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Review

Determination of aromatic hydrocarbons and their metabolites in human blood and urine

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ABSTRACT

Methods for the biological monitoring of aromatic hydrocarbons and their metabolites in the human blood and urine are reviewed. For the determination of the unchanged aromatic hydrocarbon in blood, gas chromatographic head-space analysis is recommended. The metabolites can be monitored by photometric, thin-layer chromatographic, high-performance liquid chromatographic and gas chromatographic methods. For the assessment of health risks caused by aromatic hydrocarbons, reference values and occupational limit values, expressed as biological tolerance values and biological exposure indices, have to be considered.

CONTENTS

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LIST OF ABBRAVIATIONS

BAT	Biological tolerance value for a working material
BEI	Biological exposure index
DNA	Deoxyribonucleic acid
ECD	Electron-capture detection
EKA	Exposition equivalents of carcinogenic working materials
FID	Flame ionization detection
GC	Gas chromatography
HPLC	High-performance liquid chromatography
MAK	Maximum concentration at the workplace
PID	Photoionization detection
TLC	Thin-layer chromatography
TLV	Threshold limit value
TRK	Technical guiding concentration
UV	Ultraviolet

1. INTRODUCTION

Aromatic hydrocarbons, such as benzene, toluene, ethylbenzene and the three isomeric xylenes, belong to an important category of commercial and industrial chemicals. Their common use as fuels, solvents and starting products for chemical synthesis has rendered them ubiquitous in the environment. The metabolism and toxicology of benzene [1–4] and the alkylated benzenes [2,5] have therefore been carefully studied.

Aromatic hydrocarbons are also important working materials at the workplace. The evaluation of specific and reliable methods for the biological monitoring of environmentally and occupationally exposed persons is therefore of growing importance for the surveillance of health risks caused by these compounds.

Biological monitoring is defined generally as directed systematic continuous or repetitive health-related activity for the collection of biological samples for the measurement of hazardous chemical compounds, their metabolites or their specific biochemical effect parameters. The objective is to evaluate the exposure and health risk of exposed persons by comparing the obtained data with appropriate reference values, leading to corrective actions if necessary.

Useful parameters for an estimation of the health risks of aromatic hydrocarbons are the determination of the unchanged aromatic hydrocarbons and their metabolites in blood and urine.

Benzene has been unequivocally proven carcinogenic in humans and is therefore a relevant human health problem. Automobile exhausts contain small amounts of benzene, which is thus distributed in the whole environment. Therefore benzene is the environmental carcinogen most widely distributed. At present the TRK value (technical guiding concentration) for benzene concentration at the workplace is 5 ppm (16 mg/m^3) in Germany [6].

For toluene, ethylbenzene and the xylenes an MAK value (maximum concentration at the workplace) and a TLV value of 100 ppm has been established [6]. The alkylated aromatic hydrocarbons are not suspected to be carcinogenic, as their metabolism is different to the metabolism of benzene.

Benzene is oxidized at the aromatic nucleus to phenol, whose oxidation products may be responsible for the carcinogenic potency of benzene [1–4]. Alkylbenzenes are mainly oxidized at the aliphatic side-chain. Relatively non-toxic aromatic carboxylic acids are the main metabolites. These are usually excreted free or bound to glycine in the urine. Oxidation of the aromatic nucleus of alkylbenzenes leads to alkylated phenols as their minor metabolites [2,5].

For biological monitoring of persons occupationally or environmentally exposed to aromatic hydrocarbons there are principally two approaches:

(a) The determination of the unchanged aromatic hydrocarbons in blood is a specific indicator of exposure, as they are not endogenous products. Analysis is performed mainly by gas chromatographic (GC) methods. Head-space GC is the most common method nowadays.

(b) The metabolites of the aromatic hydrocarbons, mainly phenols and aromatic carboxylic acids, can be determined both in blood and in urine. Some of the metabolites of the aromatic hydrocarbons are physiological intermediates as well, *e.g.* phenol and hippuric acid. The determination of these metabolites is diagnostically not as specific as the determination of the unmetabolized aromatic hydrocarbons. However, they are useful for biological monitoring. A multitude of photometric and chromatographic methods for biological monitoring of the metabolites has been established.

The purpose of this article is to review the determination of aromatic hydrocarbons and their metabolites in both blood and urine, as a tool for the biological monitoring of occupationally and environmentally exposed persons.

2. ANALYTICAL METHODS FOR THE DETERMINATION OF AROMATIC HYDROCARBONS IN BODY FLUIDS

2.1. Gas chromatographic methods, excluding head-space analysis

Evaluation of occupational and environmental exposure to aromatic hydrocarbons and other volatile organic solvents based on the determination of the unchanged compounds in the blood has been of growing importance in recent years. Blood is the ideal biological matrix in this case, because in urine only the metabolites are found in significant concentrations.

The analytical determination of volatile organic substances, such as the aromatic hydrocarbons, is achieved generally by GC methods. Such methods for the determination of aromatic hydrocarbons in blood are not generally recommended for use without clean-up of the biological material. Direct injection of blood into a packed column (10% dioctylsebacinate on Chromosorb W, with flame ionization, detection, FID), as described by Szadkowski *et al.* [7] causes serious contamination of the column and a high background. The detection limits are poor and the specificity mainly at lower concentration ranges.

Methods using a clean-up procedure, *e.g.* solvent extraction of the blood, are more specific. Snyder *et al.* [8] reported an extractive method for the GC determination of benzene in blood. Benzene was extracted from haemolysed blood by toluene, which contained methylene chloride as the internal standard. Separation was achieved on a packed column (10% UC-W 98, Chromosorb W AW DCMS) with FID. Tissue samples may be processed in the same way [9]. Nevertheless, the reported recovery rates were poor, *e.g.* 79% for benzene.

An improved extraction method with recovery rates of 98–100% was reported by Jirka and Bourne [10]. Hemolysed blood was extracted with purified toluene. Benzene was separated on a column packed with 10% SP-2100 on Supelcoport with FID. Thus the detection was limited by the background concentration of benzene in the toluene of 0.04 mg/l. This corresponds to a detection limit of 100 μg of benzene per litre of blood for this method.

Peterson and Bruckner [11] described a GC method for the determination of toluene in blood and tissue with methanol as the extraction solvent. The extract is placed on filter paper in a container, which is purged with nitrogen. The vapour is adsorbed on Tenax, desorbed from Tenax with heat, and injected into a gas chromatograph also equipped with a Tenax column. The procedure involves 2 h of preparation time and so is rather time consuming; the detection limit is 200 μg/l.

Both direct methods and extraction techniques suffer from limited sensitivity and selectivity. The detection limits are too high to cope with decreas-

ing occupational threshold values and environmental exposure levels in the low μg/l range. Thus they have been superseded completely by head-space analysis.

2.2. Gas chromatographic head-space analysis

As exposure levels drop, the determination of blood levels of aromatic hydrocarbons after environmental or occupational exposure requires increasingly more sophisticated methods. These methods must not only be sufficiently sensitive and specific to determine low levels of aromatic hydrocarbons, they must also be able to detect natural background levels, often in the ng/l range. Special care has to be taken to exclude contamination during work-up. Head-space analysis offers an especially elegant method for separating volatile substances from the biological matrix. It is based on Henry–Dalton's law. Thus the volatile aromatic hydrocarbons can be separated from the blood sample merely by increasing the temperature, usually to 60°C, in a sealed airtight vial. After equilibration has been reached an aliquot is taken from the head space and directly analysed by GC. As interference from the biological matrix is eliminated, head-space analysis achieves very low detection limits, as low as the limit of the ecological concentration range at the low μg/l level. Flame ionization detectors and mass selective detectors are primarily used as detection systems.

GC head-space analysis was first introduced into forensic medicine for the determination of ethanol in blood [12] in 1962. In 1971 Sato [13] published a precursor of head-space analysis of benzene, toluene and *m*-xylene in blood, using a hypodermic syringe for equilibration of the sample, a procedure that was very tedious and unpracticable. A column packed with 10% PEG 400 on Celite 545 [13,14] was used for separation. Detection limits were 20 μg/l for benzene, 40 μg/l for toluene and 100 μg/l for xylene [13].

Similar detection limits were achieved by Angerer *et al.* [15,16] by equilibrating the blood sample in an airtight vial and injection of the head-space with a gastight syringe to a column packed with Chromosorb G AW DMCS and 4% phenylsilicone oil. On a Chromosorb W NAW column with 5% bentone and 5% di-*n*-decylphthalate it was possible to separate benzene, toluene, ethylbenzene and the three isomeric xylenes in one run with detection limits from 20 μg/l to 40 μg/l [17]. Drummons *et al.* [18] achieved a detection limit of 3.9 μg/l for benzene by separation on 10% Carbowax 1500 on Chromosorb W and FID. Toluene was used for internal standardization.

A selected-ion monitoring GC head-space analysis of benzene in blood using [$^2H_3$] benzene as the internal standard was developed by Gruenke *et al.* [19]. A detection limit of 2 μg/l blood was attained by separation on a Tenax column.

Brugnone *et al.* [20] reported an even lower detection limit of 0.5 μg/l blood for benzene, achieved on a packed column coated with Apiezon L on Supelcoport and FID.

A great improvement in the specificity of head-space analysis was achieved by the use of capillary columns instead of packed columns. Better peak shapes and baseline separation led to improved sensitivity. A very important aspect is the persistence, especially of chemically bonded phases, making the routine use of head-space analysis possible. Wall-coated open tubular columns do not allow this.

Sensitive detection with enormously improved precision is possible with automatic injection systems, which are the laboratory standard nowadays. Under optimal conditions, the imprecision for manual dosage with gastight syringes is 10–20%. If samples are automatically injected, the imprecision is reduced to 5–7%.

As Angerer [21] reported, the use of a fused-silica capillary column with a bonded layer of phenylsilicone instead of packed columns improves the detection limit of toluene in blood to 5 μg/l. Using a fused-silica capillary column with bonded dimethylpolysilocane and FID, Angerer [21] showed that it is possible to separate benzene, toluene, ethylbenzene, styrene and *m*- and *o*-xylene in one run with low detection limits, from 3 μg/l for benzene to 8 μg/l for xylene. A typical chromatogram is shown in Fig. 1.

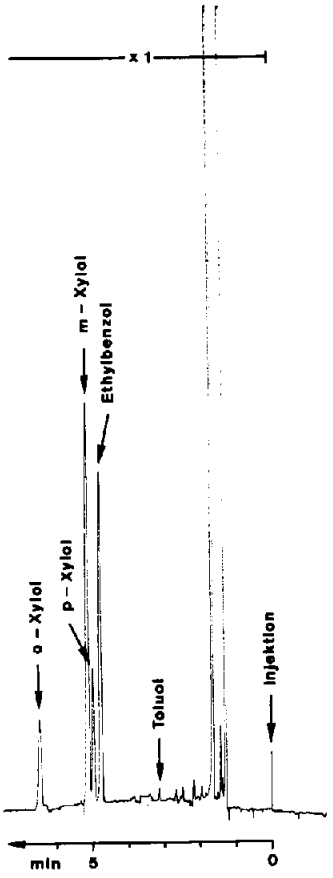

Fig. 1. Example of a capillary gas chromatogram of a blood sample of a varnish worker exposed to aromatic hydrocarbons. Toluene, 1 μg/l; ethylbenzene, 48.4 μg/l; *p*-xylene, 25 μg/l; *m*-xylene, 66 μg/l; *o*-xylene, 17 μg/l [149].

The simultaneous determination of benzene and toluene by separation on methylsilicone or phenylmethylsilicone capillary columns and photoionization detection (PID) was reported by Pekari *et al.* [23]. Interference from matrix and reagents was less marked, as PID is sensitive to double bonds and aromatic rings. Internal standardization was carried out with fluorobenzene and *o*-xylene, respectively. The detection limits were 4 μg/l for benzene and toluene.

With detection limits of this magnitude the whole concentration range of occupational exposure can be determined by GC head-space analysis using capillary columns. Even lower detection limits, reaching the ng/l range relevant in environmental exposure, can be achieved either by the purge-and-trap procedure or by the so-called dynamic head-space analysis.

Because the purge-and-trap procedure, in which the gas is passed through the sample, causes blood to foam, dynamic head-space analysis, where the carrier gas is passed over the fluid, is more commonly used nowadays. The total content of the components present in the head-space is flushed by a carrier gas over a solid collection phase (Tenax) where it is adsorbed. Usually the collection phase must be cooled. The components are desorbed by rapid heating of the adsorbant and transported by a small volume of carrier gas into the GC injector. Detection limits can be lowered by a factor of at least 10. Solvent levels in blood due to ecological exposure (ng/l level) can be detected in this way.

In 1974, Bellar and Lichtenberg [24] described a gas-stripping or purge-and-trap method for the head-space analysis of volatiles in water. Based on this method Cocheo *et al.* [25] developed a method for the determination of toluene in blood. The toluene is stripped from the blood sample by means of a helium gas stream, concentrated on Tenax, desorbed by heat, and separated on a Carbopack C column with 0.1% SP 1000. The detection limit was 7.5 μg/l blood. Antoine *et al.* [26] were able to improve the detection limits of this method to less than 50 ng/l for aromatic hydrocarbons. Separation was achieved on fused-silica SE-54, using [$^2H_{10}$]xylene as the internal standard and mass selective detection. These results were confirmed by Hajimiragha *et al.* [27].

Perbellini and co-workers [28,29] have published several studies of environmental and occupational exposure to benzene, where blood levels have been measured by dynamic head-space analysis. A silica capillary column with cross-linked 5% phenylmethylsilicone oil as the stationary phase and a mass selective detector (quadrupole) was used for benzene quantification. They reported benzene levels in blood of 1–377 μg/l.

Angerer *et al.* [30] reported the determination of benzene in blood by dynamic head-space analysis and FID. A gas chromatograph equipped

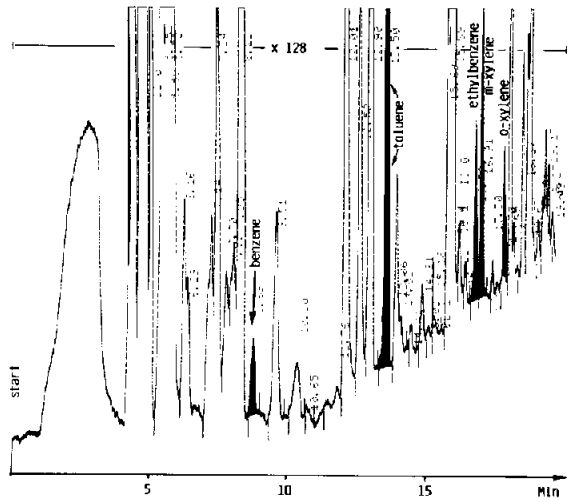

Fig. 2. Dynamic head-space chromatogram of a blood sample from an occupationally non-exposed person. Concentration of benzene in blood, 500 ng/l [30].

with a DB 1301 fused-silica column (50 m) was used for separation. The detection limit of 80 ng/l blood showed that this method is able to evaluate benzene levels in blood in the ecological concentration range. Fig. 2 shows a typical example of benzene determination in this concentration range performed by head-space analysis.

3. ANALYTICAL METHODS FOR THE DETERMINATION OF METABOLITES OF AROMATIC HYDROCARBONS IN BLOOD AND URINE

The second approach to the biological monitoring of persons occupationally exposed to aromatic hydrocarbons is the determination of their metabolites in blood and urine. The metabolites are to some extent, more responsible for the toxicity of aromatic hydrocarbons. The phenolic metabolites, in particular, are of great diagnostic value. The metabolites have a greater half-life in the organism than the unchanged aromatic hydrocarbons, thus a time-weighted assessment of exposure is possible.

The main disadvantage of this approach is that some of the parameters are diagnostically unspecific. Phenol and hippuric acid are endogenous products and are excreted in urine in the mg/l and in the g/l concentration range, respectively. Mandelic acid, phenylglyoxylic acid, methylhippuric

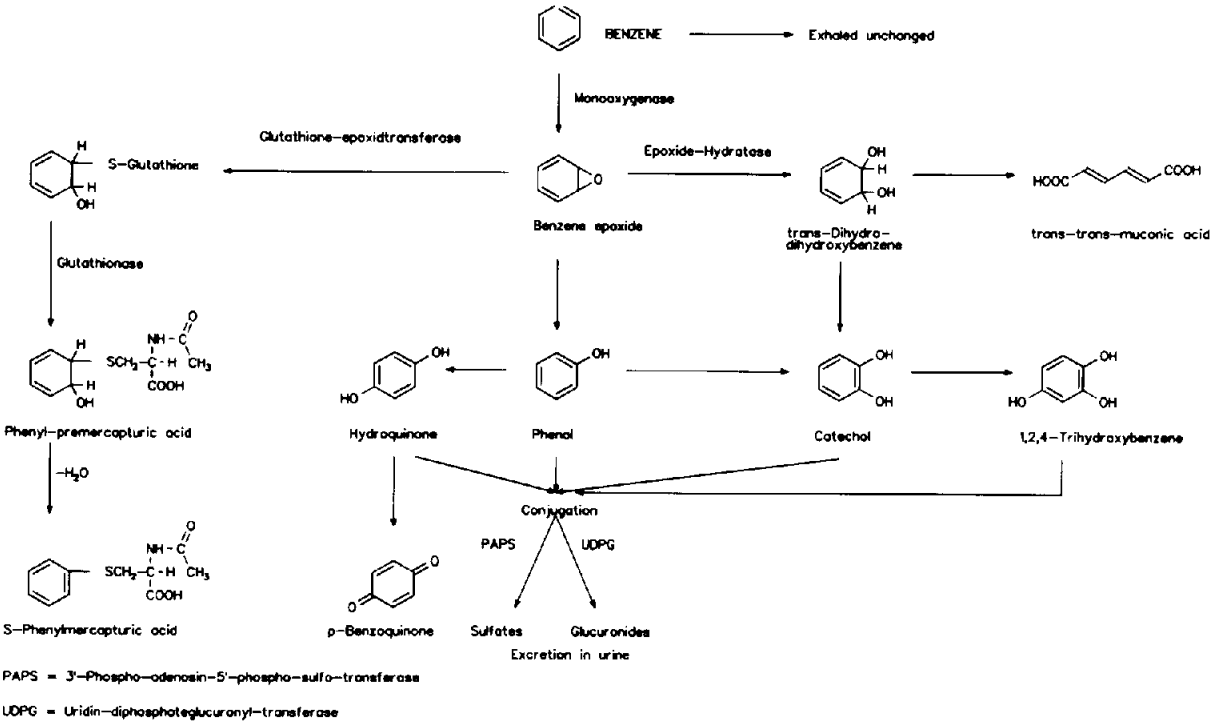

Fig. 3. Metabolism of benzene [2].

TABLE 1

AROMATIC HYDROCARBONS AND THEIR MAJOR AND MINOR METABOLITES AS PARAMETERS FOR BIOLOGICAL MONITORING

Aromatic hydrocarbon	Major metabolites	Minor metabolites
Benzene	Phenol	S-Phenylmercapturic acid
		trans,trans-Muconic acid
Toluene	Hippuric acid	*o*-Cresol
		p-Cresol
Ethylbenzene	Mandelic acid	2-Ethylphenol
	Phenylglyoxylic acid	DL-Phenylethanol
		Phenyleneglycols
Xylenes	Methylhippuric acids	Xylenols

acids and some minor metabolites of benzene are diagnostically more specific because they are not endogenously produced on a large scale. For low exposure levels or environmental exposure, the determination of the unchanged hydrocarbons or the minor metabolites is of greater diagnostic value.

The metabolism of benzene has been carefully studied [1–4]: Fig. 3 shows an overview [2]. The main metabolite is phenol. Phenol and its oxidation products, quinol and catechol, are excreted as glucuronide and sulphate conjugates in the urine. Minor metabolites, such as S-phenylmercapturic acid, and *trans,trans*-muconic acid, are nevertheless useful parameters for the estimation of occupational exposure.

The alkylbenzenes are mainly oxidized at the aromatic side-chain, leading to aromatic carboxylic acids, which are excreted in the urine bound to glycine. The oxidation products of the aromatic nucleus, leading to alkylphenols, are minor metabolites. Their importance for biological monitoring has been growing in recent years. Fig. 4a–c shows the metabolism of the alkylbenzenes.

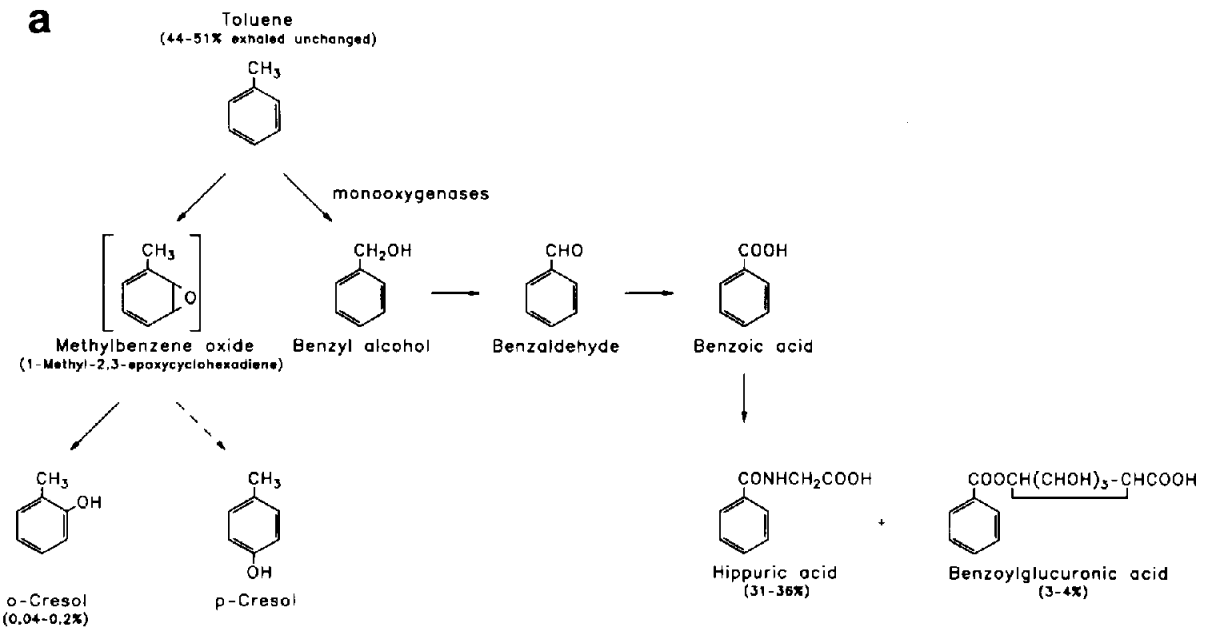

Fig. 4.

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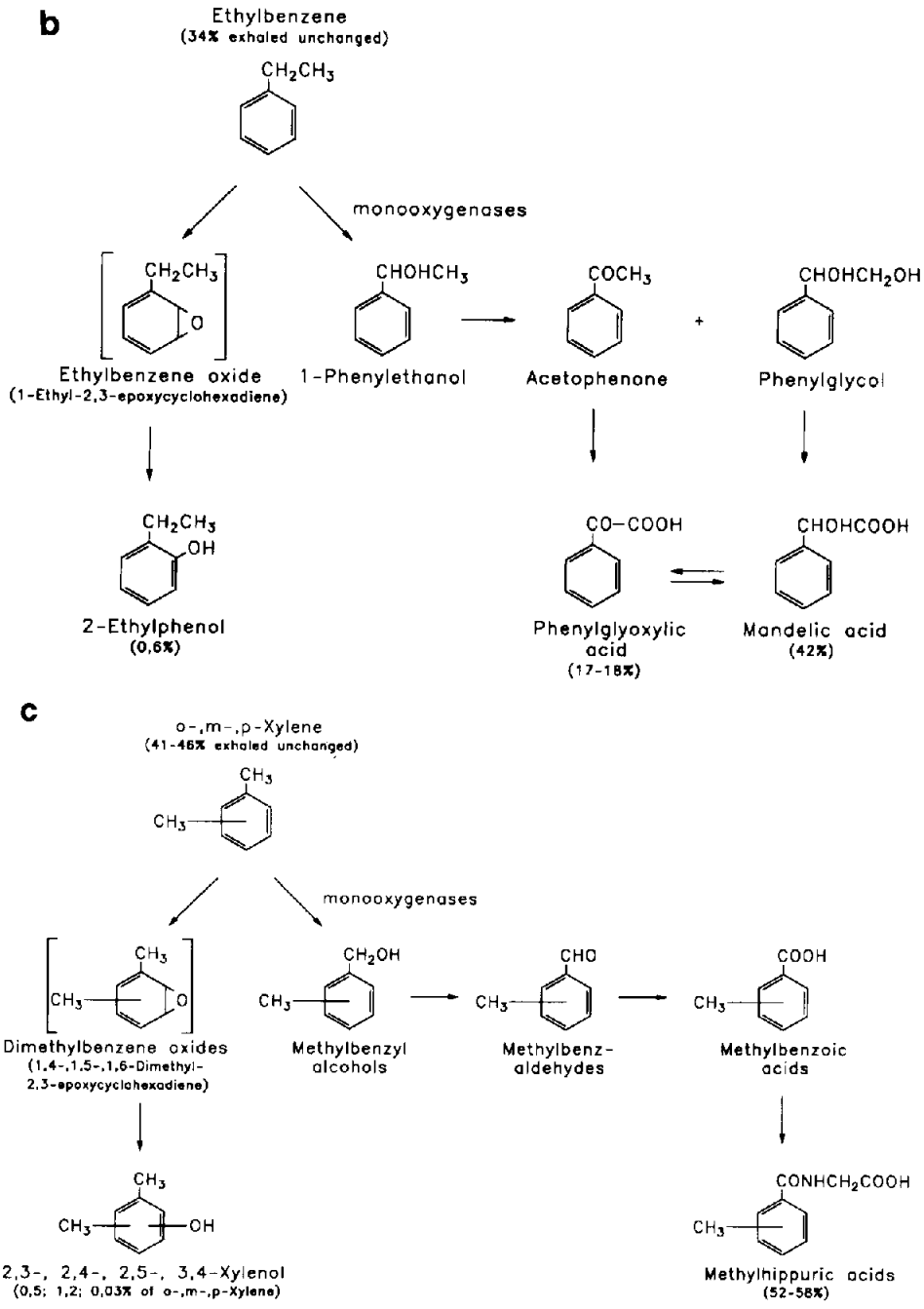

Fig. 4. Metabolism of (a) toluene, (b) ethylbenzene and (c) the xylenes [150].

A summary of the metabolites of benzene, toluene, ethylbenzene and the xylenes important for biological monitoring is given in Table 1.

3.1. Photometric methods

Photometric methods for the determination of the metabolites of aromatic hydrocarbons have

been available for a number of years [31–34]. Though the detection limits may be rather high, their practicability and relative cheapness mean that some are still in use for routine monitoring of exposed persons.

Many colour-forming reagents for the coupling of phenol, the main metabolite of benzene, have been used [31,32]. The method recommended by the German Science Foundation [34] for the photometric determination of phenol is based on the method of Bardodej [33]. Phenol is oxidized by potassium hexacyanoferrate(III) and reacts with 4-aminoantipyrine to form a red *p*-quinone dyestuff that can be determined at 492 nm. The detection limit was 2.5 mg/l urine. This method can also be fully automated [35]. It is the method of choice for routine monitoring, its analytical specificity being sufficient.

The photometric methods for the determination of hippuric acid, the main metabolite of toluene in urine, are based on solvent extraction of the analyte from the urine sample. Hippuric acid can be determined directly in diethyl ether–2-propanol [36] or by a fluorimetric method in 70% sulphuric acid [37].

A sensitive method for the determination of hippuric acid at the mg/l level was developed by Umberger and Fiorese [38], Tomokuni and Ogata [39] and Kaneko *et al.* [40]. It is based on the colour reaction of hippuric acid, pyridine and benzenesulphonyl chloride. With Tomokuni's and Kaneko's method no extraction step is necessary, and the urine sample can be processed directly.

A detection limit of 4 mg/l urine is achieved by the coupling of hippuric acid with *p*-dimethylbenzaldehyde and measurement of the coloured product at 458 nm [41,42]. Both *p*- and *m*-methylhippuric acid, metabolites of the corresponding xylenes, can be determined as well [41]. For monitoring an exposure to toluene a photometric determination of hippuric acid is sufficiently sensitive, as hippuric acid is physiologically excreted at the lower g/l level. If a mixed exposure occurs, these methods are not specific enough to distinguish between the aromatic carboxylic acids.

A rapid colorimetric method for the determination of the ethylbenzene and styrene metabolites, phenylglyoxylic acid and mandelic acid, was reported by Ohtsuji and Ikeda [43]. Diethyl ether extracts of the two acids were evaporated and mixed with sulphuric acid and formaline, forming a coloured product measurable at 450 nm. Both acids could be detected at the mg/l level.

3.2. Chromatographic methods

3.2.1. High-performance liquid chromatographic methods

3.2.1.1. Determination of benzene metabolites in body fluids. Phenol, the main metabolite of benzene, is a physiological intermediate in the biological degradation of aromatic amino acids and certain pharmaceuticals, such as phenyl salicylates. The excretion of phenol in the urine of persons occupationally not exposed to benzene can therefore reach values up to 20–35 mg/g creatinine [44]. In some cases even higher values have been reported [2].

Phenol excreted in urine is bound to glucuronic acid or to sulphuric acid. There are two analytical methods in general use. Phenol can be released hydrolytically from its conjugates, or the glucuronides and sulphates of phenol can be determined directly. Ogata *et al.* [45,46] reported a method of the latter type for the determination of urinary phenyl sulphate and phenylglucuronide. Without further treatment urine was separated on a reversed-phase column (Nucleosil 5 C_{18}) using methanol–water–acetic acid (50:50:0.2), containing 0.05 *M* tetra-*n*-butylammonium bromide, as the mobile phase, with UV detection at 254 nm.

Usually the glucuronides or sulphates are hydrolysed either by acid [47–50] or enzymically by use of a β-glucuronidase [51,52]. The released phenol can then be subjected to solvent extraction [49,52].

Extraction of the acidified urine with dibutyl ether and subsequent separation on a Spherisorb-NH_2 silica gel phase using 2-propanol–*n*-hexane (2:98) as the mobile phase and detection at 265 nm was reported by Eadsforth and Coveney [49]. As phenol is also soluble in the aqueous phase,

the extraction is insufficient and recovery rates were poor.

Brega *et al.* [52] reported a solvent extraction method after enzymatic hydrolysis. Phenol is extracted with methylene chloride and analysed on a reversed-phase column (Pecosphere 3 × 3 C_{18} RP; 3 cm × 4.6 mm I.D.; 3 μm) with methanol–water–orthophosphoric acid (30:70:0.1) as the mobile phase and detection at 210 nm. Cresols, *p*-aminophenol and *p*-nitrophenol may be processed in the same way.

The disadvantage of extraction methods is the insufficient separation of phenol from the other constituents of the urine, which results in a high analytical background.

Steam distillation of the acidified urine with simultaneous hydrolysis of the phenol conjugates had proved to be the ideal method for clean-up [47,48,50,51]. The steam-distillable phenols can thus easily be separated from the other interfering urine constituents. Artefacts do not occur. When this kind of clean-up is used, analytical background levels caused by the biological matrix are drastically reduced. An increase of specificity for the determination of phenol can thus be reached by a simple procedure that is not very time consuming in routine use.

Usually the distillate is directly injected into the reversed-phase column [48,50,51]. In general, octadecyl-silanized silica gel (LiChrosorb RP-18 [47,48], RP-8 [50], or RSil C_{18} [51]) is used as the stationary phase. UV detection is achieved at 271 nm [47], 205 nm [48], 275 nm [50], or 285 nm [51]. Mobile phases used are methanol–water (20:80) [47], acetonitrile–phosphate buffer (35:65) [48], water–acetonitrile–phosphate buffer (89:9:2) [50] and methanol–phosphate buffer (48:52) [51]. The detection limits reported were 1 mg/l urine [47,48].

The steam distillate may be purified or enriched by solvent extraction [47] or by solid-phase extraction [48]. Lower detection limits can result, which is especially important for the determination of alkylphenols.

Because of its physiological appearance in urine, phenol is a diagnostically unspecific parameter for estimating low exposure levels of benzene at the workplace. Low level exposures are more specifically indicated by the minor metabolites of benzene, such as S-phenylmercapturic acid (S-phenyl-N-acetylcysteine) resulting from conjugates with glutathione, or *trans,trans*-muconic acid. These metabolites are physiologically excreted in only small amounts.

Jongeneelen *et al.* [53] reported a method for the determination of S-phenylmercapturic acid in urine by high-performance liquid chromatography (HPLC) on a reversed-phase column (RP-18) with water–methanol phosphate buffer (80:10:10) and water–methanol (10:90) as the mobile phases for gradient elution and UV-detection at 256 nm. A physiological background level of S-phenylmercapturic acid could not be detected with this method, as the detection limit with 6 μg/l urine was too high. GC determination of S-phenylmercapturic acid therefore should be preferred. This method reaches a detection limit of 1 μg/ml. Thus a normal value of 4 μg/l could be determined in the urine of non-exposed persons.

Urinary *trans,trans*-muconic acid is also a potential candidate for the biological monitoring of benzene. Karim *et al.* [54] first reported an HPLC method for its determination in diluted urine samples by separation on a reversed-phase column. However, recovery rates were poor. A slightly changed work-up procedure, the addition of methanol to the urine, described by Inoue *et al.* [55] and Ducos *et al.* [56], causes column contamination. Separation was achieved on Spherisorb ODS (5 μm) with methanol–1% aqueous acetic acid (10:90) as the mobile phase and detection at 265 nm.

A clean-up procedure that entails passing the urine over solid-phase extraction devices and subsequent elution with aqueous acetic acid, as designed by Ducos *et al.* [56], greatly improves the sensitivity and reliability of the determination of *trans,trans*-muconic acid. Separation is achieved on a LiChrosorb C_{18} 5-μm column with detection at 259 nm. The eluent is methanol–1% aqueous acetic acid (10:90) and the detection limit 50–100 μg/l. A physiological level of 100 μg/l was observed from unexposed persons. If *trans,trans*- muconic acid turns out to be a sensi-

tive indicator for low exposures to benzene, it should be more suitable for biological monitoring than phenol.

3.2.1.2. Determination of the metabolites of toluene, ethylbenzene and xylenes. Toluene, ethylbenzene and the xylenes are mainly metabolized by the oxidation of the aromatic side-chain, leading to benzoic acid, mandelic acid or phenylglyoxylic acid and *o*-, *m*-, *p*-methylbenzoic acids, respectively. Benzoic acid and the methylbenzoic acids are usually excreted in the urine bound to glycine, yielding hippuric acid and methylhippuric acids. Mandelic acid and phenylglyoxylic acid are eliminated unconjugated.

Hippuric acid is an endogenous metabolite common in human urine in concentrations up to several g/l, dependent on nutrition. The 95th percentile of normal excretion is 2.5 g/l [2].

On exposure to toluene increased levels of hippuric acid are found. The determination of hippuric acid in urine is thus important for the estimation of the degree of exposure to toluene. The other aromatic carboxylic acids are excreted at the mg/l level in cases of occupational exposure.

A multitude of HPLC methods for the determination of aromatic carboxylic acids has been developed. Generally, it is possible to determine hippuric acid, mandelic acid, phenylglyoxylic acid and the methylhippuric acids simultaneously.

The HPLC methods used nowadays for the routine analysis of aromatic carboxylic acids have the great advantage that no derivatization is necessary. A derivatization procedure by benzoxylation of *m*- and *p*-methylhippuric acid and separation on a silica gel column is no longer practicable [57].

Ion-exchange columns as the stationary phase [58] are no longer used for routine analysis, because reversed phases have turned out to be more practicable. Reversed-phase columns are generally used for the separation of aromatic carboxylic acids. Moreover, these phases are mainly used in environmental and occupational medicine owing to their ruggedness, the long standing times and their easy applicability. UV detection is the norm. A summary of the stationary phases, mobile phases and detection wavelengths used for the HPLC separation of aromatic carboxylic acids is given in Table 2.

Acidified urine may be injected directly into the HPLC column [59–68]. It is possible to separate hippuric acid and methylhippuric acids [59,62,65,66] or hippuric acid, methylhippuric acids, mandelic acid and phenylglyoxylic acid [67] in the same run. Plasma may be processed in the same way [64]. The detection limits are sufficient for routine analysis, from 2 mg/l for hippuric acid to 10 mg/l for mandelic acid [67].

Direct injection of the unprocessed urine into the reversed-phase column may cause serious contamination of the column. This results in reduced specificity because of a high analytical background. Therefore it is recommended that the aromatic carboxylic acids are cleaned up by an extraction procedure [69–76]. Ethyl acetate [69–73], butyl chloride–2-propanol [74] and diethyl ether [75,76] have been used as extraction solvents. The extract can be injected directly, or it can be evaporated and the residue dissolved in an appropriate solvent for further analysis. The aromatic carboxylic acids can then be separated in one run with less analytical background than if solvent extraction of the urine is not used [71,73,74,76].

The method recommended by the Deutsche Forschungsgemeinschaft (German Science Foundation) for the determination of aromatic carboxylic acids [76] is a typical example of the determination of hippuric acid, methylhippuric acids, phenylglyoxylic acid, mandelic acid and benzoic acid in one run in the concentration range relevant to occupational medicine. The acidified urine was extracted with diethyl ether, evaporated and redissolved in methanol–water. 3-Hydroxybenzoic acid or 3-chloro-4-hydroxybenzoic acid was used as the internal standard. The detection limits were 15–25 mg/l urine. Separation was achieved on LiChrosorb RP-18 with 0.01 *M* phosphate buffer–methanol (75:25) with detection at 215 nm. An example is shown in Fig. 5.

Besides the oxidation of the aliphatic side-chain, alkylbenzenes are metabolized to a lesser

TABLE 2

HPLC CONDITIONS FOR THE DETERMINATION OF AROMATIC CARBOXYLIC ACIDS IN URINE

Aromatic carboxylic acid	Stationary phase	Mobile phase (ratio)	Detection wavelength (nm)	Ref.
Hippuric acid Methylhippuric acids	Nucleosil C_{18}, 5 μm	Water–methanol–acetic acid (80:20:0.33)	254	59
Hippuric acid	Partisil ODS 2, 10 μm	1% Aqueous acetic acid–acetonitrile (4:1)	250	60
Hippuric acid	LiChrosorb Si-60, 5 μm	Acetonitrile 0.2 *M* phosphate-buffer–water + 1.25 m*M* N,N,N-trimethylhexadecyl-ammonium bromide (30:5:65)	254	61
Hippuric acid Methylhippuric acids	Ultrasphere ODS, 3 μm	10 m*M* Sodium acetate buffer–methanol–THF (90.5:7:2.5)	254	62
Hippuric acid	HS 5-C8, 5 μm	Acetonitrile–water–acetic acid (95:5:0.05)	225	63
Hippuric acid	Yanapak ODS-A, 7 μm	Acetonitrile–water–acetic acid (35:63:2)	235	64
Hippuric acid Methylhippuric acids	LiChrosorb RP-18, 10 μm	Methanol–water–acetic acid (20:80:0.2)	254	65
Hippuric acid Methylhippuric acids	Silica gel having dinitrophenyl residue, 10 μm	Methanol–water–acetic acid + 0.2% tetrabutylammonium bromide (80:20:0.2)	225	66
Hippuric acid Methylhippuric acids Phenylglyoxylic acid Mandelic acid	Octadecylsilanized silica gel (TSK gel, 80TM), 5 μm	Acetonitrile–5 m*M* phosphate buffer pH 2.5 (10:90)	225 254	67
Hippuric acid Methylhippuric acids	Zorbax C_8, 5 μm	Acetonitrile–water–acetic acid + 20 g β-cyclodextrin (20:80:1.5)	272.4	68
Hippuric acid	μBondapak C_{18}, 10 μm	Methanol 0.01 *M* phosphate buffer containing 0.5% acetic acid (20:80)	254	69,70
Hippuric acid Methylhippuric acids Phenylglyoxylic acid Mandelic acid	LiChrosorob RP-8, 10 μm	Water methanol (60:40) 0.01 *M* Phosphate buffer–methanol (90:10)	212 254	71
Methylhippuric acids	μBondapak C_{18}, 10 μm	Water–acetonitrile (90:10)	254	72

TABLE 2 *(continued)*

Aromatic carboxylic acid	Stationary phase	Mobile phase (ratio)	Detection wavelength (nm)	Ref.
Hippuric acid Phenylglyoxylic acid	Supelcosil LC 18	Water–acetonitrile (90:10)	225	73
Hippuric acid Methylhippuric acids Phenylglyoxylic acid Mandelic acid	HC ODS SIL X	Water acetonitrile–acetic acid (95:5:0.02)	225	74
Hippuric acid	LiChrosorb RP-18, 5 μm	0.01 *M* Phosphate buffer–methanol (75:25)	215	75
Hippuric acid Methylhippuric acids Phenylglyoxylic acid Mandelic acid	LiChrosorb RP-18, 5 μm	0.01 *M* Phosphate buffer–methanol (75:25)	215	76

extent by oxidation of the aromatic nucleus. Although the amounts of phenolic compounds formed in the organism are much lower than those of the main metabolites, they are of value for the estimation of health risks to exposed persons [47,77,78]. Phenols might represent the leading toxicological principle better than aromatic carboxylic acids because, in the case of benzene, the intermediate epoxide seems to be the carcinogenic agent.

As cresols are usually determined by GC, only a few HPLC methods have been published [47,52,61,79]. Hansen and Dossing [61] reported the determination of *o*-cresol in urine after solvent extraction. Separation was achieved by chromatography on a reversed-phase column (LiChrosorb Si-60) with acetonitrile–phosphate buffer–water (30:5:65) containing 1.25 m*M* N,N,N-trimethylhexadecylammonium bromide as mobile phase and fluorescence detection (excitation wavelength 273 nm, emission wavelength 298 nm). Recovery rates were still very poor.

The methods developed by Angerer [47], Lewalter *et al.* [48] and Brega *et al.* [52] for the determination of phenol can also be applied to the determination of cresols, as shown in Fig. 6. However, the three isomeric cresols could not be separated under the reported conditions.

Separation of the three isomeric cresols has been achieved on a LiChrosorb RP-18 5-μm column with acetonitrile–water (15:85) containing 2.5 g of β-cyclodextrin as the mobile phase and detection at 270 nm [79].

HPLC is a practical method for the determination of the main metabolites of aromatic hydrocarbons that are eliminated in the concentration range greater than 10 mg/l. Derivatization procedures are not necessary. Phenol and the aromatic carboxylic acids, as the main metabolites of aromatic hydrocarbons, can be determined easily and with great specificity in the concentration range relevant for occupational exposure. Separation is in general achieved on reversed-phase HPLC columns with UV detection, and different mobile phases have been used.

trans,trans-Muconic acid, as a minor metabolite, can also be easily determined with high specificity by HPLC. Although it is eliminated in the lower mg/l range, an efficient clean-up and enrichment procedure makes the determination by HPLC very specific and sensitive enough for biological monitoring.

The minor metabolites S-phenylmercapturic acid and the cresols are preferably determined by GC, because the analytical specificity and sensitivity of HPLC is not sufficient.

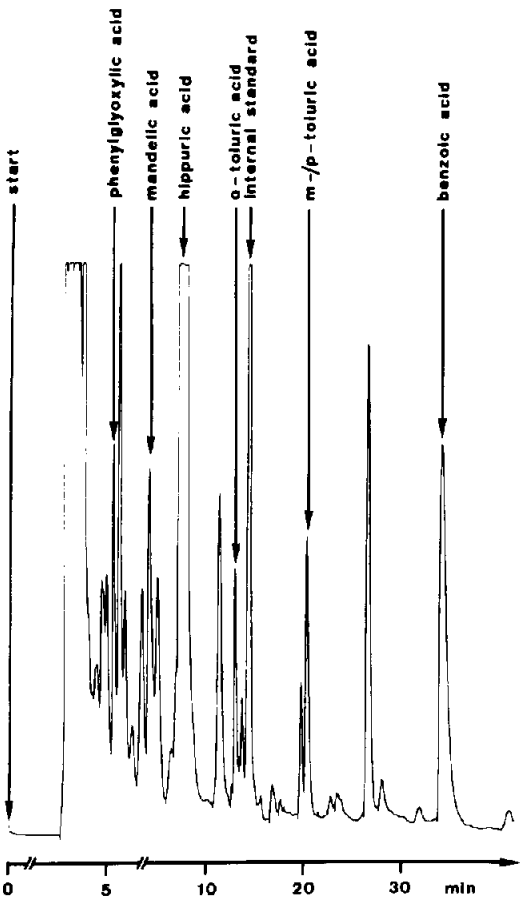

Fig. 5. HPLC of a processed urine sample spiked with aromatic carboxylic acids: phenylglyoxylic acid, 50 mg/l; mandelic acid, 50 mg/l; hippuric acid, 1060 mg/l; *o*-methylhippuric acid, 50 mg/l; *m,p*-hippuric acids, 50 mg/l; benzoic acid, 383 mg/l. Internal standard, 3-hydroxybenzoic acid [76]; column, LiChrosorb RP-18, 5 μm; UV detector wavelength, 215 nm; column temperature, 35°C; mobile phase, 0.01 *M* phosphate buffer–methanol (75:25).

3.2.2. Gas chromatographic methods

3.2.2.1. Determination of the metabolites of benzene in body fluids. As stated earlier, phenol is preferably determined in biological materials by photometric or HPLC methods. These methods are, as a rule, specific and sensitive enough to determine phenol in the mg/l concentration range relevant to occupational exposure.

Nevertheless GC methods for the determination of phenol in urine and blood have also been developed. The work-up of the urine sample is similar to that of the HPLC methods. The acidified and hydrolysed urine is subjected either to solvent extraction with diethyl ether [80], isopropyl ether [81] or methyl-*tert.*-butyl ether [82] or to steam distillation [47,38] followed by solvent extraction [83].

Formerly, separation was achieved using packed columns, and FID was used for detection. The reported detection limits were 2 mg/l [80,81] and 0.1 mg/l [83]. An on-column hydrolysis of the conjugated phenol as reported earlier [84] is not state-of-the-art. A derivatization procedure by silylation, as proposed by Niwa *et al.* [85] for the determination of phenol and its oxidation products catechol and hydroquinone in uremic serum, is very tedious but may improve the separation, analytical background and detection limit.

Nowadays, capillary columns are more and more used instead of packed columns, on account of their better resolution. The specificity of the detection of phenols and alkylphenols has been increased [86–88]. On capillary columns coated with methylsilicone [86], SE-54 [87] or Chirasil-L-Val [88], it is possible to separate phenol and the alkylphenols in one run, as discussed in the next chapter. Detection is generally achieved by FID.

If an exposure to a mixture of the aromatic hydrocarbons has to be considered, one of these capillary GC methods is preferable to any HPLC method.

For exposure levels below 2–5 ppm of benzene, phenol is not a useful parameter for biological monitoring, as it is not diagnostically specific. S-Phenylmercapturic acid has been proposed as a parameter indicating benzene exposure below 1 ppm [89,90]. The acidified urine is hereby extracted with ethyl acetate and subsequently hydrolysed with hydrochloric acid. The residue is dissolved in citrate buffer and analysed with an amino acid analyser [89,90]. Mass spectrometric detection of S-phenylmercapturic acid is still more sensitive [90]. The decisive step is enrichment on a cation-exchange column before the hydrolysis with hydrochloric acid. After the following derivatization with methanol–HCl, *n*-butanol–HCl and trifluoroacetic acid, separation was achieved

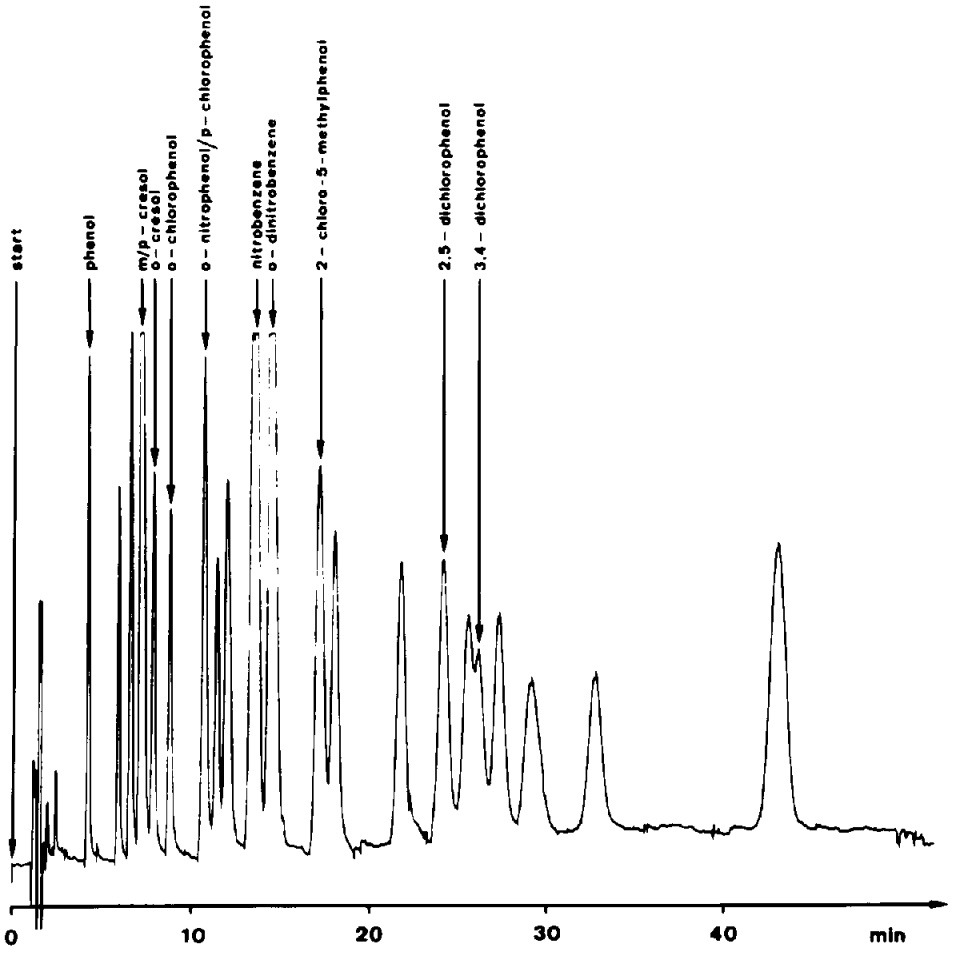

Fig. 6. HPLC of an aqueous solution containing various benzene derivatives. Column, Spherisorb ODS II; 3 μm; UV detection wavelength, 205 nm; mobile phase, acetonitrile–phosphate buffer pH 3.5 (35:65) [48].

on a PB 2 Q capillary column (WGA) with mass selective detection. The detection limit, at 1 μg/l urine, was in the ecological concentration range. For non-exposed workers a normal value of 4 μg/l urine could be determined [90].

3.2.2.2. Determination of the metabolites of toluene, ethylbenzene and xylenes. Besides a multitude of HPLC methods, GC methods for the determination of hippuric acid, methylhippuric acids, mandelic acid and phenylglyoxylic acid, the main metabolites of alkylbenzenes, have been developed [91–108].

The principal difference from HPLC methods is the need for derivatization of the aromatic carboxylic acids if a GC separation has to be considered. This makes GC a more time consuming procedure.

The aromatic carboxylic acids first have to be extracted from the acidified urine matrix with an organic solvent, as a rule ethyl acetate. Serum can be processed in the same way [94].

Different derivatization procedures following the extraction have been described. The conversion of the aromatic carboxylic acids into the corresponding methyl esters, which increases the volatility of the acids, is the most commonly reported method [91–100]. Methylation can be easily achieved by reaction with an ethereal solution of diazomethane [91–97]. The advantages of this procedure are an easy work-up, no interfering

byproducts in methylation, and reaction at ambient temperature. Nevertheless, diazomethane is explosive and carcinogenic, and must be handled carefully. Some alternatives to methylation have been proposed, *e.g.* methanol–hydrochloric acid at 60°C [98] or the use of 3-methyl-1-(*p*-toluyl)-triazene [99,100] as a mild methylating reagent at ambient temperature, liberating diazomethane. The separation of the methyl esters of hippuric acid [93,94] together with methylhippuric acids [91,92,95,97–100], mandelic acid and phenylglyoxylic acid [96] was achieved on packed columns, generally with FID. Different stationary phases, *e.g.* 10% polyethyleneglycol 1500 on Chromosorb W [91], 4% Apiezon M [99,100] or 10% Dexsil GC 300 [94] on Chromosorb G, 2% OV 225 [97], 3 10% SE 30 on Chromosorb W [92,95,98] or 10% neopentylglycolsuccinate on Chromosorb W [96] have been used. Internal standards were acetophenone [91], benzoylproline [97], fluorene [96], 3,4-dimethoxyphenylacetic acid methyl ester [94] or long-chain carboxylic acids, such as heptadecanoic acid [92,95,98], tridecanoic acid [99,100] or stearic acid [93]. The typical detection limits reported were 3 mg/l urine [100] and 0.6 mg/l serum [94].

Silylation has also been reported as a derivatization method [93,101–105]. A separation by paper chromatography before silylation is too tedious and merely of historical interest [101]. The acidified urine is extracted and silylated with the usual silylation reagents, *e.g.* Silyl-8 [105]. Separation is achieved on packed columns, preferably OV 17 [93,102,103,105]. The detection limits are comparable with those reached with the methylated aromatic carboxylic acids. However, the silylation procedure is more time consuming and has the disadvantage that the detector may become contaminated.

The use of capillary columns for the separation of urinary aromatic carboxylic acids was reported by Woiwode *et al.* [106] and Korn and coworkers [88,107]. The acids were converted into their corresponding isopropyl esters by 2-propanol hydrochloric acid. Separation was achieved on OV 225, OV 1, OV 17 and SE 30 capillary columns with high specificity. If chiral capillary columns coated with Chirasil-Val were used, the enantiomers of mandelic acid could be separated [88,107]. With the same columns, the minor metabolites of aromatic hydrocarbons could also be determined, making this method suitable for routine analysis of nearly all metabolites of aromatic hydrocarbons.

Since it was recognized that the oxidation of the aromatic nucleus is a branch of the human metabolism of alkylbenzenes [77,78], the determination of alkylphenols in urine has become of great significance. The excretion of these phenols seems to be of particular diagnostic value because of its close link with the potential damaging principle of these aromatic hydrocarbons. Except for 4-methylphenol, the alkylphenols do not seem to be endogenous products. Their appearance in urine is therefore due to exposure to alkylbenzenes. This makes them a useful parameter of greater diagnostic specificity than the physiologically appearing hippuric acid and methylhippuric acids for the biological monitoring of toluene and xylene at low exposure levels.

Though some HPLC methods have been established for the determination of alkylphenols, GC is of greater specificity and has lower detection limits, even down to the lower mg/l range and the μg/l level. Especially at low exposure levels GC determination should always be preferred to HPLC because of its greater resolving power and lower detection limits.

o-Cresol was first identified in urine as a minor metabolite of toluene [47,77], and *p*- and *m*-cresol can also be determined simultaneously [47,77,108–113]. The work-up procedure is similar to that of HPLC methods. After acidic or enzymic hydrolysis of the urine, the cresols are subjected either to solvent extraction with methylene chloride [109,113], chloroform [110] or carbon disulphide [112] or to steam distillation followed by extraction of the distillate with methylene chloride [47,77,108]. For separation, packed columns, *e.g.* 5% tri-2,4-xylenyl phosphate on Chromosorb HP-W AW MCS [47,77], 3% QF on Chromosorb W AW [109], and K-02 on Uniport HP [112], were used with FID. 2,3- [47,77], 2,4- [109] or 3,5-dimethylphenol [112] served as

the internal standard. Detection limits were 0.2–0.3 mg/l urine.

A greater specificity of analysis is gained if capillary columns are used for the separation of the three cresols. The stationary phases reported were OV 225/151 [109], Chirasil-Val [110,111], SE 54 [108] and methylsilicone SP 2100 [113]. Detection was achieved either by FID [109–111] or mass spectrometry [108,110,111,113]. As the internal standard, 2,4-dimethylphenol [109], tridecane [110,111], 2,3-dimethylphenol [108] or 3,5-dinitro-4-chlorobenzene trifluoride [113] was used. Detection limits were 0.1 mg/l.

Ethylbenzene and the xylenes are also oxidized at the aromatic nucleus, to give ethylphenols and xylenols, respectively. Oxidation at the aromatic side-chain results in DL-1-phenylethanol, 2-phenylethanol, phenyleneglycols and 3-methylbenzyl alcohol [87,88,114–116]. For the GC determination of these minor metabolites of aromatic hydrocarbons, capillary columns should be used because of their greater specificity. Methylsilicone SP 2100 [115], Chirasil-Val [88,114] or SE 54 [87,116] were reported as stationary phases with FID. On Chirasil-Val it was possible to separate the enantiomers of 1-phenylethanol after derivatization with pentafluoropropionic anhydride [88,114].

Two methods were reported for the simultaneous GC determination of the phenolic metabolites of aromatic hydrocarbons. Korn *et al.* [88] reported the separation of phenol and alkylphenols after methylene chloride extraction of the hydrolysed urine and separation on a Chirasil-Val capillary column. *p*-Chlorophenol served as the internal standard. With the method reported by Angerer and co-workers [87,116] it is also possible to separate phenol, cresols, xylenols, 2-ethylphenol, 1- and 2-phenylethanol and 3-methylbenzyl alcohol in one run. The acidified urine sample is subjected to steam distillation and subsequent extraction with methylene chloride. The analytical background can thus be reduced, increasing the specificity of the method. Separation is achieved on an SE 54 capillary column with FID. 3-Ethylphenol is used as the internal standard. The detection limit is 0.3 mg/l urine in the concentration range of interest in occupational medicine, and to some extent in environmental studies. An example of a gas chromatogram of a processed urine sample is shown in Fig. 7.

Because of lower detection limits and great specificity of analysis, capillary GC is the method of choice for the determination of the minor metabolites of both benzene and the alkylbenzenes.

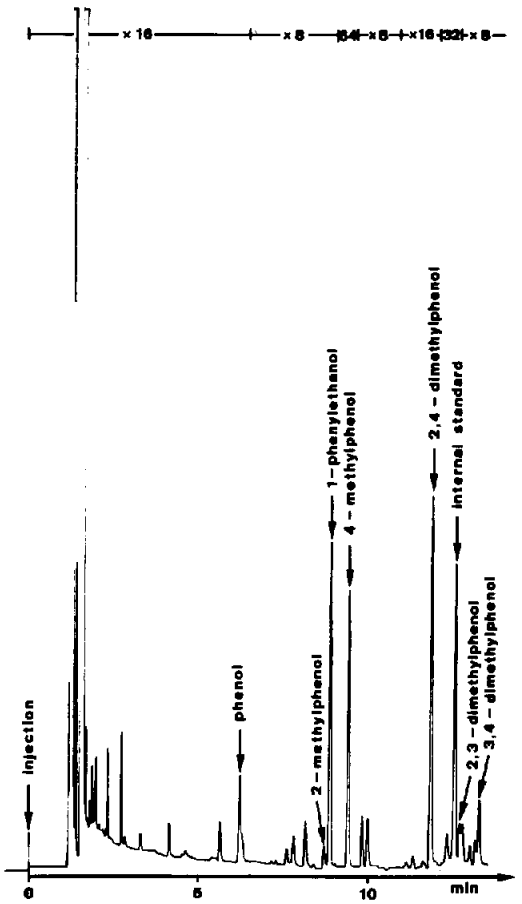

Fig. 7. Gas chromatogram of a processed urine specimen from an individual exposed to aromatic hydrocarbons. Concentrations: phenol, 3.4 mg/l; 2-methylphenol, 0.3 mg/l; DL-1-phenylethanol, 2.8 mg/l; 4-methylphenol, 18.3 mg/l; 2,4-dimethylphenol, 6.6 mg/l; 2,3-dimethylphenol, 0.3 mg/l; 3,4-dimethylphenol, 0.7 mg/l [87]. Column, quartz capillary column (30 m × 0.25 mm I.D.); stationary phase, SE 54, 0.25 μm; detector, flame ionization; temperature programme, 5 min at 60°C, 5°C/min to 110°C, 10 min at 200°C [87].

3.3. Miscellaneous methods

Sophisticated methods such as HPLC and GC are not needed for the determination of hippuric acid, which is excreted at the g/l level. Photometric methods or thin-layer chromatography (TLC), which do not need expensive equipment and are also very simple to perform, have been proposed for the determination of hippuric acid in urine [117–122]. Urine samples can be directly applied to the TLC plates [117], usually coated with silica gel, or subjected first to a solvent extraction [118–122]. Development of the plates is achieved with mixtures of organic solvents with acetic acid. The separated spots are treated with colour-forming substances. They can then be extracted and determined photometrically [119–122] or they can be measured densitometrically [117,118]. The detection limit of this method was 50 mg/l urine, which is sufficient for routine monitoring of hippuric acid, if no HPLC or GC apparatus is available.

A method that is not routine use, because it needs special equipment, is the determination of aromatic carboxylic acids by isotachophoresis [123–126]. The acidified urine is extracted with diethyl ether or diluted and separated by electrophoresis using 5 m*M* hydrochloric acid, 20 m*M* β-alanine and 0.4% hydroxypropylmethylcellulose as the leading electrolyte and 5 m*M* capronic acid as the terminating electrolyte. Quantification is carried out by an UV detector. Detection limits are 5–8 mg/l urine.

3.4. Adducts of aromatic hydrocarbons to DNA and haemoglobin

Benzene is a proven carcinogen and is a cause of leukaemia [1,2]. The common feature of chemical carcinogens is their covalent binding to DNA or other macromolecules such as haemoglobin. The stability of these adducts could allow the monitoring of an exposure long after it has ceased and also of damage not detectable by common analytical methods brought about by a low chronic exposure.

Andrews *et al.* [127] reported in 1977 that benzene metabolites accumulated in bone marrow *in vivo*. The binding of benzene metabolites to hepatic DNA was first reported by Lutz and Schlatter [128]. They estimated that one in 10^6 nucleotides were bound by DNA metabolites. Kalf *et al.* [129] and Snyder *et al.* [130] showed that the metabolites of radiolabelled benzene were binding covalently to the mitochondrial DNA *in vitro*, using mitoplasts of rat liver and rabbit bone marrow. The adducts were characterized after isolation and hydrolysis of the DNA by UV, fluorescence, mass spectrometry and nuclear magnetic resonance spectroscopy. Liver microsomes were also used for studies on the metabolism of radiolabelled benzene and toluene [131,132].

Bauer *et al.* [133] described a ^{32}P-postlabelling assay for an *in vivo* study of benzene metabolite DNA adducts formed in rabbit liver. The formation of (3′-OH)-benzethenol-(1,N_2)deoxyguanosine was suggested. However, none of these methods can determine the molecular structure of the DNA adducts.

Norpoth *et al.* [134] reported the excretion of the benzene adduct N-7-phenylguanine in the urine of rats after benzene exposure. The adduct was studied by HPLC and GC–MS in comparison with a synthetic N-7-phenylguanine reference substance. It is suggested that these adducts may become a new class of indicator metabolites of benzene exposure, as they could be a better approach to the carcinogenic principle of benzene.

The formation of adducts of benzene with haemoglobin could also be used as a biomarker for benzene exposure. So far, only a few studies have been published. Radiolabelled benzene was fed to mice and rats and the amount of ^{14}C bound to isolated globin was measured [135,136]. The structure of the adducts has not been investigated. In this field, much research work still has to be done, if haemoglobin adducts are to be used in the biological monitoring of benzene.

4. BIOMEDICAL APPLICATIONS

It is the duty of occupational and environmental medicine to evaluate the health risk posed by hazardous chemicals in order to guard against

impairment of health. The determination of hazardous substances and their metabolites in blood, urine and expired air, the so-called biological monitoring, is an excellent instrument for the estimation of the individual doses of hazardous substances. An evaluation and thus a reduction of the individual health risk based on environmental and occupational exposure is thus made possible.

The levels of hazardous substances in blood and urine assessed by biological monitoring have still to be interpreted by occupational and environmental medicine, and in this context limit values are helpful. Such limit values are the physiologically or environmentally occurring reference values in the body fluids and, in case of occupational exposure, the occupational limit values. Both values have to be considered when the health effects of hazardous chemicals are discussed.

4.1. Environmental exposure to aromatic hydrocarbons

The aromatic hydrocarbons are ubiquitous in the human environment, chiefly as fuels and solvents. The Total Exposure Assessment Methodology Study of the US Environmental Protection Agency showed that the personal indoor air exposure to benzene, for example, exceeded the outdoor air concentration [137]. The major indoor exposure source is tobacco smoke [138].

For the assessment of total exposure the blood level of aromatic hydrocarbons in the occupationally non-exposed population is a suitable indicator for biological monitoring. Dynamic head-space analysis has made it possible to determine blood levels of aromatic hydrocarbons in the ng/l range, which is relevant to environmental exposure. Tables 3 and 4 show normal values of aromatic hydrocarbons in blood for occupationally non-exposed persons.

The benzene concentrations found in blood range from 10 to 455 ng/l for non-smokers and from 109 to 1657 ng/l for smokers. The median levels found were 0.57–2.0 μg/l for toluene, 0.43–1 μg/l for ethylbenzene and 1.4–5.2 μg/l for xylene. The levels are significantly higher for smokers than for non-smokers. This must be explained by the well known fact that cigarette smoke contains significant amount of these compounds [139].

Some of the metabolites of aromatic hydrocarbons are endogenous products and are excreted in the urine of occupationally non-exposed persons as well. For biological monitoring in case of occupational exposure these normal values have to be considered if health risks are to be estimat-

TABLE 3

BENZENE CONCENTRATIONS IN BLOOD OF SMOKERS AND NON-SMOKERS NOT OCCUPATIONALLY EXPOSED TO BENZENE

Smoking habit	*n*	Benzene concentration (ng/l of blood)			Ref.
		Median	Mean ± S.D.	Range	
Non-smokers	13	190	218 ± 96	112 455	27
Smokers	14	493	547 ± 195	287–947	
Non-smokers	8	127	127 ± 54	49–191	28
Smokers	11	578	584 ± 300	109–1136	
Non-smokers	8	165	176 ± 62	80–300	30
Smokers	2	180	211 = 85	130–430	
Non-smokers			130 ± 96		29
Non-smokers plus smokers	58	235	332 ± 320	10–1657	
Non-smokers plus smokers	250	–	800	n.d.–1657	26

TABLE 4

CONCENTRATION OF ALKYLATED BENZENES IN BLOOD OF SMOKERS AND NON-SMOKERS NOT OCCUPATIONALLY EXPOSED TO TOLUENE, ETHYLBENZENE AND XYLENE

Aromatic hydrocarbon	Smoking habit	n	Concentration (μg/l of blood)		Ref.
			Median	Range	
Toluene	Non-smokers	13	1.1	0.5–4.6	27
	Smokers	14	2.0	1.3–3.8	
	Non-smokers plus smokers	37	0.57	0.02–5.0	29
	Non-smokers plus smokers	250	1.5	0.2–38	26
Ethylbenzene	Non-smokers	13	0.43	0.18–2.3	27
	Smokers	14	0.53	0.98–2.7	
	Non-smokers plus smokers	37	1	–	29
	Non-smokers plus smokers	250	1.0	n.d.–59	24
Xylene	Non-smokers	13	1.4	0.68–7.1	25
	Smokers	14	1.85	1.15–41	
	Non-smokers plus smokers	250	5.2	0.5–160	26

ed. Normal values in the mg/l and even the g/l level are found in urine for phenol and hippuric acid. This makes these constituents diagnostically unspecific in the case of low exposure levels to aromatic hydrocarbons and for the individual person. On the basis of group data [150], however, these parameters are specific and sensitive. Fig. 8 gives an example. It shows the cumulative frequencies of phenol excretion in three groups of workers exposed to benzene and one group of persons not exposed to solvents. The normal excretion of phenol overlaps great areas of phenol concentration in the urine of the workers. Regarding median values, however, an exposure of 0.2 ppm already causes a dramatic increase of the median concentration of phenol in urine.

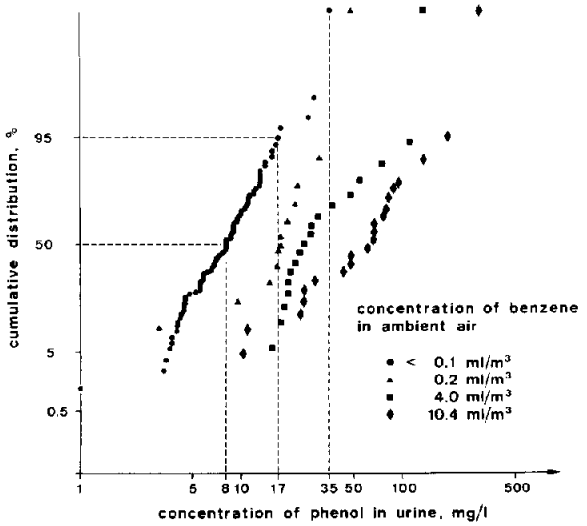

Fig. 8. Cumulative distributions of concentrations of phenol in urine (mg/l) for non-exposed persons and persons exposed to different concentrations of benzene in the ambient air [150].

The determination of phenol in urine is, therefore, though diagnostically unspecific for the individual, a diagnostically specific indicator for an exposed group. These considerations are also valid for the determination of hippuric acid in urine.

For the metabolites of ethylbenzene, mandelic acid and phenylglyoxylic acid, normal values in the lower mg/l level have been discussed. These normal values, however, are mainly due to the detection limits of the analytical method used.

For the minor metabolites of benzene, S-phenylmercapturic acid [90] and *trans,trans*-muconic acid, normal values in the μg/l range have been found. Epidemiological studies, however, have still to prove these results. A summary of normal values is given in Table 5.

TABLE 5

REFERENCE VALUES OF METABOLITES OF AROMATIC HYDROCARBONS OF NON-EXPOSED PERSONS

Aromatic hydrocarbon/metabolite	Average	Range	95th percentile	Ref.
Benzene/phenol		5–10 mg/l	–	140
	8.2 mg/24 h	–	20 mg/l (97.5%)	141
	7.8 mg/l	–	16 mg/l (97.5%)	142
	7.5 mg/l	2–18 mg/l	–	80
	10.4 mg/24 h, 8.6 mg/l	–	12.4 units (95%)	143
	4.7 mg/g creatinine, 9.4 mg/l	–	14.7 mg/g creatinine (97.5%)	144
	7.6 mg/l	3.2–14.7 mg/l	–	145
	6.75 mg/24 h	–	–	146
	4.6 mg/l	0.6–12.8 mg/l	7.7 mg/l	47
	3.4 mg/l	0.5–17.3 mg/l	–	50
	11.3 mg/24 h	–	–	51
	6.9 mg/l	–	–	55
Benzene/S-phenylmercapturic acid	4 μg/g creatinine	–	–	90
Benzene/*trans,trans*-muconic acid	0.16 mg/l, 24 h	0.1–0.5 mg/l	–	56
Toluene/hippuric acid	–	0.3–3.2 g/l	–	37
	0.44 ± 0.2 g/l	–	–	39
	0.8 g/l	–	–	36
	0.8 g/l	–	–	33
	1.6 g/l	–	–	147
	0.8 g/g creatinine	Up to 0.6 g/l		92
	0.79 g/l	–	–	118
	0.4 g/l	0.16–2.01 g/l	1.25 g/l	58
	0.35 g/l	0.2–0.62 g/l		43
	0.47 g/l	0.15–1.09 g/l	–	63
	0.27 g/l	–	–	60
	–	0.04–1.42 g/l	–	148
	0.72 g/l	–	–	110
	0.27 g/l	–	–	61
Toluene/*p*-cresol	89 mg/l	20–200 mg/l	–	80
	47.6 mg/l	–	–	134
	45.7 ± 31.0 mg/l	4–123.1 mg/l	–	47
	29.0 mg/l	–	–	109

o- and *p*-cresol and ethylphenols, the minor metabolites of toluene and ethylbenzene, cannot be detected in the urine of non-exposed persons with current analytical methods. Also the xylene metabolites, the methylhippuric acids and the xylenols, could not be determined in the occupationally non-exposed population. These parameters are therefore useful instruments for the biological monitoring of exposed persons, but they are not yet in wide use.

4.2. Occupational exposure to aromatic hydrocarbons

In cases of occupational exposures, limit values in biological materials have been evaluated. The Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the German Science Foundation evaluated biological tolerance values for working materials (BAT values) in 1981. A BAT value is defined as the maximum permissible amount of a chemical compound, its metabolites or any deviation from the norm of biological parameters induced by these substances in exposed humans. It would be emphasized that, in contrast to the biological exposure indices (BEI values), according to current knowledge these conditions do not impair the health of the employee, even when exposure is repeated and of long duration. As with the BAT and BEI values, the maximum period of exposure is generally given as 8 h daily and 5 days a week [6].

BEI values, established by the American Conference of Governmental Industrial Hygienists (ACGIH), are reference values intended as guidelines for the evaluation of potential health hazards in the practice of industrial hygiene. BEI values represent the levels of chemicals or their metabolites in exhaled air, blood or urine collected from a healthy worker who has been exposed to these chemicals. An 8-h exposure for 5 days a week is assumed. BEI values do not indicate a sharp distinction between hazardous and unhazardous exposures [132].

A summary of the established BAT and BEI values for aromatic hydrocarbons is given in Table 6.

For benzene as a carcinogenic working material no BAT value was evaluated, because at present no biological value which can be regarded as safe can be specified. Instead, so-called exposition equivalents for carcinogenic working materials (EKA values) have been established. Correlations between the concentration of benzene in the air of the work area and the concentration of benzene in blood and of its metabolite phenol in urine have been evaluated.

Table 7 lists BAT and BEI values for the metabolites of aromatic hydrocarbons.

If an occupational exposure has to be considered it is necessary to consider both the normal values of the analysed parameter and the occupational limit values. Diagnostic specificity, both for individual and for group exposure, has to be considered as well. An evaluation of results is necessary from an analytical as well as a medical viewpoint, and should be based on collaborative efforts.

TABLE 6

BAT AND BEI VALUES OF AROMATIC HYDROCARBONS IN BLOOD

Aromatic hydrocarbon	BAT/EKA value	BEI	Sampling time
Benzene	54 μg/l	–	End of exposure
Toluene	1.7 mg/l	1 mg/l	End of shift
Ethylbenzene	(1.5 mg/l, planned)	–	End of exposure
Xylene	1.5 mg/l	-	End of exposure

TABLE 7

BAT AND BEI VALUES OF THE METABOLITES OF AROMATIC HYDROCARBONS IN URINE

Aromatic hydrocarbon	Metabolite	BAT/EKA value	BEI	Sampling time
Benzene	Phenol	45 mg/l (6 ppm)	50 mg/g creatinine	End of shift
Toluene	Hippuric acid	–	2.5 g/g creatinine	End of shift
	o-Cresol	–	1 mg/g creatinine (planned)	End of shift
Ethylbenzene	Mandelic acid	–	1.5 g/g creatinine	End of shift at end of workweek
Xylene	Methylhippuric acids	2 g/l	1.5 g/g creatinine	End of shift

5. CONCLUSION

Two approaches have been discussed for the biological monitoring of aromatic hydrocarbons in blood and urine.

(a) For the determination of the unchanged aromatic hydrocarbons, blood is the preferred matrix for biological monitoring. The method of choice is head-space GC on capillary columns in the case of occupational exposure (μg/l range). For the determination of reference values in the ecological concentration range (ng/l level) dynamic head-space analysis is preferred.

(b) The determination of the major metabolites of aromatic hydrocarbons, phenol and the aromatic carboxylic acids, in routine monitoring of occupational exposure, is performed on urine samples. Photometric methods, TLC and HPLC can be used, as normal values of these metabolites are in the mg/l and the g/l region, respectively. HPLC on reversed phases is the method preferred by most laboratories.

(c) The minor metabolites of aromatic hydrocarbons, such as S-phenylmercapturic acid and the alkylphenols, are determined preferably by capillary GC because of its greater specificity and lower detection limits. *trans,trans*-Muconic acid, however, can be determined by HPLC.

(d) The minor metabolites of aromatic hydrocarbons seem to be diagnostically more specific than the main metabolite for the estimation of internal exposure.

(e) Haemoglobin and DNA adducts of benzene, and possibly of alkylbenzenes, may in future give a better basis for the estimation of carcinogenic risks.

(f) Evaluating the results of biological monitoring on a group basis gives valuable information, even if the parameter is not diagnostically specific on an individual basis.

(g) For the evaluation of biological monitoring results, both reference and occupational limit values, such as BAT and BEI values, have to be considered.

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