enhances percutaneous uptake of *o*-toluidine.

Tobacco smoke as a source of human exposure to *o*-toluidine was first shown by El-Bayoumy *et al.* (58) who reported a 1.5.-fold higher excretion of free *o*-toluidine in smokers compared to nonsmokers. This was confirmed in a series of follow-up studies summarized in Table 1 (54,59-65). The differences between smokers and nonsmokers range between 1.3- and 5.1-fold. Clearly, other environmental sources of *o*-toluidine exposure also play an important role in total *o*-toluidine exposure. Seidel (64) in his report to the German Umweltbundesamt confirmed earlier reports that nutrition could add to the human burden of arylamine exposure including exposure to *o*-toluidine (66,67). In 10 nonsmokers, excretion of *o*-toluidine in urine increased 3-fold from 31 to 102 ng/24 h after one day on a controlled diet rich in eggs, fat and meat. Seidel detected *o*-toluidine in µg/kg amounts in meat and dairy products and in upper ng/kg amounts in salads, vegetables, eggs, alcoholic beverages, cereals and fish. Even higher concentrations in the upper µg/kg range were reported for ice cream powders, food colors and soft drink concentrates (68). Particles from rubber tires in road dust could be another significant source of exposure to *o*-toluidine since extracts from shredded used tires contains on average 58.2 mg/kg (range 0.07-130) of *o*-toluidine (64).

As already mentioned, one should be aware that free *o*-toluidine in urine only accounts for a minor percentage of *o*-toluidine uptake. This has been clearly demonstrated in human studies with prilocaine which date back to the 1970s (69). Prilocaine was introduced in 1960 and is the only amide ester used as a local anesthetic that on metabolism is hydrolyzed to release *o*-toluidine (70). Within 24 h after subcutaneous administration of 20 mg prilocaine/kg body weight to human volunteers, only a minor amount of the initial prilocaine dose was excreted in urine as *o*-toluidine (0.75%), whereas 4- and 6-hydroxy-*o*-toluidine accounted for 34% and 2.7% of the dose, respectively (69).

Finally, analysts should be aware of possible artifactual formation of *o*-toluidine when analyzing urine samples which are highly contaminated with 2,5-toluylenediamine from personal application of hair dyes (71).

In plasma, *o*-toluidine has only been determined in humans after subcutaneous administration of prilocaine (69) or after treatment with eutectic mixtures of prilocaine and lidocaine (EMLA®, Oraqix®) (72-74). The average half-life of *o*-toluidine elimination from plasma was 4 h after administration of the anesthetic periodontal gel Oraqix® (74).

Monocyclic arylamines including *o*-toluidine and 2,6-dimethylaniline, the primary metabolite of lidocaine and other local anesthetics (70), have also been detected in human milk (75,76).

4.1.2. Hemoglobin adducts

Results on hemoglobin adducts of *o*-toluidine published up to the year 2000 have been summarized in a previous review (30). Although the role of *o*-toluidine for bladder cancer is well established and high environmental exposure has been demonstrated even in nonsmokers, no additional data have been published, except for in a dissertation by Tobias Weiss (65). Weiss analyzed blood from 46 smokers and 154 nonsmokers and confirmed the much lower contribution of smoking to hemoglobin adduct levels of *o*-toluidine (median 158 vs. 140 pg/g hemoglobin) compared to 4-aminobiphenyl (median 50 versus 13 pg/g hemoglobin). Regional differences related to traffic density in Bavarian children from Munich, Augsburg and Eichstätt (77) have not been confirmed by Weiss (65).

Singular extremely high hemoglobin adduct levels of *o*-toluidine in nonsmokers of our earlier studies (unpublished observation) as well as by Weiss (65), and the high urinary excretion of *o*-toluidine by one nonsmoker in the study of Riedel *et al.* (62), finally let us to consider treatment with prilocaine as the source of high adduct levels from *o*-toluidine. In 20 head and neck surgery patients and 6 healthy volunteers 24 h after receiving a standard dose of 100 mg for prilocaine local anesthesia, hemoglobin adducts releasing *o*-toluidine increased on average 40-fold (78). As shown in Figure 4, the *o*-toluidine hemoglobin adduct level after prilocaine treatment was in the same order of magnitude as in exposed workers from a rubber manufacturing company with

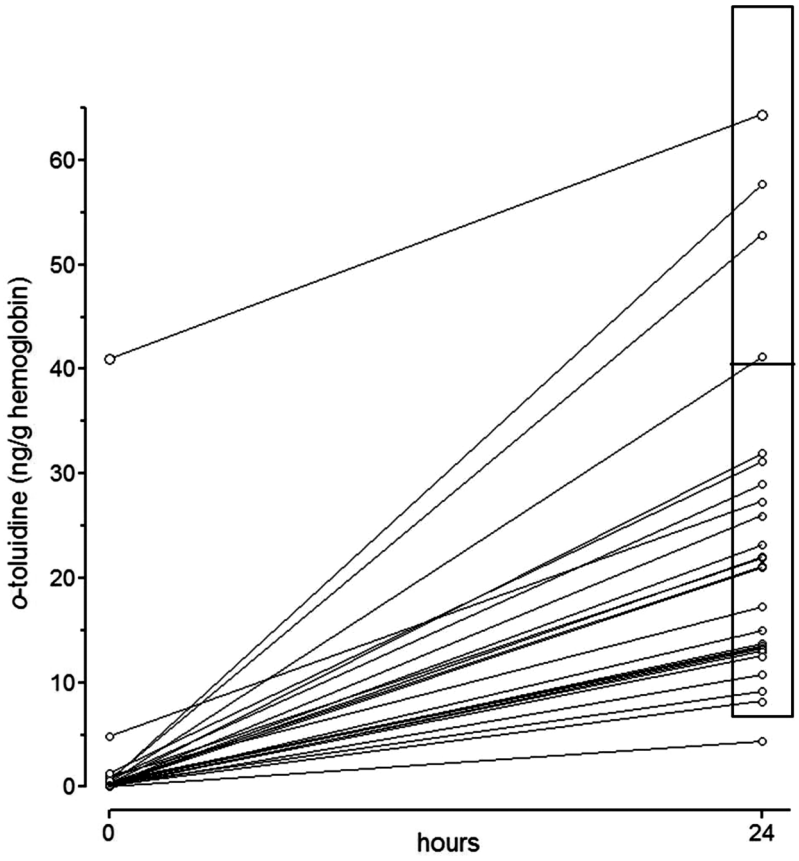

Figure 4. Increase of o-toluidine hemoglobin adducts in patients 24 h after subcutaneous injection of 100 mg prilocaine. For comparison, the box given on the right edge represent mean ± standard deviation of results for workers of a rubber manufacturing company (57); modified from Gaber *et al.* (78).

an increased risk of bladder cancer (79-81). It has to be kept in mind that treatment of patients with a single dose of prilocaine is of far less concern than cumulative exposure to lower doses of *o*-toluidine in rubber workers which may last for many years. However, for tumescent liposuction, prilocaine is given by subcutaneous infiltration at much higher doses in the range of 10-30 mg/kg body weight, i.e. gram amounts of prilocaine (82). Eutectic mixtures of prilocaine and lidocaine are frequently used in children as pain- and distress-reducing intervention for venipuncture (83,84). Recently, the European Medicines Agency granted a marketing authorization valid throughout the European Union for Lidocaine/Prilocaine Plethora (TEMPE®) for treatment of premature ejaculation (85). This drug formulation consists of a metered-dose aerosol spray releasing 7.5 mg lidocaine and 2.5 mg prilocaine per actuation and each dose consists of three spray actuations (86-88). Clearly, this treatment will lead to long-term exposure to *o*-toluidine in addition to 2,6-dimethylaniline which is released from lidocaine (89). Authorization of drugs which are metabolized to *o*-toluidine is in sharp contrast to efforts of the pharmaceutical industry to minimize exposure of patients to impurities in pharmaceuticals which may be genotoxic and potentially carcinogenic (90). It is also contrary to strict EU regulations banning from commerce azo dyes derived from a variety of carcinogenic arylamines including *o*-toluidine (18,91) and the long-standing EU law prohibiting use of *o*-toluidine in cosmetics (92). During non-clinical evaluation of TEMPE®, dermal application of 1, 4 and 40 mg daily for 28 days to rats led to a dose-dependent increase of hemoglobin adducts releasing *o*-toluidine and 2,6-dimethylaniline. Interestingly, low levels of *o*-toluidine were present in untreated rats (88) confirming earlier observations published for 4-aminobiphenyl (93,94) and our unpublished observations for *o*-toluidine-releasing hemoglobin adducts.

A remarkable degree of variation of *o*-toluidine-releasing hemoglobin adducts (6- to 360-fold, Figure 4) was observed in patients and volunteers who received 100 mg by subcutaneous infiltration (78). This may indicate a high degree of polymorphism in enzymes involved in the primary metabolism of prilocaine as well as in the metabolism of *o*-toluidine. Metabolism studies using human liver microsomes have shown that prilocaine is hydrolyzed to *o*-toluidine by recombinant human carboxylesterases (CES) 1A and CES2 (95). General inhibitors of CES significantly decrease methemoglobin formation by prilocaine in mouse erythrocytes co-incubated with microsomes. An anti-CYP3A4 antibody further decreased the residual formation of methemoglobin. In the same study *o*-toluidine mediated methemoglobin formation was only inhibited by an antibody to CYP2E1 (95). This is in accord with data of Stiborová for *o*-anisidine (96,97). The absence of any effect by smoking status on the increase of *o*-toluidine adducts after prilocaine treatment does not support a significant role of CYP1A2 for the metabolic activation of *o*-toluidine (78). This is further supported by studies in rats showing no increased formation of *o*-toluidine hemoglobin adducts after induction with the CYP1A2 inducer ß-naphthoflavone (98). Polymorphic *N*-acetyltransferase 2 is regarded to be a key enzyme in arylamine metabolism (99,100) and has been suggested to modulate 4-aminobiphenyl-releasing hemoglobin adducts in smokers (45). However, *o*-toluidine is much less efficiently *N*-acetylated by recombinant human *N*-acetyltransferase 2 compared to 4-aminobiphenyl (101-103).

4.1.3. DNA adducts

According to Klaene *et al.* (35) "Exposure to carcinogens can lead to the formation of DNA adducts, a key step towards the onset of disease such as cancer. This is a well-established mechanism and therefore detection and monitoring DNA adducts can serve as an indicator of exposure and disease. While there is great debate as to whether the presence of DNA adducts will lead to disease, it is imperative that early detection methods be developed to improve prognosis and early treatment. DNA adducts are an ideal target for human screening, biomarker discovery, and measurement of exposure, as adducts are often present well before the onset of disease." Although this statement is supported by most scientists working in molecular and occupational epidemiology, studies concerning arylamines have been mostly restricted to exposure to 4-aminobiphenyl (34). Three most common approaches, in decreasing order of specificity, have been used, mass spectrometry, immunoassays and immunohistochemistry, and ^{32}P-postlabeling. Only in 1994 was the first study using mass spectrometry published which confirmed the presence of DNA adducts from 4-aminobiphenyl in human bladder (104).

DNA adduct formation by *o*-toluidine in rat liver was first shown in 1990 by Brock *et al.* (105). This was later confirmed by two other groups (106,107) but not by Jones and Sabbioni (108). In addition to the detection of DNA adducts by ^{32}P-postlabeling in liver of both *o*-toluidine- and 2,6-dimethylaniline-treated rats, Duan *et al.* (107) were also able to detect DNA adducts in nasal mucosa. Interestingly, adducts in bladder were only detected after treatment with 2,6-dimethylaniline. In this study (107), the same DNA adducts were detected in lidocaine- and prilocaine-treated rats, thus confirming the genotoxicity of these drugs.

In a preliminary study including only 6 volunteers, DNA adducts releasing *o*-toluidine were detected by gas chromatography/mass spectrometry in DNA from urinary sediments (78). Finally, the presence of *o*-toluidine adducts was confirmed in epithelial tissue of sudden death victims as well as in tumor samples from patients with bladder cancer (109). Because of unavoidable background problems when analyzing *o*-toluidine, adducts could be verified in only 13 of 46 samples from sudden death victims. However, 11 of 12 tumor samples, having significantly higher adduct levels compared to tumor free samples (> 30-fold on average), tested positive for *o*-toluidine adducts. In all positive samples adducts from 4-aminobiphenyl were considerably lower than those from *o*-toluidine.

5. RECOMMENDATION FOR FUTURE STUDIES ON *O*-TOLUIDINE

The detection of o-toluidine-releasing DNA adducts in bladder tissue by gas chromatography/mass spectrometry calls for confirmation by up-to-date techniques for determination of the major guanine adduct using liquid chromatography/mass spectrometry which have been successful used for determination of adducts from 4-aminobiphenyl (47,110). This should be feasible in view of the much higher adduct levels of *o*-toluidine compared to 4-aminobiphenyl (109).

In view of the large variation in hemoglobin adducts after a single dose of prilocaine, the human metabolism *o*-toluidine needs to be investigated as to the role of polymorphic or environmentally modulated enzymes. Information on the involvement of cytochromes P450 and *N*-acetyltransferases are still insufficient and other enzymes such as glucuronosyl transferases, sulfotransferases and glutathione *S*-transferases have not yet been studied. The elevated expression of cyclooxygenase 2 in high-grade bladder cancer but not in normal bladder (111) could be responsible for the much high levels of *o*-toluidine-releasing DNA adducts in tumor tissue (109). A series of carcinogenic aromatic and heterocyclic amines, not including *o*-toluidine, have been shown to be activated by cyclooxygenases to DNA binding species (112,113).

Finally, environmental sources of o-toluidine other than tobacco smoke need to be studied in more detail.

6. SUMMARY

According to Golka *et al*. (114), "*o*-toluidine is the only Group I carcinogenic aromatic amine which is still used in the industry and in the workplace". Even worse, the local anesthetic prilocaine which releases *o*-toluidine after amide bond hydrolysis is still in use in large amounts for pain management and treatment of premature ejaculation.

In his review on monocyclic arylamines Skipper *et al*. (33) emphasized the potential significance of *o*-toluidine as human environmental carcinogen. This has been supported by detection of DNA adducts of *o*-toluidine in urinary sediments and samples of human bladder which closed an important gap in the chain of evidence that *o*-toluidine is a human bladder carcinogen (78,115) and supports the classification of *o*-toluidine as human carcinogen by the IARC (7) which has been mainly based on results from epidemiology, animal experiments and biomonitoring of hemoglobin adducts in humans. Nonetheless, our knowledge on the mechanism of action of *o*-toluidine as well as its environmental sources is sparse when compared with other well studied arylamines such as 4-aminobiphenyl.

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