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Review

Biomonitoring of exposure to aromatic amines: haemoglobin adducts in humans

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Abstract

Haemoglobin (Hb) adducts from aromatic amines (AAs) are well established biomarkers of exposure. Tobacco smoking and occupational exposure are major sources of AA Hb adducts. The origin of background levels in non-smokers and non-occupationally exposed humans are largely unknown. Here we examine the determination of AA Hb adducts, focussing on the analytical strategies for Hb isolation, removal of unbound AAs from Hb solutions, hydrolysis of the Hb bound AAs, extraction, preconcentration, clean-up and derivatisation of the free amines for determination by gas chromatography–mass spectrometry. Finally, a detailed summary of available results on the determination of AA Hb adducts is given.

Keywords: Reviews; Aromatic amines; Hemoglobin adducts

Contents

1. Introduction

Aromatic amines (AAs) and nitroarenes are widespread occupational and environmental pollutants [1,2]. Commercial production of these two classes of compounds started in the middle 1800s. At the end of the nineteenth century they were among the first chemicals recognised as human carcinogens being responsible for an increased incidence of urinary

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bladder cancer in German dye stuff workers [3,4]. Up to date the following compounds have been classified as carcinogens in humans: 4-aminobiphenyl, benzidine, 4-chloro-2-methylaniline and 2-naphthylamine [1,5]. The main exposures of the general population to AAs is through cigarette smoke or to products which contain products synthesised from AAs. Among other compounds AAs as 2-, 3-, and 4-methylaniline, 3- and 4-aminobiphenyl, and 2-naphthylamine are present in the cigarette smoke with substantially higher concentrations in side stream compared to main stream smoke [6]. These compounds may account for the positive correlation between cigarette smoking and the incidence of bladder cancer in humans [2,7]. As with most chemical carcinogens, AAs and nitroaromatic hydrocarbons need to be metabolised into reactive electrophiles in order to exert their carcinogenic effects. This activation involves typically *N*-oxidation of arylamines and nitroreduction of nitroarenes to yield *N*-hydroxy arylamines (Fig. 1). Within erythrocytes *N*-hydroxy arylamines are further oxidised by haemoglobin (Hb) to the nitrosoarenes, which react with the thiol group of cysteine to semimercaptals. Elimination of water to the nitrenium ion and subsequent reaction of sulfur with water yields the sulfinamide. Sulfinic acid amide adducts are readily hydrolysed under mild conditions, yielding the parent amine. In experiments with radiolabelled AAs it could be shown that the Hb binding correlates with DNA binding. Therefore, Hb adducts can be taken as surrogate markers for the biologically active dose of these genotoxins [7–9]. For most of the AAs and nitroarenes other metabolic pathways leading to either activation or detoxification are less well characterised. *N*-acetylation by NAT2 in liver is generally regarded as detoxification and this has been well established for 4ABP.

This paper reviews and summarises the current chromatographic methods for the determination of Hb adducts from AAs and nitroarenes (Fig. 2) and gives an overview of the results obtained with these methods (Tables 2–4).

2. Chromatographic methods for the determination of Hb adducts of AAs

Ten years after the proposal of Lars Ehrenberg and his co-workers to use protein adducts as surrogate for DNA adducts [10], based on the pioneering experimental work of Neumann [8] the first results on the determination of Hb adducts of AAs in humans have been published [11–13]. The major steps in the analysis involve isolation of Hb from separated erythrocytes, removal of unbound AAs, release of the free amines by hydrolysis, determination of the free amines, mostly after extraction into an apolar

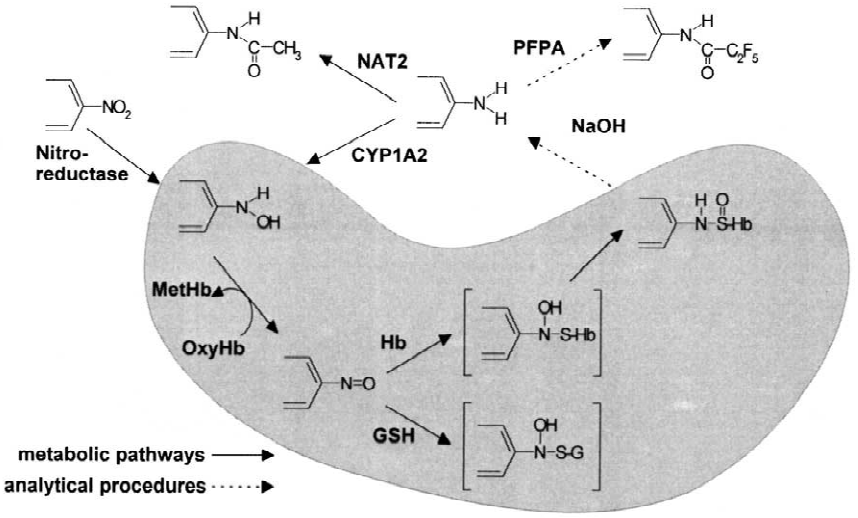

Fig. 1. Metabolic pathways of aromatic amines in liver and erythrocytes and analytical procedures for determination of haemoglobin adducts. CYP1A2: cytochrome P450 isozyme 1A2, GSH: glutathione, NAT2: *N*-acetyltransferase 2, PFPA: pentafluoropropionic anhydride.

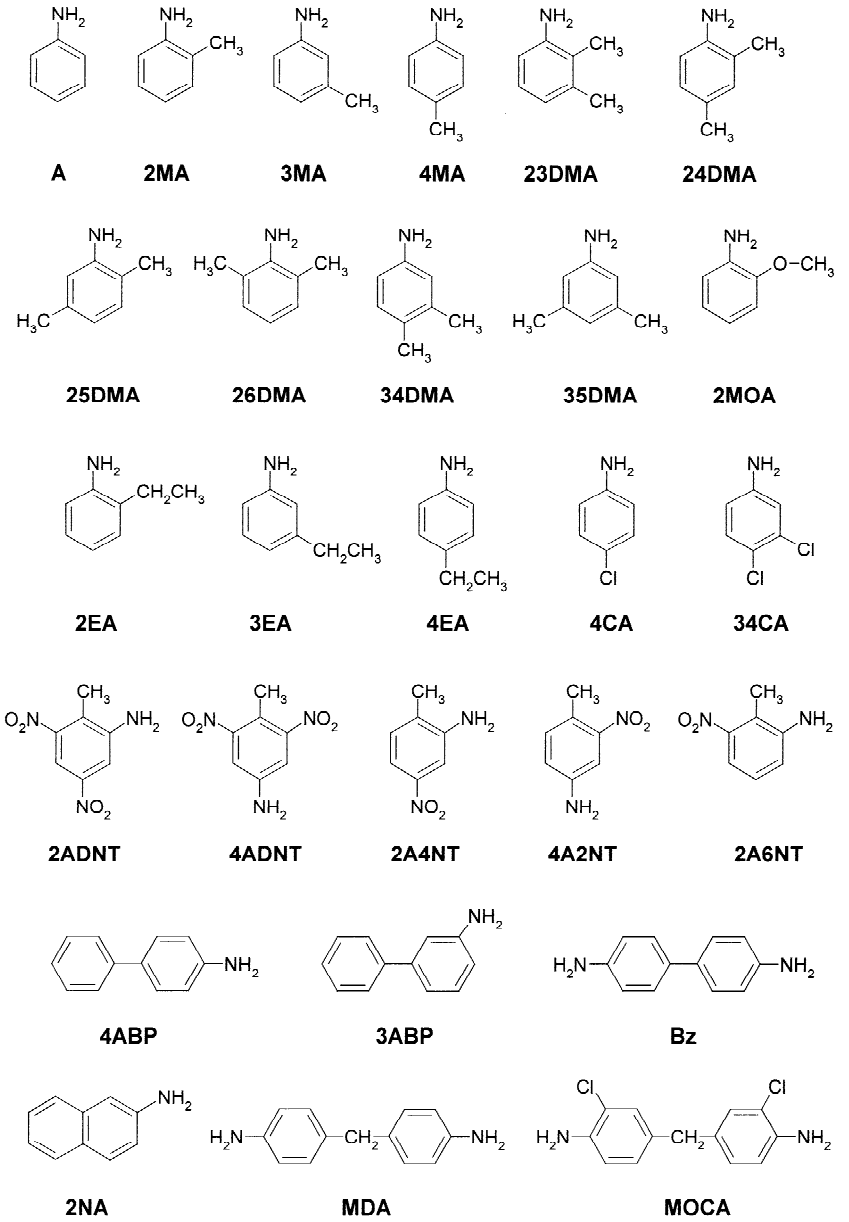

Fig. 2. Aromatic amines found as haemoglobin adducts in humans. A: aniline, 2MA: 2-methylaniline, 3MA: 3-methylaniline, 4MA: 4-methylaniline, 24MA: 2,4-dimethylaniline, 25MA: 2,5-dimethylaniline, 26MA: 2,6-dimethylaniline, 34MA: 3,4-dimethylaniline, 35MA: 3,5-dimethylaniline, 2MOA: 2-methoxyaniline, 2EA: 2-ethylaniline, 3EA: 3-ethylaniline, 4EA: 4-ethylaniline, 4CA: 4-chloroaniline, 34CA: 3,4-dichloroaniline, 2ADNT: 2-aminodinitrotoluene, 4ADNT: 4-aminodinitrotoluene, 2A4NT: 2-amino-4-nitrotoluene, 4A2NT: 4-amino-2-nitrotoluene, 2A6NT: 2-amino-6-nitrotoluene, 4ABP: 4-aminobiphenyl, 3ABP: 3-aminobiphenyl, Bz: benzidine, 2NA: 2-naphthylamine, MDA: 4,4′-methylenedianiline, MOCA: 4,4′-methylenebis(2-chloroaniline).

solvent and derivatisation, by GC–MS methods (Fig. 3). The established analytical procedures differ mainly in two steps. In order to remove unbound AAs either the Hb solution is dialysed against water or Hb is precipitated and washed with apolar solvents. After hydrolysis free AAs are enriched and purified by either liquid–liquid extraction or solid-phase extraction (SPE). The different approaches have been

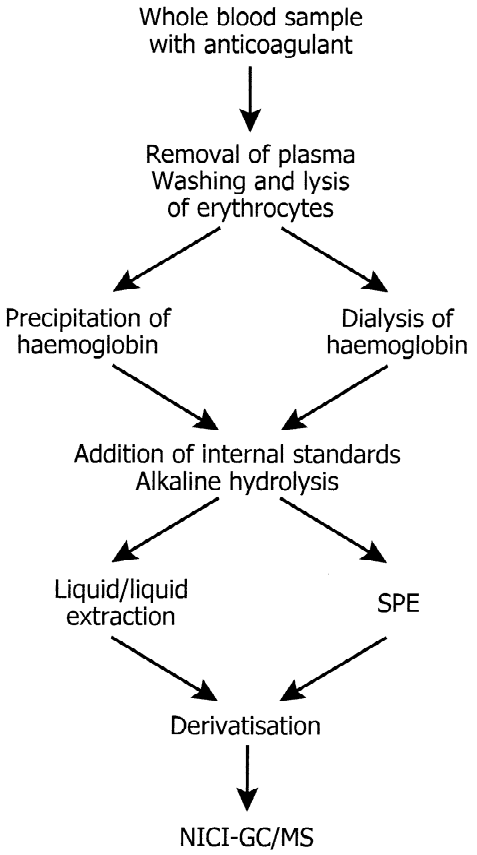

Fig. 3. Major steps in the analysis of haemoglobin adducts from aromatic amines. NICI-GC–MS, capillary gas chromatography mass spectrometry in the negative-ion chemical ionization mode, SPE: solid-phase extraction.

described in detail in the literature [12,14–27]. In the following advantages and disadvantages of the methods will be discussed. Emphasis will be given on possible pitfalls when using the method in the lowest ranges of detection sensitivity used for environmental samples.

2.1. Preparation of Hb solution

Blood samples are collected by venipuncture into vessels containing an anticoagulant. There is no indication from literature that the type of anticoagulant, e.g. heparin or EDTA, is of any importance. The blood samples can be stored for 24 hours at room temperature and for up to a maximum of 3 days at 4–6°C. No suitable Hb solutions for adduct analyses can be obtained from clotted or frozen blood samples [24]. Erythrocytes are sedimented by low speed centrifugation at 1200–3000 *g* and the plasma is drawn off. The packed cell layer is washed two to three times with equal volumes of physiological (0.9% NaCl in water) or phosphate buffered saline to remove plasma proteins. The saline is mixed thoroughly with the cells and after centrifugation drawn off and discarded. Subsequently, the red cells can be either stored deep frozen (≤20°C) or lysed to obtain a haemoglobin solution. No loss of adducts has been observed after storage of frozen cells at −20°C for up to 2 years [16]. Cell lysis is accomplished by adding 2–3 volumes of distilled water per volume of packed red blood cells. The addition of approximately 10% (v/v) of toluene as proposed by Bryant et al. [12] has not proven to be essential [17,21,24]. The sample is centrifuged to remove cell debris.

2.2. Removal of unbound amines from Hb

Dialysis of the Hb solution for 2–3 days against three changes of water is the most simple and straightforward method to remove unbound amines and amines bound to low molecular mass peptides such as glutathione [12,15,16,21–23,26]. Other groups prefer to precipitate Hb which may be subsequently washed by organic solvents [17–20,24,25,27]. Most of the environmental samples with low adduct levels of AAs have been measured using the dialysis method [12,15,22,26,28–48]. Laboratories with ample experience in Hb handling prefer to precipitate the Hb. This shortens the overall time of analysis but not necessarily the time for processing the samples. More handling and the use of additional solvents, e.g. ethanol, ether or acetone, may increase the risk of introducing background contamination during the Hb precipitation. It is strongly recommended to initiate an interlaboratory test to compare both methods with low contaminated human blood samples.

2.3. Adduct hydrolysis

Prior to adduct hydrolysis aliquots of the dialysed Hb solutions are removed for determination of the Hb concentration [12,16,21]. Subsequently, one tenth the volume of 1 *M* NaOH [21] or equally one hundredth the volume of 10 *M* NaOH [12,16] is

added to the Hb solutions. Precipitated Hb is weighed and subsequently dissolved directly in 0.1 *M* NaOH [17,24]. At this point internal standards (see below) are added. After addition of NaOH the solutions are either held for 2–3 h [12,16], mechanically shaken [17,24] or sonically dispersed [21] for 1 h at room temperature. In order to avoid significant losses, Hb adducts from 1-nitropyrene should be hydrolysed under a nitrogen atmosphere [25].

2.4. Extraction of free amines

Most analytical procedures use liquid–liquid extraction to transfer the hydrolysed amines into organic solvents for derivatisation. According to Skipper and Stillwell [16] hexane is the optimum solvent for amine extractions. Many other groups used hexane as well [12,15,17,23,26] or alternatively dichloromethane [18,19,22,35] partly depending also on the amines to be analysed. For liquid–liquid extraction the tubes should be shaken gently to avoid the formation of unbreakable emulsions. The emulsions can be broken by freezing. Such problems can be avoided by absorption of the Hb solution onto Chem Elut [20] or Extrelut [27,49] with subsequent elution with ether [20,27] or dichloromethane [49]. However, this is only feasible with low volumes of Hb solutions which are only obtained with the precipitation methods. For larger volumes of Hb solutions we have developed a SPE method using C_{18} cartridges which allows the simultaneous determination of Hb adducts from AAs and tobacco-specific nitrosamines [21,48]. This method has been further developed and validated for aniline, 2MA, 3MA, 2MOA, 4CA 1NA, 2NA, 4ABP, Bz, MDA and 3,3′-dichlorobenzidine by the Working Group Analytical Chemistry of the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area [24]. For 2,4- and 2,6-toluenediamine and monoacetylated diamines an alternative SPE method based on acid hydrolysis with sulphuric acid is proposed as well as the liquid–liquid extraction method of Sabbioni and Beyerbach [19]. A low extraction efficiency by both SPE and liquid–liquid extraction has been reported for 1NA [17,24].

2.5. Derivatisation

The solvents from either liquid–liquid extraction or SPE are dried over $Na_2SO_4/MgSO_4$ and then derivatised by addition of trimethylamine and pentafluoropropionic anhydride (PFPA) [12,16,21–23]. Alternatively, PFPA or heptafluorobutyric anhydride (HFBA) is used for derivatisation without addition of trimethylamine [17,19,20,24,50]. The reaction takes less than 10 min and some authors add methanol [24] or a methanolic solution of 4MA [19,20,50] to stop the reaction after 10 min. Traces of water may have crucial influence on the yield of derivatisation and should be excluded as far as possible.

2.6. Internal standards

The use of labelled internal standards reduces the variability due to extraction efficiencies or changes in instrument performance and assures the accuracy of quantification. For GC–MS AA marked with isotopes such as [^{2}H] or [^{13}C] can be used. For analysis of AA Hb adducts several deuterated, e.g. 4ABP-d_9 and 2MA-d_4 [24], and some [^{13}C]-labelled compounds, e.g. [$^{13}C_6$]4CA [17], are commercially available. Skipper and Stillwell [16] proposed Hb adducted with the amine of interest labelled with a stable isotope as the best internal standard. They are using routinely 4ABP-d_9 adducted Hb in their analyses. With this standard the extent of alkaline hydrolysis is additionally full in control. However, this is not a critical step in the analysis of Hb adducts from AAs. According to experience by us and others analysing duplicate or more samples from one blood donor, the same precision (coefficient of variation $<10\%$) can be achieved using unadducted stable isotopes [17,21,24]. When analysing different classes of AAs at least one representative amine should be used; e.g. aniline-d_5 because of its higher volatility is not a suitable internal standard for analysis of methylated or ethylated amines [17,24,48]. Consequently, Sabbioni and coworkers use deuterated internal standards for every single amine which they synthesised mostly by themselves [17,19,20].

2.7. Chromatographic analysis

The majority of analyses of Hb adducts from AAs and nitroarenes have been performed using capillary

GC–MS in the negative-ion chemical ionization (NICI) mode. Some typical settings are summarised in Table 1. For analysis of PFPA or HFBA derivatives together with stable isotope-labelled internal standards capillaries with non-polar stationary phases are to be preferred. More polar phases have been necessary when monofluoro internal standards such as 4′-F-4ABP were used [16]. In the analysis of trace

Table 1
GC–MS methods

Analytes[a]	Column (m/mm/μm)[b]	Injection mode	Temperature (°C)				Carrier gas	Ionization mode	Reagent gas	Emission current	Electron energy	References
			Injector	Column	Source	Interface						
b-A	Supelcowax 10 20/0.25/0.25	Splitless, valve open for 0.5 min	240–250	60: 1 min, 20/min to 240, 240: 5 min	150	240–250	Helium, 2 ml/min	NICI	Methane	400–450 μA	60–150 eV	[12]
b-A	DBWAX 15/0.32/-	Moving-needle-solid inlet system	250	180: 0 min, 8/min to 240			Helium, 1 ml/min	NICI	Isobutane	500 μA	50 eV	[35]
b-A	DBWAX 20/0.18/0.3	Splitless	200	100: 1 min, 20/min to 240, 240: 15 min	180		Helium, 21 kPa	EI				[22]
b-A	SGE BPX35 25/0.32/0.25	Splitless	260	60: 2 min, 30/min to 320	230		Helium, 1 ml/min	NICI	Methane		200 eV	[26]
m-A	DB5 30/0.25/0.25	On-column		60: 1 min, 10/min to 180				EI				[40]
m-A, b-A	Supelcowax 10 20/0.25/-	Splitless, valve open for 0.5 min		60: 1 min, 20/min to 230, 5/min to 250, 250: 5 min	150	240	Helium, 2 ml/min	NICI	Methane	400–450 μA	150 eV	[15]
m-A, b-A	DB5 15/0.25/-	Splitless, valve open for 0.5 min		80: 1 min, 20/min to 280, 280: 5 min	150	240	Helium, 2 ml/min	NICI	Methane	400–450 μA	150 eV	[15]
m-A, b-A	DB5.ms 30/0.25/0.25	Splitless, valve open for 1 min	220	60: 2 min, 20/min to 260, 260: 4 min	150	250	Helium, 2 ml/min	NICI	Methane	300 μA	70 eV	[21]
m-A, b-A	DB1701[c] 15/0.25/1	Splitless	200	50: 0 min, 50/min to 200, 200: 1.2 min, 50/min to 240, 240: 3.2 min	200	200	Helium, 1.5 ml/min	NICI	Methane	300 μA	100 eV	[17]
m-A, b-A, acb-A	Rtx5-MS[c] 15/0.25/0.25	Splitless	320	80: 1 min 50/min to 200, 200: 1 min, 30/min to 260, 260: 1.5 min, 50/min to 300, 300: 1.5 min	280	250	Helium, 90 kPa	NICI	Methane	300 μA	240 eV	[19]
m-A, b-A, acb-A	DB5.ms 25/0.2/0.33	Splitless, valve open for 1 min	280	80: 1 min, 12/min to 210, 15/min to 320	190	250	Helium, 80 kPa	NICI	Methane	300 μA	70 eV	[24]

[a] b-A, bicyclic amines; m-A, monocyclic amines; acb-A, acetylated bicyclic amines.
[b] Length/ID/film thickness.
[c] With a 1 m×0.25 mm methyl-silyl deactivated retention gap.

levels of adducts, such as those produced by tobacco smoke exposure, the organic extracts obtained after AA derivatisation have to be concentrated to volumes in the low μl range. This is achieved either in a rotary evaporator [16], by careful evaporation under a gentle stream of N_2 [17,24] or by concentration in a vacuum centrifuge at room temperature [21]. Evaporation prior to derivatisation could lead to significant losses due to the higher volatility of the free amines.

2.8. Pitfalls

Absolute verification of the identity of the compounds which are quantified under GC–MS with NICI conditions is not possible. In most cases there is not enough material available to obtain a full mass spectrum under electron ionization mode. The qualitative information usually consists of GC retention times and the fact that the chromatograms are generated by characteristic ions. These ions are at least unequivocal but not sufficient. The retention times are only of value when appropriate internal standards are used and when the time of analysis is not too short. A major problem is the ubiquitous presence of trace levels of aromatic amines in the environment. With aniline, we and others never achieved a zero background level when analysing blank water samples instead of Hb solutions. It is of utmost importance to control regularly the cleanliness of the equipment and materials and we strongly recommend to run blank water samples without addition of internal standards with every batch of analyses. Water is preferred to Hb solutions for this purpose because no Hb free from low amounts of Hb adducts may be available due to the ubiquitous environmental occurrence of AAs [12,51].

As pointed out by Skipper and Stillwell [16], rubber is one of the most significant potential source of contamination. The water can also be critical. With our SPE method for extraction and clean-up of free amines released from Hb by alkaline hydrolysis, a rather large amount of water is used, e.g. for conditioning and washing the SPE cartridges. We found that water from our MilliQ reagent water system introduced a high background. HPLC water from Merck (Darmstadt, Germany) was even worse producing a high background peak at the retention time of 3ABP [21]. When using SPE extraction of hydrolysed free AAs one should avoid any multiple used parts such as needle adapters in vacuum manifolds. For removal of water prior to elution of the AAs from the C_{18} cartridges by apolar solvents such as $CHCl_3$, laboratory air should not be drawn through the wet SPE tubes. It is recommended to either centrifuge the cartridges or flushing them with nitrogen [21,24].

3. Levels of haemoglobin adducts of AAs in humans

The structures of amines determined as Hb adducts in humans are shown in Fig. 2. By far the most data are available for 4ABP (Table). Tobacco smoke has been recognised as a significant source of contamination. Smokers usually have 3–8-fold higher Hb adduct levels of 4ABP compared to non-smokers [12,15,26,33,36,39,52]. Extreme values for the relation of adduct levels in smokers and non-smokers have been reported for mothers at delivery: In Louisville, KY, smoking women had 16-fold higher adduct levels compared to non-smoking women [22], whereas in Homburg, Germany smoking women had only 2.8-fold higher 4ABP Hb adduct concentrations than non-smoking women [46]. The important contribution of smoking to 4ABP adduct levels is further substantiated by the dose response [29,42,45] and by the significant decrease of adduct levels in controlled smoking cessation trials [28,43]. Conflicting results have been published on the contribution of exposure to environmental tobacco smoke (ETS), so-called passive smoking. Whereas in some studies a significant 1.3–1.6-fold elevation of background levels of 4ABP by passive smoking was found in adults [31], children [53] and mothers at delivery [36] from New England, we did not see any influence in pregnant women from Homburg, Germany [46], and only a marginal non-significant increase in children from Upper Bavaria, Germany [48]. In any case, the major contributors to environmental background levels of 4ABP have not been elucidated. Possible sources of 4ABP have been discussed in most papers. However, no convincing data have been presented. One possible source could be the diet [51,54]. Another source may be traffic exhaust containing 4-nitro-

Table 2
Haemoglobin adducts from 4-aminobiphenyl

Area of residence	Gender	Subgroups	Concentration (pg/g Hb)[a]		Reference
			Smoker	Non-smoker	
New York, NY, USA	♂♀		162±11	33±3	[52]
Boston, MS, USA	♂		154±11	28±3	[12]
Cambridge, MS, USA	♂♀		170±60	25±11	[15]
Los Angeles, CA, USA	♂	All: (1–19/≥20 cigarettes/day)	58/90[b]	24[b]	[37]
		whites	67/87	28	
		blacks	50/86	24	
		Asians	63/100	20	
Maastricht, Netherlands	♂♀		202±11	57±9	[26]
Munich, Germany	♂♀		126±21	29±3	[39]
Upper Bavaria, Germany	♂	Nasal snuff users		15±3	[39]
New York, NY, USA	♂♀	Children			[53]
		non-smoking home		24±3	
		smoking home		34±3	
Upper Bavaria, Germany	♂♀	Children: All		26±1	[48]
		Eichstätt		21±2	
		Augsburg		27±2	
		non-smoking home		23±2	
		smoking home		29±5	
		Munich		31±1	
Worcester, MS, USA	♀	Pregnant women: All	184±28	22±1	[36]
		weekly exposure ($\mu g/m^3$)<0.5		18±2	
		0.5-1.9		21±2	
		≥2.0		28±1	
Louisville, KY, USA	♀	Mothers at delivery	488±38	30±4	[22]
	♂♀	Newborn	244±20	14±1	
Louisville, KY, USA	♀	Mothers at delivery		18±1	[42]
		cigarettes, pack/day: <1	144±6		
		1	250±8		
		1–2	394±15		
		>2	633±20		
	♂♀	Newborn		9±1	
		Mothers cigarettes, pack/day: <1	74±4		
		1	123±6		
		1–2	196±9		
		>2	319±11		
Homburg, Germany	♀	Mothers at delivery	28±3	10±1	[46]
		ETS 0[c]		11±2	
		ETS 1		11±1	
		ETS 2		9±1	
		ETS 3		10±1	
Boston, MS, USA	♀	Mothers at delivery	183±28	22±1	[33]
	♂♀	Newborn	92±14	17±2	
Boston, MS, USA	♂	Cot−/ETS−[d]		50±24	[31]
		Cot−/ETS?		42±15	
		Cot−/ETS+		45±31	
		Cot+/ETS+		45±14	
		Cot+/ETS++		54±21	
Danvers, MA, USA	♂♀	Smoking cessation: Initial	120±7		[28]
		days quit: 20–23	82±6		
		65–80	34±5		
		120–160	33±6		

Table 2. Continued

Area of residence	Gender	Subgroups	Concentration (pg/g Hb)[a]		Reference
			Smoker	Non-smoker	
New York, NY, USA	♂♀	Smoking cessation: Initial	50±6		[43]
		months quit: 2.5	21±3		
		8	12±2		
		14	13±2		
Turin, Italy	♂			51±4	[30]
		Blond tobacco	176±12		
		Black tobacco	288±34		
Turin, Italy	♂	All:		28±3	[29,32,38,41,44]
		slow acetylator		27[b]	
		fast acetylator		13	
		slow CYP1A2		29±5	
		fast CYP1A2		30±4	
		Blond tobacco: all	103±10		
		slow acetylator	112±13		
		fast acetylator	86±15		
		slow CYP1A2	87±17		
		fast CYP1A2	114±12		
		Black tobacco: all	146±11		
		slow acetylator	175±11		
		fast acetylator	118±14		
		slow CYP1A2	154±12		
		fast CYP1A2	108±30		
Baltimore, MD, USA	♂♀	Patients: all	126±12	86±7	[34]
		men	128±12	79±7	
		women	87±50	123±23	
		with lung tumours	122±13	74±9	
		with COPD[e]	152±34	81±10	
		with other tumours	98±14	115±17	
Florence, Italy	♂♀	Patients without tumours	65±12		[35]
		Bladder tumour patients	103±13		
Copenhagen, Denmark	♂♀	Epileptic patients[f]			[45]
		(Pb−)		23±4	
		(Pb+)		25±3	
		<20 g tobacco/d (Pb−)	85±10		
		<20 g tobacco/d (Pb+)	55±6		
		≥20 g tobacco/d (Pb−)	224±22		
		≥20 g tobacco/d (Pb+)	130±17		
New York, NY, USA	♂	Unexposed control	119±18	48±13	[59]
		Rubber workers	98±22	73±23	
Essen, Germany	♂	Exposed workers: all	126±10	46±5	[27]
		slow acetylator	98±5	47±11	
		fast acetylator	186±9	46±3	

[a] Mean±SE.
[b] Median.
[c] No ETS exposure (0), partner stopped smoking (1), exposure at home or at work (2), exposure at home and at work (3).
[d] Detectable cotinine in plasma: no (−), yes (+)/self reported exposure to ETS: no (−), questionable (?), yes (+), non-smoking bartender (++).
[e] Patients with chronic obstructive pulmonary disease.
[f] Patients treated chronically with phenobarbital or primidone (Pb+) or other drugs (Pb−).

biphenyl [12]. In children, regional differences could be seen with 1.5-fold higher adduct levels in children from Munich, a large city with high traffic density, compared to Eichstätt, a small city where most children came from a rural environment [48]. However, one has to be careful in the interpretation of these studies because there are many additional factors which influence the 4ABP adduct levels. As pointed out in Fig. 1, acetylation of 4ABP by the polymorphic *N*-acetyltransferase 2 (NAT2) can be regarded as a detoxification reaction and competes in liver with the activation of 4ABP by *N*-hydroxylation which is mediated mainly by the cytochrome P450 isozyme CYP1A2. Indeed, both the NAT2 and CYP1A2 status modulate the adduct levels with slow acetylators and extensive *N*-hydroxylators having higher 4ABP adduct concentrations [27,29,32,37, 38,41,44,55]. The ethnic/racial differences in adduct levels in men from Los Angeles, CA, which where highest in whites, intermediate in blacks and lowest in Asians could be largely explained by the different proportions of slow acetylators in these ethnic groups [37]. Epileptic patients treated with phenobarbital, were found to have lower levels of 4ABP adducts than patients on other treatment. This effect was only apparent in smokers [45]. *N*-Acetylcysteine treatment has been shown to lower 4-ABP adduct levels in non-smokers [56]. This effect is possibly mediated by an increase of glutathione within the erythrocyte where it competes with Hb for reaction with nitrosobiphenyl (Fig. 1) [57]. An association between the 4ABP adduct levels and the glutathione *S*-transferase *M1* genotype (*GSTM1*) was found in the ethnic/racial study from Los Angeles, CA, with higher 4ABP Hb adduct levels in subjects possessing the *GSTM1*-null versus *GSTM1*-non-null genotype [58]. This effect could not be confirmed in smokers and non-smokers from Turin, Italy [44].

Hb adducts from 3ABP have been reported only by two groups (Table 3) [30,31,37,39,46]. Differences between smokers and non-smokers tend to be higher for 3ABP compared to 4ABP. However, it has to be pointed out that in contrast to 4ABP, 3ABP is not a carcinogen in animals and is not considered to

Table 3
Haemoglobin adducts from 3-aminobiphenyl

Area of residence	Gender	Subgroups		Concentration (pg/g Hb)[a]		Reference
				Smoker	Non-smoker	
Los Angeles, CA, USA	♂	All: (1–19/≥20 cigarettes/day)		2.50/4.41[b]	0.29[b]	[37]
		whites		2.94/5.72	0.26	
		blacks		3.84/4.92	0.32	
		Asians		1.28/2.09	0.28	
Turin, Italy	♂				1.2±0.4	[30]
		Blond tobacco		14±2		
		Black tobacco		13±3		
Munich	♂♀			13.6±1.9	1.3±0.4	[39]
Homburg, Germany	♀	Mothers at delivery		3.0±0.5	1.4±0.1	[46]
			ETS 0[c]		1.2±0.2	
			ETS 1		1.8±0.3	
			ETS 2		1.3±0.2	
			ETS 3		1.2±0.2	
Boston, MS, USA	♂	Cot−/ETS−[d]			1.3±1.5	[31]
		Cot−/ETS?			1.2±0.6	
		Cot−/ETS+			1.2±0.7	
		Cot +/ETS+			1.5±1.3	
		Cot +/ETS++			2.4±1.1	

[a] Mean±SE.
[b] Median.
[c] No ETS exposure (0), partner stopped smoking (1), exposure at home or at work (2), exposure at home and at work (3).
[d] Detectable cotinine in plasma: no (−), yes (+)/self reported exposure to ETS: no (−), questionable (?), yes (+), non-smoking bartender (++).

be genotoxic for humans. A significant increase of 3ABP adduct levels by ETS exposure was reported in one study but could not be confirmed in our study with pregnant women [31,46]. As with 4ABP, Hb adduct concentrations of 3ABP depend on the NAT2 acetylator status explaining largely the observed ethnic/racial differences in the adduct levels [37,55,58]. After adjustment for race and smoking the effect on 3ABP adduct levels was even stronger than on 4ABP adduct levels with significant 47% (3ABP) versus 17% (4ABP) increases in slow compared to rapid acetylators [55]. The effect of the *GSTM1* genotype on 3ABP Hb adduct levels was only of borderline significance [58]. No association was found between the *NAT1*10* genotype and 3ABP or 4ABP adduct levels [55]. As with 4ABP, the smoke adjusted increase of 3ABP Hb adduct levels was significantly lower in phenobarbital treated epileptic patients than in patients on different medications which are presumably no CYP2B type inducers [45].

The Hb adduct levels of monocyclic aromatic amines are summarised in Table 4. Adduct levels are generally highest for aniline and are about 100-fold higher than 4ABP adduct levels in non-smokers [15,30,39,46,59]. Adduct levels of methylanilines are the next highest exceeding the 4ABP Hb adduct concentrations about 10-fold. Exceptionally high values have been reported for 2MA in samples from New York [59]. Dimethyl- and ethylaniline adducts are lowest approaching the background levels of 4ABP in non-smokers. Overall, smoking has only small effects of borderline significance on the concentrations of Hb adducts of monocyclic amines. Therefore, other yet unknown environmental sources are more important than tobacco smoke exposure. As with 4ABP, adduct levels from methylanilines were highest in children from Munich, intermediate in children from Augsburg and lowest in children from Eichstätt which correlates with the size of the cities having 1 300 000, 250 000 and 13 000 inhabitants, respectively [48]. In occupationally exposed workers, adduct levels of both aniline and 4CA depended on the individual acetylation capacity as determined by the proportions of free and acetylated aniline and 4CA in the urine [27]. The antiarrhythmic and local anaesthetic drug lidocaine leads to a significant increase of background Hb adduct levels of 26DMA which may explain episodes of methaemoglobinaemia in cardiac patients who where administered i.v. lidocaine [40]. Propanil, a major herbicide used on rice-paddies gives rise to 34CA Hb adducts in exposed agricultural workers. No background levels of 34CA adducts were detected in laboratory staff [49]. Conflicting results have been reported for Hb adducts stemming from exposure to nitrotoluene explosives, 2ADNT, 4ADNT, 2A4NT, 4A2NT, 2A6NT (see Fig. 2). Whereas Neumann et al. [60] reported the presence of all these adducts in both exposed and non-exposed adults, Sabbioni et al. [18] found 2ADNT and 4ADNT only in exposed workers and not in laboratory control personnel. Recently, very high Hb adduct levels (3.5–12 ng/g Hb) stemming from musk xylene, a fragrance component in toiletries, detergents and skin care products, have been reported [61]. The structure released from human Hb was identified as 1-*tert*-butyl-3,5-dimethyl-4-amino-2,6-dinitrobenzene.

Hb adduct levels in humans from bicyclic aromatic amines other than 3ABP and 4ABP have been reported for Bz [19], 2NA [15,30,45], MDA [20,50] and MOCA [23] (see Fig. 2). 2NA adduct levels are in the same concentration range as 3ABP adduct levels. They are about 2-fold higher in smokers than non-smokers [15,30] and slightly higher in smokers using cigarettes with black compared to blond tobacco [30]. As with 3ABP and 4ABP adducts, smoke adjusted Hb adduct levels of 2NA were significantly lower in phenobarbital treated epileptic patients than in patients on different medications [45]. Hb adducts from Bz and its monoacetylated derivative, 0–26 and 2.5–712 ng/g Hb, respectively, were detected in 33 exposed workers but not in 15 controls [19]. Hb adducts from MDA and its monoacetylated derivative have been reported in workers exposed to 4,4′-methylenediphenyl diisocyanate [20,50]. In the case of MOCA, 0.2–12 ng/g Hb were detected in five workers [23]. The contribution of *N*-acetyl-MOCA, 0.016–0.05 ng/g Hb, to the total Hb adduct burden was negligible. No increase in MOCA Hb adduct levels were observed when the Hb solution were not dialysed prior to alkaline cleavage of the bound MOCA.

Hb adducts resulting from exposure to polycyclic nitroarenes, 1-aminopyrene, 2-aminofluorene, 3-aminofluoranthene, 9-aminophenanthrene and 6-

Table 4
Haemoglobin adducts from monocyclic aromatic amines

Area of residence	Gender	Amine	Subgroups	Concentration (ng/g Hb)[a]		Reference
				Smoker	Non-smoker	
Boston, MS, USA	♂♀	**A**		4.400±0.660	3.800±0.632	[15]
		2MA		0.100±0.009	0.034±0.003	
		3MA		0.490±0.049	0.680±0.063	
		4MA		0.130±0.014	0.070±0.013	
Turin, Italy	♂	**2MA**	Blond tobacco	0.290±0.019	0.188±0.019	[30]
			Black tobacco	0.329±0.022		
		3MA	Blond tobacco	1.097±0.108	1.141±0.138	
			Black tobacco	1.140±0.138		
		4MA	Blond tobacco	0.306±0.035	0.209±0.024	
			Black tobacco	0.415±0.073		
		25DMA	Blond tobacco	0.067±0.010	0.050±0.006	
			Black tobacco	0.070±0.014		
		24DMA	Blond tobacco	0.073±0.010	0.040±0.005	
			Black tobacco	0.114±0.017		
		26DMA	Blond tobacco	0.086±0.015	0.264±90	
			Black tobacco	0.098±0.030		
		23DMA	Blond tobacco	0.056±0.011	0.052±0.008	
			Black tobacco	0.064±0.019		
		35DMA	Blond tobacco	0.112±0.031	0.093±0.012	
			Black tobacco	0.135±0.031		
		34DMA	Blond tobacco	0.046±0.011	0.047±0.011	
			Black tobacco	0.065±0.019		
		2EA	Blond tobacco	0.070±0.010	0.038±0.003	
			Black tobacco	0.080±0.012		
		3EA	Blond tobacco	0.115±0.022	0.102±0.016	
			Black tobacco	0.129±0.034		
		4EA		0.077±0.013	0.092±0.017	
				0.111±0.041		
Munich	♂♀	**A**		0.930±0.613	0.725±0.330	[39]
		2MA		0.310±0.185	0.142±0.085	
		3MA		0.675±0.549	0.258±0.171	
		4MA		0.409±0.284	0.162±0.110	
		24DMA		0.039±0.018	0.008±0.004	
		2EA		0.028±0.017	0.007±0.004	
		2MOA		0.045±0.039	0.038±0.023	
Upper Bavaria, Germany	♂	**A**	Nasal snuff users		1.226±0.889	[39]
		2MA			0.123±0.068	
		3MA			0.273±0.194	
		4MA			0.141±0.078	
		24DMA			0.007±0.005	
		2EA			0.005±0.004	
		2MOA			0.028±0.022	
Homburg, Germany	♀	**A**	Mothers at delivery	1.190±0.122	1.240±0.097	[46]
		2MA		0.289±0.025	0.237±0.065	
		3MA		0.672±0.666	0.706±0.055	
		4MA		0.315±0.032	0.197±0.013	
		24DMA		0.026±0.003	0.019±0.002	
		2EA		0.025±0.003	0.019±0.002	
		2MOA		0.080±0.012	0.097±0.009	

Table 4. Continued

Area of residence	Gender	Amine	Subgroups	Concentration (ng/g Hb)[a]		Reference
				Smoker	Non-smoker	
Upper Bavaria, Germany	♂♀	**2MA**	Children: all		0.571±0.019	[48]
			Eichstätt		0.487±0.037	
			Augsburg		0.598±0.029	
			Munich		0.632±0.036	
		3MA	all		1.106±0.028	
			Eichstätt		0.935±0.053	
			Augsburg		1.115±0.038	
			Munich		1.384±0.045	
		4MA	all		0.992±0.030	
			Eichstätt		0.866±0.045	
			Augsburg		0.983±0.044	
			Munich		1.254±0.048	
		2MOA	all		0.251±0.009	
			Eichstätt		0.254±0.022	
			Augsburg		0.241±0.012	
			Munich		0.284±0.013	
New York, NY, USA	♂	**A**	Unexposed control	3.240±2.86	3.118±0.367	[59]
			Rubber workers	19.776±2.607	16.072±1.378	
		2MA	Unexposed control	3.510±2.234	3.518±1.354	
			Rubber workers	40.494±5.365	41.028±6.997	
Essen, Germany	♂	**A**	Exposed workers: all	4.296±1.346	6.468±9.039	[27]
			slow acetylator	5.138±1.076	5.746±3.788	
			fast acetylator	3.903±1.278	7.431±13.035	
		4CA	all	6.151±2.587	8.454±3.467	
			slow acetylator	9.937±1.505	9.043±3.144	
			fast acetylator	4.574±8.492	3.155	

[a] Mean±SE.

aminochrysene have been detected in bus garage workers, urban hospital workers and rural council workers [62]. The Hb adducts were not different between the groups. Therefore, it was questioned whether these adducts are suitable biomarkers to assess exposure to diesel exhaust.

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