

WHO/HPR/OCH 96.2

Distr.: General

**Biological Monitoring of
Chemical Exposure
in the Workplace**
Guidelines

Volume 2

World Health Organization
Geneva 1996

Contribution to the International Programme on Chemical Safety (IPCS)

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This publication has been published with the support of the Finnish Institute of Occupational Health.
ISBN 951-802-167-8

Geneva 1996

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Preface

This is the second in a series of volumes on 'Guidelines on Biological Monitoring of Chemical Exposure in the Workplace', produced under the joint direction of WHO's Office of Occupational Health (OCH) and Programme for the Promotion of Chemical Safety (PCS).

The objectives of this project was to provide occupational health professionals in Member States with reference principles and methods for the determination of biomarkers of exposure, with emphasis on promoting appropriate use of biological monitoring and assisting in quality assurance.

For readers' information, it should be mentioned that Volume 1 included a description of the general principles of biological monitoring in occupational health, quality assurance, followed by biological monitoring of exposure to selected metals (cadmium, chromium, inorganic lead, inorganic mercury); selected solvents (carbon disulphide, N,N-dimethylformamide, 2-ethoxyethanol and 2-ethoxyethyl acetate, hexane, styrene, toluene, trichloroethylene and xylene); selected pesticides (organophosphorus pesticides); other selected compounds (carbon monoxide, fluorides).

The material in this volume has been discussed and finalized at the WHO meeting of experts, which took place in Geneva, 31 May to 3 June 1994, with the participation of international and industrial organizations concerned (see attached list of participants). All information available for the chemicals included in this volume was assessed and validated by this meeting. Contributions made by all participants are highly appreciated and acknowledged.

The second volume includes additional chemicals and provides an additional cadre of methods for the assessment of occupational exposure to a variety of important metals, solvents, pesticides and other chemicals. However, the amount of information available for each chemical varies depending on the specific chemical. In some cases, a lack of sufficient data on the external exposure – internal dose relationship in humans, resulted in the absence of published Occupational Biological Reference Values (OBRV). Nevertheless, these chemicals and the methods for biological monitoring have been included in this volume, since all of these methods can be useful in the assessment of occupational exposure, particularly by routes of exposure other than by inhalation or in the case of multi-route exposure (inhalation, skin absorption, ingestion). It is also thought that inclusion of these chemicals will stimulate research and the development of OBRV for these chemicals.

The introduction section for each chemical provides a summary of the available information, gaps and limitations for routine use of the proposed specific biological monitor-

ing methods identified in the specific chapter. For example, methods for biological monitoring of exposure to polyaromatic hydrocarbons using the urinary metabolite, 1-hydroxypyrene, must be applied after documenting the presence of pyrene in the work environment. In those instances where a biological action limit, which is called in this series an Occupational Biological Reference Value (OBRV), is available, the basis for the action limit (health-based or exposure-based) is stated.

In practical terms, it should be underlined that the application of biological monitoring in conjunction with a programme of occupational hygiene to control workplace exposures, can provide significant information on the effectiveness of workplace control in reducing exposures of the worker to toxic chemicals.

Contributions to this volume made by Dr A. Aitio (Chapters 1 and 2), Dr J. Cocker (Chapter 4), Professor M. Ikeda (Chapter 2), Dr F. Jongeneelen (Chapter 4), Dr L.K. Lowry (Chapter 2), Dr B. Nutley (Chapter 3), Dr A.F. Pelfrène (Chapter 3), Dr D. Templeton (Chapter 1), and Dr Y. Wu (Chapter 3) are highly acknowledged, as well as the efforts of Professor Fengsheng He and Dr R. Plestina in the role of Project Coordinators.

Substantive editing was completed by Dr K. Wilson, Head, Biomedical Sciences Group, Health and Safety Executive, United Kingdom, who is kindly acknowledged.

When biological monitoring of chemical exposure at work is used in practice or research, it should be stressed again, as for the first volume, that risk to health should be avoided and the confidentiality of data should be protected, as it is required by the International Code of Ethics for Occupational Health professionals.

Implementation of this project component, Volume 2, became possible due to the technical and financial contribution of the National Institute for Occupational Safety and Health (NIOSH), USA (Project No. U60/CCU008636-02), and the European Commission (DG V – Public Health and Safety at Work Directorate). Both are kindly acknowledged.

Financial support for publishing this book, was also provided by the Japan Chemical Industry Association, International Council of Metals and the Environment (ICME), and Nickel Producers Environmental Research Association (NiPERA). Their support is highly appreciated and hereby acknowledged.

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Chapter 1. Biological monitoring of selected metals

1.1 Aluminium

1.1.1 Introduction

Aluminium has traditionally been considered as non-toxic, but recently a serious, sometimes life-threatening disease and strong accumulation of aluminium in the bones and central nervous system have been described in patients with renal failure. This is caused by elevated aluminium intake in the diet (drugs decreasing phosphorus availability) and via dialysis fluids, together with decreased aluminium excretion in the urine. Recently, aluminium accumulation, and possible nervous system effects have also been described after occupational aluminium exposure. Because of such systemic effects, biological monitoring could markedly enhance the possibilities of assessing the health risks caused by aluminium at the workplace. The biochemistry, health effects of occupational exposure, and biological monitoring of aluminium have been reviewed (1–6).

1.1.2 Physical-chemical properties (2, 4, 7, 8)

The relative density of metallic **aluminium** (Al, CAS 7429-90-5) is 2.70 g/cm³, melting point 660°C and boiling point 2467°C. Pure aluminium is a light, ductile metal and a good conductor of electricity and heat. Aluminium is insoluble in water, but soluble in alkali and mineral acids. When aluminium is exposed to air, a thin film of **aluminium oxide** (CAS 1344-28-1) is formed, and this makes aluminium resistant to corrosion: aluminium oxide is very slightly soluble in water, acid and alkali.

Aluminium sulphate (CAS 10043-01-3) is used in water treatment; it is soluble in water but forms **aluminium hydroxide** (CAS 21645-51-2) in water, which is amphoteric: insoluble at neutral pH (and coprecipitates humic materials from raw water) and readily soluble both in acid (as octahedral hexahydrate, $\text{Al}(\text{H}_2\text{O})_6^{3+}$) and in alkali (as tetrahedral $\text{Al}(\text{OH})_4^-$).

Aluminium phosphate (CAS 7784-30-7) is practically insoluble; this is the basis for the use of aluminium hydroxide as a phosphate scavenger for patients with renal failure: aluminium hydroxide given orally forms insoluble phosphate, which is not absorbed from the gastrointestinal tract.

Aluminium silicates (CAS 27-36-2, 12141-46-7) are insoluble in cold and hot water.

The relative atomic mass of aluminium is 26.98; thus 1 mmol = 26.98 mg, and 1 mg = 37.1 μ mol.

1.1.3 Possible occupational and non-occupational exposures

Aluminium is used extensively in metallurgy. A large variety of packaging materials and containers are made of aluminium. Its use is extensive in aerospace industry, automotive industry, construction, and in the building of ships, train carriages, and other corrosion-resistant structures. Aluminium silicate is used in washing agents; aluminium sulphate in raw and waste water treatment and aluminium oxide in abrasives. Elevated aluminium concentrations in blood/urine have been reported in aluminium welding, grinding, melting aluminium powder production, aluminium sulphate production, corundum production, as well as electrolytic aluminium production (4, 9).

Aluminium is a common element, comprising approximately 8% of the earth's crust. It is present in most dietary items, and the daily ingestion of aluminium from food and drink has been estimated to be 1–30 mg/d. A large but variable part of this aluminium comes from food additives such as leavening agents in baked goods, emulsifying agents in cheese, acidifying agents, dyes and colours, as well as anti-caking agents (10). Dissolution of aluminium from kitchen utensils, pots, pans, coffee percolators, etc. represents probably a minor contribution to the daily dietary aluminium: the aluminium content of acid food items may reach 1 mg/L (11). Tea and soft drinks may also contain aluminium at levels between 0.1 and 1 mg/L (11, 12). Some herbs, such as thyme, oregano, celery seed, and basil may contain aluminium at levels of approximately 0.1–1 mg/g (10).

Antacid drugs used in the treatment of duodenal or gastric ulcer may contain large amounts of aluminium (daily dose may be in the order of grams). Several studies have shown that plasma and urinary aluminium concentrations are elevated after ingestion of aluminium containing antacids, or sucralfate (aluminium sucrose sulphate) (13–23).

1.1.4 Summary of toxicokinetics

1.1.4.1 Absorption

(a) *Inhalation*

Elevated blood and urinary aluminium levels have been observed after occupational exposure to different aluminium compounds; this indicates absorption via inhalation, but no quantitative data are available.

(b) *Dermal*

There are no data indicating significant dermal absorption.

(c) *Gastrointestinal*

The gastrointestinal absorption of aluminium is very limited, in the order of 0.005% of the ingested amount of aluminium hydroxide (15, 16). However, citric acid and prob-

ably other dietary components may greatly enhance this proportion (15, 24, 25). On the other hand, elevated gastric pH after ranitidine therapy was accompanied by decreased aluminium absorption (17). Bicarbonate, administered simultaneously with aluminium, did not enhance the gastrointestinal absorption of aluminium (25). After ingestion of aluminium-containing antacid tablets, the peak serum concentration was observed after 30 min (23).

1.1.4.2 Metabolic pathways and biochemical interactions

Not applicable.

1.1.4.3 Distribution

Aluminium is evenly distributed between the cellular elements of blood, and plasma (26). In plasma, aluminium is bound to proteins, notably transferrin and albumin; part of it remains ultrafiltrable, chelated mostly with citrate. The total amount of aluminium in the body is estimated at 30–330 mg. Most of the aluminium in the body is accumulated in the bones; lungs may also show high concentrations (10).

1.1.4.4 Elimination

Of the aluminium that has been absorbed, most is excreted in the urine. Biliary excretion is likely to be a minor route of exposure (10). Immediately after exposure to aluminium-containing welding fumes, the urinary aluminium decreased with a half-time of 8 h (27). Upon longer follow-up, it was noted that among welders exposed for less than 1 year, the half-time for urinary concentration of aluminium was about nine days, whereas welders exposed for more than 10 years had half-times six months or longer (28). This indicates that aluminium is stored in several compartments in the body. In workers exposed to aluminium flakes, the half-time of urinary aluminium was approximately 5–7 weeks; no dependence on the length of the preceding exposure time was observed (29).

1.1.5 Summary of toxic effects

Aluminium is the causative agent in dialysis dementia, a disease observed in patients with long-term dialysis therapy for renal failure. This disease entity comprises central nervous system damage of varying (serum aluminium concentration-dependent) intensity, together with osteomalacia, and microcytic anaemia (30).

Some studies suggest that even occupational exposure to aluminium or aluminium compounds may interfere with neurophysiological and -psychological functions. An eventually fatal neurological disease was described in a worker who had been heavily exposed to aluminium dust; on autopsy it was noted that the concentration of aluminium in the brain was twenty times higher than the levels considered normal (31). Miners treated with aluminium/aluminium oxide powder by inhalation (to prevent silicosis) showed an exposure duration-dependent impairment in cognitive functions, when studied after an exposure that had lasted for 6 months to 36 years; no increased risk of clinical neuro-

logical disease was observed (32). Aluminium-exposed workers in electrolytic aluminium production (9), aluminium foundries (9, 33–35) and in aluminium smelters (36) produced impaired test results in neuropsychological tests. Welders exposed to aluminium exhibited aluminium exposure-related impairment in memory tests (37) but no change in the frequency of subjective symptoms. In another study, aluminium welders had more subjective symptoms (problems in concentrating, depressive moods) than welders not exposed to aluminium (38).

Aluminium-induced pulmonary diseases have also been described after inhalation exposure, but they seem to be rare and rather mild (39–41).

1.1.6 Biological monitoring indices

Table 1.1.1. Biological monitoring of exposure to aluminium

Indicator	Suitability	Reference
Urinary aluminium	Specific, sensitive	(27–29, 42–44)
Serum aluminium	Specific, less sensitive, limited information	(27–29, 42–44)
Bone aluminium	Insensitive, no experience	(45)

At present, the best validated approach for the biological monitoring of aluminium exposure is the analysis of aluminium in the urine (table 1.1.1).

1.1.7 Aluminium in urine

1.1.7.1 Toxicokinetics

After occupational exposure to aluminium-containing welding fumes, the elevated concentration of aluminium in the urine decreases following several consecutive half-times, approximately 8 h, 9 days, and six months or more. In long-term welders, the urinary aluminium concentration is closely related to the exposure time in years (27).

1.1.7.2 Biological sampling

(a) Sampling time

At the beginning of the workweek, before the day's exposure or alternatively, after the working day.

(b) Specimen

Spot urine. At present no information is available, whether correction to a common relative density or creatinine excretion is appropriate.

(c) Contamination possibilities

Very marked risk of contamination at sample collection, from the sample collection vials, and during the analysis. Dust from the workplace constitutes a major contamination hazard: The specimen has to be collected outside the working area, and after a shower, and changing into street clothes.

(d) Sampling device and container

Several procedures for the cleaning of sampling vials for trace elements have been published (46). For example, the following procedure has proved successful. Samples (50 mL) are collected in polyethylene vials which have been kept overnight in a detergent, rinsed twice with tap water, machine washed using detergent and rinsing chemical, kept in 5% HNO_3 for 12 hours, rinsed with 18 M Ω water several times, dried at room temperature in a closed cupboard for 2–3 days, capped with acid-washed caps, and stored in plastic bags.

(e) Preservative

Acidification by e.g. nitric acid, in order to prevent adsorption on vial walls, has been recommended for dilute aqueous specimens (47), but does not seem to be necessary for urine specimens.

(f) Shipment

At room temperature shipment just before a weekend should be avoided; rather, the samples should be kept refrigerated and sent after the weekend.

(g) Stability

The sample is stable for several days at room temperature; it can be stored in the refrigerator for two weeks. For longer storage, freezing at -20°C is recommended.

1.1.7.3 Recommended analytical method

The method described below has been used in the authors' laboratory and follows the guidelines of the manufacturer of the instrument.

(a) Principles of the method

Sample is diluted with nitric acid, and analysed with graphite furnace atomic absorption spectrometry using Zeeman background correction.

(b) Reagents required

Nitric acid (suprapur), 18 M Ω water, Triton X-100 (as autosampler flushing solution only), aluminium standard solution (e.g. Spectrosol®, BDH); control specimens: pooled mixture of specimens sent to the laboratory for aluminium analysis and freeze-dried commercial reference material (BioRad Lyphocheck urine metals control).

(c) *Equipment required*

Graphite furnace atomic absorption spectrophotometer with Zeeman background correction and automatic sampler; pyrolytically coated graphite tubes and the platform facility; HEPA filtered air hood.

(d) *Procedures*

The sample preparation is performed in a HEPA filtered air-hood. Powder-free gloves are worn throughout. The test tubes and autosampler cups, as well as pipette tips are acid washed, and rinsed with 18 M Ω water. The analysis is performed by the standard addition method: to 200 μ L of the sample, 50, 100, 150 and 200 μ L of the standard (200 μ g aluminium/L 0.2% nitric acid), and 150, 100, 50 and 0 μ L of 0.2% nitric acid are added, and the volume is made to 800 μ L with water. The liquid, after thorough mixing, is transferred into autosampler cups.

Details of the programme of the instrument are given in table 1.1.2, but have to be determined for each instrument, and may vary with the condition of the tube.

Table 1.1.2. Details of the programme of the instrument

Step	Temp °C	Ramp(s)	Hold(s)	Gas flow
Dry	105	1	20	300
Dry	145	40	1	300
Char	570	5	20	300
Char	1500	1	20	300
Stabilise	20	1	5	0
Atomise	2500	0	3	0
Clean	2700	1	5	300

(e) *Analytical reliability*

i) *Trueness*

The mean result ($n=28$, CV = 7.8%) obtained in successive analytical runs of a batch of the BioRad Lyphocheck urine metals control no 1 was 4.056 μ mol/L, when the target value set by the manufacturer was 3.954 μ mol/L.

ii) *Precision*

Relative standard deviation is 5% and the between-day imprecision 15.7% at the level of 0.25 μ mol/L and 7.2% at the level of 4.0 μ mol/L.

iii) *Detectability*

The detectability (signal to noise ratio 2) is 0.07 μ mol/L.

(f) Quality assurance

Before each analytical run, a blank, and the two different internal quality control specimens are analysed. A control specimen is analysed after every 10 specimens. The external quality control scheme of the German Society of Occupational Medicine includes urinary aluminium (one sample annually).

(g) Sources of possible errors

i) Preanalytical

The analysis of aluminium is extremely prone to contamination. Absence of contamination has to be verified by repeated analyses of blanks in each series. Aluminium phosphate tends to precipitate in urine, therefore the specimen has to be mixed thoroughly before sampling.

ii) Analytical

Because of the varying composition of the urine matrix, the method of standard addition is used.

(h) Reference to the most comprehensive description of the method

This method has not been published earlier but is very similar to that of Schaller and co-workers (42).

1.1.7.4 Other analytical methods

Other graphite furnace AAS methods for the analysis of aluminium in the urine have been published. D'Haese et al. used standard addition, and added acetic acid to the specimens, which they found to improve the precision of the method, probably by dissolving the aluminium phosphate that is only slightly soluble in urine (48). Magnesium nitrate (49–51), Triton X-100 (52); magnesium nitrate together with Triton X-100 (53) and nitric acid alone (42) have been used as matrix modifiers in the analysis of urinary aluminium. Bradley and Leung recently reported that use of the transversely heated graphite atomiser allowed use of a low atomization temperature (2200°C) which resulted in an improved analytical throughput (51). Analytical methods for aluminium in blood, serum, tissues, and urine have recently been reviewed (47).

1.1.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

From the published studies it appears that the upper reference limit for the urinary aluminium in occupationally non-exposed people is approximately 0.6 $\mu\text{mol/L}$ (table 1.1.3).

Table 1.1.3. Measured values in groups without occupational exposure ($\mu\text{mol/L}$)

n	Median/Mean (SD)	97.5 Percentile/Range	Reference
78	0.15/NR	NR	54
766	NR/0.40	0.72/NR	55
63	NR/0.24 (0.13)	0.50/NR	52
8	NR/0.25	NR/0.19–0.34	49
44	NR/0.33	NR/0.07–0.82	56

NR = not reported

(b) Published biological action levels

The German BAT value (based on the occupational exposure limit) for urinary aluminium is 200 $\mu\text{g/L}$ (7.4 $\mu\text{mol/L}$) (not corrected to relative density) in an after shift specimen (57). Based on toxicity data of aluminium, the Finnish Institute of Occupational Health has recommended 6.0 $\mu\text{mol/L}$ (corrected to a relative density of 1.024) as the biological action level in a specimen collected in the morning after two days without exposure (58).

(c) Non-analytical interferences

- i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

High concentrations (in excess of 10 $\mu\text{mol/L}$) of aluminium may be observed in the urine after ingestion of aluminium-containing antacid drugs.

- ii) Diet and the environment

Acid food cooked using utensils containing aluminium may in exceptional cases increase the urinary aluminium concentration to levels exceeding 2.5 $\mu\text{mol/L}$ (56).

(d) Sampling representative of recent or long-term exposure

The urinary aluminium content is affected both by short- and long-term exposure. Samples collected immediately after the exposure are most closely related to actual exposure, especially in workers with relatively short work history. The concentration of aluminium in specimens collected in the morning after the weekend shows closest relationship with the body burden of aluminium, but is also affected by exposure of the preceding days.

(e) Ethnic differences

None are known.

1.1.8 Aluminium in serum

1.1.8.1 Toxicokinetics

After exposure to aluminium-containing welding fumes, the elevated serum aluminium concentrations showed a rapid decrease (approximate half-time 16h) (43). However, even long-term exposure has an effect on the level of aluminium in the serum (44).

1.1.8.2 Biological sampling

(a) Sampling time

After the working day or shift.

(b) Specimen

Blood specimen is allowed to clot at room temperature, and serum is separated by centrifugation.

(c) Contamination possibilities

The risk of contamination is very marked. Heparin and citrate may contain aluminium (48, 59). Several brands of commercial syringes, blood collection tubes, and catheters leached appreciable amounts of aluminium in dilute nitric acid (60, 61).

(d) Sampling device and container

Whole blood is collected in acid-washed glass tubes, allowed to clot, and serum is transferred using an acid washed Pasteur pipette into acid washed plastic tubes for transportation.

(e) Anticoagulant

Several anticoagulants have been shown to contain aluminium; therefore, serum is preferred over plasma for aluminium analysis.

(f) Preservative

None.

(g) Shipment

At room temperature. Shipment just before a weekend should be avoided; rather, the samples should be kept refrigerated and sent after the weekend.

(h) Stability

Serum is stable for several days at room temperature; it can be stored in refrigerator for 1–2 weeks. For longer storage, freezing at -20°C is recommended.

1.1.8.3 Recommended analytical methods

The method described below has been used in the authors' laboratory and follows the guidelines of the manufacturer of the instrument.

(a) Principles of the method

Serum is diluted in nitric acid, and the aluminium concentration determined using electrothermal atomic absorption with Zeeman background correction.

(b) Reagents required

Nitric acid (suprapur); Triton X-100 (for autosampler flushing only); aluminium standard solution (e.g. Spectrosol, BDH); 18 M Ω water.

(c) Equipment required

Graphite furnace atomic absorption spectrometer with Zeeman background correction; pyrolytically coated tubes, platform technique. HEPA-filtered air flow hood. All lab ware used is washed with 10% suprapur nitric acid, and rinsed several times with 18 M Ω water.

(d) Procedures

Standard curve, prepared in serum from non-exposed people, with 5 points (0.4–2 $\mu\text{mol/L}$) is used in the quantitation. Sera with high aluminium concentrations are reanalysed using a standard curve covering the concentration range 0.9–7.4 $\mu\text{mol/L}$. Nitric acid concentration in the standards and samples is adjusted to 0.05%. The analysis is performed in triplicate.

Details of the programme of the instrument are given in table 1.1.4 but have to be verified for each instrument, and may vary with the condition of the tube.

Table 1.1.4. Details of the programme of the instrument

Step	Temp °C	Ramp	Hold	Gas flow
Dry	140	1	20	300
Dry	160	40	1	300
Char	550	5	20	300
Char	1550	1	20	300
Stabilise	20	1	5	0
Atomise	2500	0	3	0
Clean	2700	1	5	300

(e) Analytical reliability

i) Trueness

During one year, 38 specimens from the external quality assurance scheme of the Robens Institute (Guildford, UK), were analysed. The results averaged 100.5% (SD 12.8) of the target value; the target concentrations varied between 0.3 and 2.38 $\mu\text{mol/L}$.

ii) Precision

Relative standard deviation (RSD) is 3%, and day-to-day RSD is 6.0% at a level of 4.0 $\mu\text{mol/L}$.

iii) Detectability

The detectability was (signal to noise ratio 2) 0.02 $\mu\text{mol/L}$.

(f) Quality assurance

In each analytical series, a reference sample (e.g. Seronorm Trace Elements Serum (Nycomed, Oslo, Norway) or Lyphecheck (BioRad ECS Division, California)) is analysed. External quality assurance schemes exist for serum aluminium analysis (The Robens Institute of Health and Safety, UK, The German Society of Occupational Medicine, Centre de Toxicologie du Québec).

i) Special precautions

Contamination is the main problem of the serum aluminium analysis. All sample preparation is done in HEPA-filtered air hood. Every analytical series is begun with the analysis of a reagent blank.

ii) Interferences

The molecular absorption is not a major problem. The use of nitric acid alleviates the problem of the volatility of aluminium chloride (47).

(g) Sources of possible errors

The analysis is extremely vulnerable to contamination from the airborne dust. Heparin and citrate may contain enough aluminium to disturb the analysis at levels close to the range observed in non-exposed persons (59).

(h) Reference to the most comprehensive description of the method

This method has not been published in detail, but is similar to that of D'Haese and co-workers with the exception that nitric acid was used as the diluent instead of water (48). With the exception of the use of external standardization, the method is similar to that used for the analysis of aluminium in the urine.

1.1.8.4 Other analytical methods

Several other graphite furnace atomic absorption methods have been published using water dilution (48), nitric acid + Triton X-100, (20, 59), magnesium nitrate (59, 62), magnesium nitrate plus nitric acid (63), magnesium nitrate and Triton X-100 (49–51,

53), Triton X-100 alone (52, 64), ammonium hydroxide + sulphuric acid (65) or potassium dichromate as matrix modifiers (66). Inductively coupled plasma-atomic emission spectrometric methods have been developed (67–69), but do not seem to be sensitive enough for the measurement of serum aluminium at the levels observed in healthy persons (47, 69). Two laboratories participating in the Québec external quality assurance scheme report that they use inductively coupled plasma-mass spectrometry; no details on the methods are available (70). Methods for serum aluminium analysis have been reviewed (47).

1.1.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Table 1.1.5. Measured values in groups without occupational exposure ($\mu\text{mol/L}$)

n	Mean $\pm$ SD	97.5 Percentile/Range	Ref.
46	0.27	NR / 0.07–0.56	64
28	0.24 $\pm$ 0.15	NR / 0.07–0.52	50
10	0.074 $\pm$ 0.015	NR / NR	48
916	0.22	0.18 / 0.04–0.40	55
22	0.044 $\pm$ 0.030	NR / NR	59
63	0.06 $\pm$ 0.05	0.28 / NR	52
13	0.27 $\pm$ 0.013	NR / 0.18–0.32	71
164	0.25 $\pm$ 0.17	0.65	49
59	0.099 <i>a</i>	0.175 <i>b,c</i>	19
172	0.16 $\pm$ 0.11	NR / 0.01–0.63	20
21	0.06 $\pm$ 0.025	0.1 <i>b</i>	56

NR = Not reported; *a* = Geometric mean; *b* = 95 percentile; *c* = Outliers and antacid-users excluded.

Although several recent studies report rather high average values, and especially maximal values, it seems that in occupationally non-exposed people that do not consume antacid drugs the serum aluminium concentration does not exceed 0.2 $\mu\text{mol/L}$ (table 1.1.5) (47).

(b) Published biological action levels

No action levels for occupational exposure have been proposed. There are, however, guidelines for the serum aluminium concentrations of patients on continuous dialysis therapy (72).

(c) *Non-analytical interferences*

i) Diet and the environment

Elevated concentrations of aluminium may be observed in the serum after ingestion of aluminium-containing antacid drugs. Slightly elevated serum aluminium concentrations may be caused by dietary aluminium.

(d) *Sampling representative of recent or long-term exposure*

Both short-term and long-term exposure affect the concentration of aluminium in the serum.

(e) *Ethnic differences*

None are known.

1.1.9 Aluminium in bone and hair

The aluminium content in bone has been measured in vivo using neutron activation analysis. However, the method, although capable of measuring aluminium in bone of dialysis patients, is not sensitive enough for the biological monitoring of occupational exposure (45). Aluminium in hair has also been measured in dialysis patients, but it was concluded that this analysis is of no value as an indicator of body aluminium accumulation (73).

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1.2 Arsenic

1.2.1 Introduction¹

Biological monitoring of arsenic exposure has long traditions; it is probably the only chemical for which data exist on the relationship between the results of biological monitoring, and cancer risk (1, 2). Biological monitoring of arsenic is also rather unique in that it is possible and necessary to perform the analysis in a compound-specific way – analysis of total arsenic – as is done for other trace elements, may give rise to misleading results. Toxicity, kinetics, metabolism and biological monitoring of arsenic have been reviewed (3–10).

1.2.2 Physical-chemical properties (3–5, 7, 8, 11)

Arsenic (CAS no 7440-38-2, relative density 5.7, melting point 817°C (28 atm), boiling point (sublimation) 613°C, insoluble in water) is a metalloid, i.e., exhibits both metallic and non-metallic properties. It occurs mainly in valence states -3, 0, +3, and +5. In nature, arsenic occurs in some 150, primarily sulphidic, minerals, such as arsenopyrite (FeAsS), realgar (As₄S₄), and auripigment or orpiment (As₂S₃) but also as arsenic trioxide (arsenolite) and lead arsenate (sultenite). Arsine may be produced, when arsenic-containing materials are in contact with nascent hydrogen (e.g. zinc + sulphuric acid). Several marine organisms extract arsenic from the water and methylate it to dimethylarsinate, arsenobetaine, arsenocholine, or different arsenosugars.

From the point of view of occupational exposure, the most important arsenic compounds are arsenic trioxide, arsenic pentoxide, arsenic sulphide, gallium arsenide as well as arsine. Dietary exposure comprises methylated arsenic compounds, such as arsenobetaine, arsenocholine, as well as dimethylarsinate (crustaceans and fish) and a variety of arsenosugars (seaweeds).

Arsenic trioxide (As₂O₃ or As₄O₆; CAS 1327-53-3), is soluble in cold water (12–37 g/L, depending on the crystalline structure), and even more readily soluble in hot water, as well as in alkali and hydrochloric acid. In an aqueous solution arsenic trioxide forms ortho- or meta-arsenite (As₃³⁻ or AsO₂⁻). Arsenate (AsO₄³⁻, HAsO₄²⁻, or H₂AsO₄⁻) may be generated from arsenite in water through oxidation at pH >7.

Arsenic pentoxide (As₂O₅; CAS 1303-28-2) is readily soluble (1500 g/L) in cold water, acid and alkali.

¹ Abbreviations used: As³⁺ = Trivalent inorganic species; As⁵⁺ = Pentavalent inorganic arsenic species; Hydride generating arsenic species = As³⁺, As⁵⁺, Monomethylarsonate (MMA), and Dimethylarsinate (DMA)

Arsenic trisulphide (As_2S_3 ; CAS 1303-33-9) is very sparingly soluble in water, but is soluble in acid and alkali.

Gallium arsenide (GaAs ; CAS 1303-00-0) is sparingly soluble in distilled water, but 100–200 mg As/L will dissolve in a phosphate buffer pH 7.

Arsine (hydrogen arsenide, AsH_3 ; CAS 7784-42-1) is a colourless, inflammable gas with a slight garlic odour.

Arsenobetaine ($((\text{CH}_3)_3\text{As}^-\text{CH}_2\text{COO}^-)$, arsenocholine ($((\text{CH}_3)_3\text{As}^+\text{CH}_2\text{CH}_2\text{OH X}^-)$, as well as dimethylarsinate ($((\text{CH}_3)_2\text{AsO}(\text{OH}))$ and methylarsonate ($(\text{CH}_3\text{AsO}(\text{OH})_2)$ are readily soluble in water.

Conversion factors (Arsenic, As): 1 mol = 74.92 g; 1 mg = 13.34 μmol

1.2.3 Possible occupational and non-occupational exposures

Greatest worker exposure to arsenic (mainly arsenic trioxide) occurs in the smelting of non-ferrous metals, in which arseniferous ores are commonly used (3, 4, 12–20). Arsenic exposure may also occur in arsenic refining and production of arsenic containing insecticides and other chemicals (3, 4, 18–22), timber treatment with copper-chromium-arsenic preservatives (20, 22–24), in semiconductor and electronics industry (17, 20, 22), production of sulphuric acid (25), and in the glass manufacturing industry (20, 22). Elevated urinary arsenic concentrations have also been reported among people living in the vicinity of copper smelters (26–28). Use of arsenic containing pesticides also poses the hazard of exposure to arsenic (29).

In some areas in the world, drinking water may contain large amounts of arsenic: concentrations in excess of 0.5 mg/L have been reported in some areas in Japan, New Zealand, Argentina, Taiwan, California, Romania, and Russia (5). Concentrations up to 200 $\mu\text{g/L}$ have been reported in some mineral waters (30).

Some marine species contain large amounts of arsenic, up to 300 mg/kg wet weight. In fish and crustaceans, this is mostly arsenobetaine and tetramethylarsonium ion, but also arsenocholine, and trimethylarsine oxide (31). In some species also dimethylarsinate and inorganic arsenic, with usually a smaller share of monomethylarsonate have been detected (7, 20, 32–34). Seaweeds that are an important part of the diet, e.g. in Japan, contain large amounts of different arsenosugars (31, 35–41).

1.2.4 Summary of toxicokinetics

1.2.4.1 Absorption

(a) Inhalation

Arsenic compounds are absorbed in inhalation exposure; the compounds that are soluble in body fluids (e.g. arsenic trioxide) are cleared rapidly from the lungs, whereas com-

pounds with limited solubility (calcium arsenate, lead arsenate, gallium arsenide, arsenic sulphide) are retained in the lungs (7, 8).

(b) Dermal

The dermal absorption of arsenic compounds has not been fully characterized, but cases have been published, in which systemic effects of arsenic have been described after dermal exposure (5). In Rhesus monkeys, 2–5% of a trace amount of H_3AsO_4 was absorbed through a 12 cm^2 area of abdominal skin in 24 hours, as judged from the urinary excretion of arsenic (42).

(c) Gastrointestinal

Arsenic trioxide is effectively (>90%) absorbed from the gastrointestinal tract, while the less soluble arsenic compounds (arsenic trisulfide, gallium arsenide, lead arsenate) are absorbed only partially (<30%) (7).

1.2.4.2 Metabolic pathways and biochemical interactions

Trivalent inorganic arsenic is oxidized in the body to pentavalent arsenic; also the reverse reaction takes place. Inorganic arsenic, probably after reduction to trivalent state, is methylated in the human body to yield methylarsonate, and dimethylarsinate. After exposure to arsenic trioxide, the two metabolites comprise approximately 80–90% of the arsenic excreted in the urine. Although the methylation reaction is saturable at very high exposure levels, the proportions of different arsenic metabolites are rather similar in occupationally non-exposed, and in people exposed at work (5, 7, 9, 19).

Methylarsonate is further methylated to a limited extent, dimethylarsinate is mostly excreted unchanged (a few per cent may be transformed into trimethylarsinic oxide, 43). Arsenobetaine and arsenocholine are excreted unchanged (7, 9, 44, 45), while arsenosugars are in part converted to dimethylarsinate and As^{5+} (41).

1.2.4.3 Distribution

Inorganic arsenic is accumulated in keratinous tissues in the body, that is, skin, hair, and nails. Relatively high concentrations have also been observed in the lungs (8).

1.2.4.4 Elimination

Once absorbed, arsenic is mostly excreted in the urine. Small amounts are excreted in keratinized formations, such as hair, nails, and dying epidermal cells. After exposure to inorganic arsenic, inorganic pentavalent and trivalent arsenic compounds are excreted first, followed by monomethylarsonate and thereafter by dimethylarsinate. After oral administration of a tracer amount of ^{74}As as arsenic acid, whole body counting revealed three consecutive half-times of the disappearance of arsenic: 2.09d (65.%), 9.5 d (30.4%), and 38.4 d (3.7%) (46).

The biological half-time of arsenobetaine and arsenocholine is less than 20 h (5, 47).

1.2.5 Summary of toxic effects

The toxicity of inorganic arsenic compounds depends on the valence state: Arsine is most toxic, As^{3+} intermediate, and As^{5+} least toxic. Monomethylarsenate and dimethylarsinate are even less toxic, and the LD_{50} values of arsenobetaine and arsenocholine are $> 5\text{g/kg}$ (4).

Humans are more sensitive to arsenic trioxide than several animal species; lethal dose is approximately 1–2 mg/kg orally. Ingestion of large doses of arsenic leads to abdominal colics, vomiting and diarrhoea, eventually followed by vascular shock and death. Dermal exposure gives rise to local irritation; and inhalation exposure may lead to nasal septal perforations. Systemic arsenic gives rise to increased skin pigmentation and palmo-plantar keratosis. Long-term oral exposure to arsenic has led to vascular damage, Raynaud-phenomen and black-foot disease. Arsenic exposure may also affect the central and peripheral nervous systems, bone marrow function, as well as liver and kidney function. Inhalation exposure to arsenic trioxide increases the risk of lung cancer, and oral exposure may lead to skin cancer (5, 6, 8). There are also data that suggest arsenic as a cause of liver, kidney and bladder cancer (48).

Arsine has caused a large number of fatal intoxications. The symptoms include nausea, abdominal colics, vomiting and shortness of breath. The cause of death is usually extensive haemolysis, which leads to renal failure because of blocking by haemoglobin casts of renal tubuli, and jaundice. In non-fatal cases, residual kidney dysfunction, liver dysfunction, and polyneuritis may occur (5).

1.2.6 Biological monitoring indices

Table 1.2.1. *Biological monitoring of arsenic*

Indicator	Suitability	Reference
Inorganic arsenic (As^{3+} and As^{5+}) in urine	Specific; fairly sensitive	18, 25
Hydride generating arsenic compounds ^a in urine	Sensitive; limited specificity	13, 16, 18, 25, 44, 47, 49
Total arsenic in urine	Unspecific; fairly sensitive	1, 2, 13, 16, 18, 20, 25, 31, 41, 44, 47, 49, 50
Arsenic in hair	Not useful	7, 51–55

a = As^{3+} , As^{5+} , monomethylarsonate and dimethylarsinate

In people eating Western-type diets, analysis of inorganic arsenic seems to be the optimal approach for the biological monitoring of arsenic. When the diet does not contain dimethylarsinate-containing fish species, the best approach probably is the hydride-generating arsenic species. When the diet contains large amounts of inorganic arsenic or arsenosugars, the choice between inorganic arsenic and hydride generating arsenic must

be made based on levels of urinary arsenic compounds observed in the local non-occupationally exposed populations.

1.2.7 Inorganic arsenic in urine

1.2.7.1 Toxicokinetics

Inorganic arsenic in the urine decreases after the exposure with a half-time of approximately 10–24 h (18, 44, 56).

1.2.7.2 Biological sampling

(a) Sampling time

After the workday.

(b) Specimen

Spot sample.

(c) Contamination possibilities

As the unchanged chemical is measured, dust from the workplace constitutes a major contamination hazard. The specimen has to be collected outside the working area, and after a shower, and changing into street clothes.

(d) Sampling device and container

Sampling directly in a clean, acid-washed 50 mL polyethylene bottle.

(e) Preservative

No preservative, but the bottle should be completely filled to prevent oxidation of As^{3+} to As^{5+} from the air in the bottle.

(f) Shipment

At room temperature. Shipment just before a weekend should be avoided; rather, the samples should be kept refrigerated and sent after the weekend.

(g) Stability

The sample is stable at room temperature at least for one week, and at -20°C for more than 6 months.

1.2.7.3 Recommended analytical methods

(a) Principles of the method

Different arsenic compounds are separated by ion-pair liquid chromatography, and inorganic tri- and pentavalent arsenic are quantitated through hydride-generation atomic absorption spectrometry.

(b) Reagents required

Standards. Arsenic trioxide, sodium arsenate, sodium methylarsonate, and sodium dimethylarsinate are separately dissolved in 50 mL of 0.1 mol/L sodium hydroxide and diluted to 1L with water to give stock solutions of the standards (1000 mg As/L). The working standards (0, 0.17, 0.33, 0.67, and 1.3 $\mu\text{mol/L}$ of each arsenic compounds), are prepared daily by diluting in water.

HPLC mobile phase. Stock solution: Tetrabutylammonium hydroxide (final concentration, 0.1 mol/L) and $\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}$ (final concentration, 0.2 mol/L) are dissolved in water and the pH is adjusted to 6.0 with phosphoric acid. This solution is used to adjust the pH and ion concentration of the samples for the HPLC separation, and to prepare the working mobile phase by diluting 1+9 with water. If methylarsonate and dimethylarsinate are analysed, in addition to As^{3+} and As^{5+} , the pH of the working mobile phase is fine-adjusted daily by phosphoric acid or sodium hydroxide to give baseline separation of the methylated arsenic standards. The mobile phase is filtered before use.

For hydride generation: a 1.5% m/v solution of sodium tetrahydroborate (III), stabilized with 0.7 % m/v sodium hydroxide, is prepared daily.

Other chemicals used include 1.5 mol/L hydrochloric acid (for hydride generation), methanol for preconditioning the disposable C_{18} -cartridges, and argon to be used as the purge gas.

(c) Equipment required

Single-use C_{18} -type Sep-Pak Cartridge columns.

For the separation of arsenic compounds a high-pressure solvent pump, a syringe injection valve, and a system of two C_{18} reversed phase columns in series (10 cm x 3 mm i.d., 5 μm), with a guard column (1 cm x 2.1 mm i.d.) dry packed with 30–40 μm C-18 resin) are used.

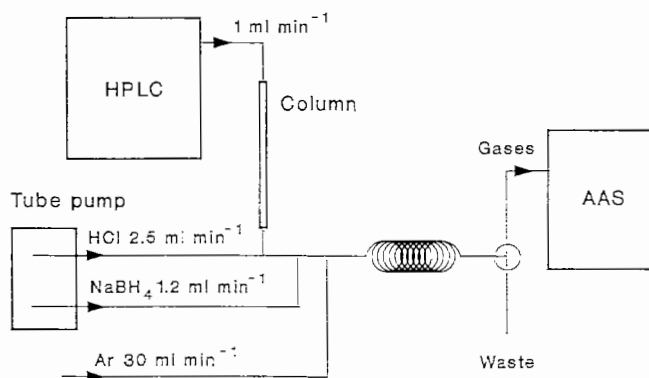


Figure 1.2.1. Diagram of the flow system for the generation and analysis of hydrides of different arsenic compounds by HPLC-HGAAS (21).

The column outlet is connected (all tubing is made from polytetrafluoroethylene) to the hydrochloric acid line of a multichannel tube pump, which pumps the reagent solutions (HCl and NaBH₄) and the purge gas (argon) into a glass reaction coil (150 cm x 2.2 mm i.d.). The gaseous hydrides generated are separated from the waste liquid in a gas-liquid separator, atomised in an air-acetylene-heated quartz atomization tube, and quantitated by an atomic absorption spectrometer equipped with an electrodeless discharge lamp for arsenic (figure 1.2.1).

(d) Procedures

Twelve mL of urine samples and reference standard solutions are passed through the C₁₈-type cartridge columns. The last 2 mL are collected and 0.1 mL of the stock mobile phase is added to an aliquot of 0.9 mL to match the ionic composition of the samples with the mobile phase. Injections into the 50 µL loop of HPLC are made through a 0.45 µm on-line filter. From the chromatograms obtained the concentrations of arsenic compounds are calculated by using the peak heights. Finally, the results are corrected to a relative density of 1.024.

(e) Analytical reliability

i) Trueness

The recovery for As³⁺ was between $93.8 \pm 0.5\%$ (at 12.5 µg/L) and $90.1 \pm 1.0\%$ (at 100 µg/L), and between 93.6 ± 1.9 and $94.9 \pm 2.4\%$ for As⁵⁺, respectively. The yield in the analysis of commercial reference materials was: 102% (Seronom Trace Elements, human urine), 83% (Lanonorm Metals 1, synthetic urine), and 90% (Lyphocheck Control Urine 1 human urine). Baseline separation of As³⁺, As⁵⁺, methylarsonate, and dimethylarsinate was achieved.

ii) Precision

Repeatability 3% (relative standard deviation) or better at average levels of 12.5, 25, 50, and 100 µg/L for both As³⁺ and As⁵⁺.

iii) Detectability

When using an injection volume of 50 µL, the detectability (signal to noise ratio >2) was 1.0 and 1.6 µg/L for As³⁺ and As⁵⁺, respectively.

(f) Quality assurance

Commercial urine specimens for internal quality assurance are available (Seronom/Nycomed, Norway, BioRad LyphoCheck, California, USA). No external quality assurance scheme reports separately the concentrations of inorganic arsenic compounds only.

(g) Sources of possible errors

As indicated above, contamination is a distinct possibility, since As³⁺ which is usually the chemical at the workplace, is measured.

(h) *Reference to the most comprehensive description of the method*

Hakala E, Pyy L. Selective determination of toxicologically important arsenic compounds in urine by high-performance liquid chromatography-hydride generation atomic absorption spectrometry. *Journal of Analytical Atomic Spectrometry* 1992;7:191–6 (21).

1.2.7.4 Other analytical methods

Another ion-pair liquid chromatography-hydride generation AAS method, using heptane sulfonate as the pairing agent, has recently been published (57). Braman and Foreback (58) described a method to speciate arsenic in environmental samples, and also analysed some urine specimens. Their method was based on pH selective reduction of various arsenic forms, and separation of the volatile arsines from a cold trap. This method was further developed (59) and has been used for the biological monitoring of occupational arsenic exposure (13, 25). Ion-exchange chromatographic separation coupled with hydride generation AAS has also been used to analyse the inorganic arsenic compounds in the urine (20, 22, 60, 61). Methods employing ion chromatography or HPLC separation and inductively coupled plasma mass spectrometric detection have also been described (57, 62), but do not seem to be superior to the much less expensive HPLC-hydride generation AAS (57).

1.2.7.5 Guide to interpretation

(a) *Measured values in groups without occupational exposure*

Table 1.2.2. *Measured values in groups without occupational exposure*

n	Median/Mean $\pm$ SD		Range	Ref.
	$\mu\text{g/L}$	nmol/L		
8	1.2 ± 1.1	16 ± 15		47
14	NR/1	NR/13		61
40			$< 1.5 \mu\text{g/g creat}$ ($< 2.2 \mu\text{mol/mol creat}$)	22
148	$\text{NR}/1.9 \pm 1.2$	$\text{NR}/25 \pm 16$	$0.5\text{--}10 \mu\text{g/L}$ ($7\text{--}13 \text{ nmol/L}$)	60
11			$< 2.6 \mu\text{g/L}$ (35 nmol/L)	21
195 ^a	$1.2/1.6 \pm 1.6$	$16/21 \pm 21$		27
208 ^b	$1.0/1.1 \pm 0.8$	$13/15 \pm 11$		27
41	$\text{NR}/2.6 \pm 2.2$	$\text{NR}/35 = 29$		13
102	$\text{NR}/12.7 \pm 7.08$	$\text{NR}/170 \pm 95$	$2.62\text{--}22.7 \mu\text{g/L}$ ($35\text{--}303 \text{ nmol/L}$)	33
102	$\text{NR}/11.4 \pm 5.85$	$\text{NR}/152 \pm 78$		17
18	$\text{NR}/10.7 \pm 6.59$	$\text{NR}/143 \pm 88$		17

^a = males; ^b = females; NR = not reported

(b) Basis for biological action levels

It was estimated (18) that 8-h TWA exposure to $10\mu\text{g}/\text{m}^3$ arsenic trioxide leads to an arsenic concentration in after-shift urine of $0.07\mu\text{mol}/\text{L}$ ($5\mu\text{g}/\text{L}$). Another study came to a very similar conclusion: air-borne concentrations of 12.5, 25, and $50\mu\text{g}/\text{m}^3$ were estimated to lead to urinary arsenic concentrations of 8, 10, and $13\mu\text{g}/\text{g}$ creatinine, in an after-shift specimen (25).

(c) Published biological action levels

The Finnish Institute of Occupational Health has issued an occupational exposure limit-based biomonitoring action level of $0.07\mu\text{mol}/\text{L}$ for the sum of As^{3+} and As^{5+} in an after-shift specimen (63).

(d) Non-analytical interferences

- i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

At usual levels of occupational exposure, interferences by other chemicals with arsenic metabolism or disposition are not expected to occur.

- ii) Diet and environment

The method is insensitive to the arsenic compounds in fish and crustaceans, arsenobetaine and arsenocholine, and dimethylarsinate. However, some seaweeds may contain appreciable amounts of arsenite and arsenate, which would interfere with the interpretation.

(e) Sampling representative of recent or long-term exposure

As the half-time of inorganic arsenic in the urine after occupational exposure to arsenic trioxide is approx 12–24 h (18, 44), the sampling represents exposure over a few last days.

(f) Ethnic differences

No ethnic differences are known.

1.2.8 Arsine generating arsenic compounds in urine

In acid conditions, inorganic arsenic³⁺, arsenic⁵⁺ and monomethylarsonate and dimethylarsinate may be reduced by borohydride to volatile arsine derivatives, and analysed as a group. After occupational exposure, 80–90% of the arsenic absorbed is excreted in methylated form, mainly as dimethylarsinate.

1.2.8.1 Toxicokinetics

After oral administration of a small dose of arsenite, the half-time of the arsenic concentration in the urine was 30–60h. In the beginning most of the excreted arsenic was inorganic, but after about 8 hours, dimethylarsinate predominated (47, 64).

1.2.8.2 Biological sampling

(a) *Sampling time*

After the working day towards the end of the week.

(b) *Specimen*

Spot urine specimen.

(c) *Contamination possibilities*

Arsenic-containing dust from the workplace may contaminate the specimen. However, when a method separating different arsenic hydrides is used, contamination would be apparent from the result as an overrepresentation of AsH_3 (in comparison to the hydride from dimethylarsinate).

(d) *Sampling device and container*

Sampling directly in a clean, acid-washed polyethylene bottle.

(e) *Preservative*

No preservative is required, but acidification may be used.

(f) *Shipment*

At room temperature. Shipment just before a weekend should be avoided; rather, the samples should be kept refrigerated and sent after the weekend.

(g) *Stability*

The sample is stable at room temperature at least for one week, and at -20°C for more than 6 months.

1.2.8.3 Recommended analytical methods

The same method described for the analysis of inorganic arsenic in the urine, can be applied for the analysis of the sum of inorganic arsenic, and monomethylarsonate and dimethylarsinate. The pH of the mobile phase in the liquid chromatography has to be fine-adjusted daily by phosphoric acid or sodium hydroxide to give baseline separation of the methylated arsenic standards. The precision, trueness, and detectability are very similar to those reported for As^{3+} and As^{5+} also for the mono- and dimethylated arsenic compounds.

1.2.8.4 Other analytical methods

All other methods described above for the analysis of inorganic arsenic, can also be applied for the analysis of the sum of inorganic plus mono- and dimethylated arsenic in the urine (13, 20, 22, 25, 57–62).

In addition to the methods, which measure separately the individual arsenic compounds, methods have been described that reduce arsenic³⁺, arsenic⁵⁺, monomethylarsonate, and dimethylarsinate, to their arsine derivatives, and measure these hydrides together (47, 49, 50, 65, 66). Until recently, however, it did not seem possible to generate conditions, in which the response was identical to all the different arsenic compounds, and the trueness achieved thus depended on how well the composition of the specimen and the standard used, could be matched (50, 66–68). However, Le and coworkers have recently published a variation of the method, where addition of cysteine seems to alleviate this problem, and which was reported to give good trueness (recovery not less than 96% ± 6%) for all hydride forming arsenic compounds (68).

A cation exchange separation of As³⁺, As⁵⁺, and mono- and dimethylarsenic compounds from arsenobetaine and arsenocholine, followed by graphite furnace atomic absorption analysis, has also been described (69).

1.2.8.5 Guide to interpretation

(a) *Measured values in groups without occupational exposure*

Table 1.2.3. *Measured values in groups without occupational exposure*

n	Median/Mean ± SD (geometric mean)		Range	Ref.
	µg/L	nmol/L		
8	NR/12 ± 7	NR/160 ± 90		47
40	NR/(4.4) ^f	NR/(6.6) ^g		22
148	NR/5.9 ± 2.9	NR/79 ± 39	2–21 µg/L (27–280 nmol/L)	60
11	NR/8.7 ± 4.5	NR/116 ± 60	3.4–18.9 µg/L (45–252 nmol/L)	21
149 ^a		NR/110 ± 130 (70)		70
195 ^b	7.9/10.2 ± 10.1	105/136 ± 135		27
208 ^c	6.4/8.4 ± 6.6	85/112 ± 88		27
70	5.5/NR	73/NR	<29 µg/L (<390 nmol/L)	49
41	NR/17.5	NR/234		13
49 ^d	7.9/12.4 ± 11.3	105/166 ± 151	2.3–52.0 µg/L (31–694 nmol/L)	71
50 ^e	7.4/9.7 ± 7.3	99/130 ± 97	1.7–40.3 µg/L (23–538 nmol/L)	71
300		NR/0.11 ± 0.11	0.35 µg/L ^h (4.7 nmol/L) ^h	50
102	NR/49.7	NR/663		33
102	NR/50.1 ± 24.2	NR/669 ± 323		17

a = 10 weeks pregnant women; b = males; c = females; d = residents of Stockholm; e = residents of Västerås; f = µg/g creatinine; g = µmol/mol creatinine; h = 95th percentile; NR = Not reported.

(b) Basis for biological action levels

A relationship has been observed in several studies between exposure to arsenic trioxide, and urinary concentration of volatile-hydride-forming arsenic compounds. However, this relationship has been different in different studies. Vahter and coworkers (16) estimated that 8-TWA exposure to $50 \mu\text{g}/\text{m}^3 \text{As}_2\text{O}_3$ leads to an arsenic concentration of $190 \mu\text{g}/\text{L}$ ($2.53 \mu\text{mol}/\text{L}$) urine in an after-shift specimen. A considerably lower ($< 100 \mu\text{g}/\text{L}$) [$< 1.33 \mu\text{mol}/\text{L}$] urinary arsenic concentration can be approximated from the study of Smith and coworkers (13). In the study of Offergelt and coworkers (25) the corresponding figure was $55 \mu\text{g}/\text{g}$ creatinine, and in the study of Hakala and coworkers (18), $47 \mu\text{g}/\text{L}$ ($0.63 \mu\text{mol}/\text{L}$). The relationship between exposure and the urinary concentration of inorganic arsenic was closer than that between exposure and total volatile-hydride-forming arsenicals (18, 25).

(c) Published biological action levels

The German Senate Commission has published EKA values for these arsenic compounds of $50, 90, \text{ and } 130 \mu\text{g}/\text{L}$ ($0.67, 1.2, 1.7 \mu\text{mol}/\text{L}$) that are purported to correspond to exposures of $10, 50, \text{ and } 100 \mu\text{g}/\text{m}^3$ of arsenic trioxide (63). The BEI value of ACGIH for arsenic (72) is $50 \mu\text{g}/\text{g}$ in a specimen collected at the end of a workweek.

(d) Non-analytical interferences

- i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

At usual levels of occupational exposure, interferences by other chemicals with arsenic metabolism or disposition are not expected to occur.

- ii) Diet and environment

The main dietary arsenic compounds, arsenobetaine and arsenocholine, are not analysed in this assay. However, dimethylarsinate, which is present in some fish species and may be generated in humans from arsenosugars in seaweeds, may lead into erroneous assessment of occupational exposure.

(e) Sampling representative of recent or long-term exposure

As the half-time of inorganic arsenic in the urine after occupational exposure to arsenic trioxide is approximately 30–60 h (44, 47), the sampling represents exposure over a few last days.

(f) Ethnic differences

No ethnic differences are known in the methylation or disposition of arsenic.

1.2.9 Total arsenic in urine

Total arsenic in urine has been used extensively in the biological monitoring of arsenic in the past. Urinary arsenic values have been shown to be related to the risk of lung cancer in smelter workers (1, 2). However, because of the very large contribution of

trimethylarsenic from dietary sources to the total urinary arsenic, this approach can only be applied, when it is known that the diet does not contain seafood.

1.2.10 Arsenic in hair and nails

Arsenic is concentrated in keratinous tissues, such as hair, and on a group level, hair arsenic concentrations have been reported to be related to dietary arsenic intake (51, 52). In case reports, the concentrations of arsenic in nail and hair have been shown to reflect changes in dietary exposure to inorganic arsenic (53, 54). However, separating the arsenic that was directly deposited from the air – or from shampoos, hair or nail colouring chemicals, etc. – from the arsenic that came from the circulation is practically impossible. Therefore, use of analysis of hair and nail arsenic is limited to follow-up of cases with dietary exposure (7, 55).

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1.3 Cobalt

1.3.1 Introduction

Cobalt exposure is widespread in mining and refining, numerous industrial settings, and in metal carbide production and usage. The general public ingests cobalt daily and generally maintains body balance. Cobalt is essential for humans, occurring in vitamin B₁₂. The acute toxicity of cobalt is low; adverse effects from occupational exposure are mainly skin sensitization from dermal exposure and pulmonary disorders arising from inhalation of cobalt-containing dusts. There are numerous reviews of cobalt biochemistry. Its occurrence, major uses, and carcinogenic potential have been extensively reviewed by IARC (1).

1.3.2 Physical-chemical properties

Cobalt (Co, atomic number 27, atomic wt. 58.9332, CAS Registry Number 7440-48-4) is a hard bluish white metal of the first transition series. It is one of four ferromagnetic elements, along with Fe, Ni, and Gd. It forms salts and compounds in oxidation states +2 (d^7) and +3 (d^6). In aqueous solution, the reduction potential of fully aquated Co^{3+} is +1.84 V.

Conversion factor: 1 $\mu\text{g/L}$ = 1 ppb = 16.97 nmol/L

1.3.3 Possible occupational and non-occupational exposures

The widespread use of cobalt in industrialized society precludes an exhaustive listing of exposures. These have been summarized (2) and more extensively reviewed (1, 3) elsewhere. Obvious exposures to cobalt metal and fumes occur in the metal production and refining processes and the production of cobalt-based salts (e.g. CoCl_2 , CoSO_4) and insoluble compounds (e.g. oxides) in the chemical industry. Cobalt is used as a matrix or binding agent in the fabrication of cemented metal carbides ('hard metals') such as tungsten carbide, which usually contain 5–10% cobalt but occasionally up to 30% (3). Their use in drilling and machining is a major source of exposure to cobalt dusts. Here exposure is by inhalation and dermal abrasion and is associated with mixed metal carbide dusts, chiefly tungsten, titanium and tantalum. Exposed individuals in tool production industries include hard and soft grinders, machinists, and technical and clerical staff (4). Dermal exposures to cobalt salts, pigments, and other organic cobalt compounds occur in the rubber industry and tire manufacture, and in the manufacture and use of paints and varnishes, pottery decoration, and inks for offset printing. Soluble and insoluble cobalt compounds in cement are a source of dermal exposure in the construction industry. Diamond polishing with microdiamonds cemented in high purity cobalt powder results in inhalation and dermal exposure to cobalt-containing dusts. Several classes of cobalt alloys result in dermal contact. These include magnetic alloys used chiefly in the telecommunications and electronics industries, the so-called super alloys used for jet

and gas turbine engines, and high strength steels used in the aerospace industry and weapons manufacture. Dental technicians are also exposed to cobalt alloys.

Non-occupational exposure to cobalt arises from surgical implants and dental prostheses, and contact with metallic objects, such as jewelry. Individual intake from food is somewhat variable, but typically 10–100 $\mu\text{g}/\text{day}$ (1, 2). Formerly, treatment of refractory anemia by administration of CoCl_2 and addition of cobalt salts to beer as foam stabilizers resulted in fatalities from cardiotoxicity; these are now of historical interest only.

1.3.4 Summary of toxicokinetics

1.3.4.1 Absorption

(a) Inhalation

Exposure occurs to cobalt fumes and aerosols of soluble cobalt compounds, as well as to cobalt-containing dusts and powders. The latter generally represent mixed exposures to a number of potentially harmful substances, such as metal carbides. Approximately 30% of inhaled inorganic cobalt is absorbed in the lungs and gives rise to a rapid increase in blood cobalt concentrations (5). Intratracheal installation of cobalt dust, tungsten carbide dust, or both together, in rats resulted in more severe lung pathology for the mixture (6). Urinary cobalt was higher when the same dose of cobalt was given together with tungsten carbide, indicative of a greater absorption.

(b) Dermal

Transdermal absorption of cobalt metal and salts is expected to be minimal (7).

(c) Gastrointestinal

Gastrointestinal absorption of inorganic cobalt is dose-dependent but the exact relationship is uncertain. Doses of a few $\mu\text{g}/\text{kg}$ body weight have been reported to be nearly completely absorbed (8). On the other hand, when $^{60}\text{CoCl}_2$ was given to humans, absorption was 5% at a dose of 1 μg cobalt and 20% at 1.2 mg (9). Iron and cobalt compete for absorption in the gut (10) and iron deficiency increases absorption of cobalt (11). Protein-deficient diets decrease the binding of cobalt to protein in the gut and may increase its absorption. In healthy adults, about 70% of an oral dose of Vitamin B_{12} (cyanocobalamin) is absorbed.

Solubility of cobalt compounds also affects gastrointestinal absorption (12). Soluble CoCl_2 was compared with Co_3O_4 , each given at 500 $\mu\text{g}/\text{day}$ for 10 days in a cross-over study with an intervening 10-day placebo period (13). CoCl_2 produced urinary levels up to 2280 $\mu\text{g}/\text{g}$ creatinine; Co_3O_4 only up to 7.6 $\mu\text{g}/\text{g}$ creatinine. With CoCl_2 the median urinary concentration in women was 57.2 $\mu\text{g}/\text{g}$ creatinine; that in men only 20.0 $\mu\text{g}/\text{g}$ creatinine, suggesting a sex difference in absorption of soluble cobalt compounds from the gut (13).

1.3.4.2 Metabolic pathways and biochemical interactions

Cobalt is an essential element whose only known role in humans is as a component of Vitamin B₁₂, required as a co-factor in i) the conversion of methyl malonic acid to succinic acid, ii) synthesis of methionine, and iii) production of methionine-derived formate for purine metabolism and folate synthesis. Vitamin B₁₂ deficiency leads to megaloblastic anemia and neuropathy. Vitamin B₁₂ accounts for 10–20% of the approximately 1 mg of the element present in the body (14). The remainder of the element is probably bound non-specifically to proteins and protein thiols, amino acids (especially histidine), and thiol-containing organic co-factors, such as lipoic acid (2). It may also substitute for iron in the porphyrin nucleus of hemes. In erythrocytes, cobalt is present both in low molecular mass complexes and bound to hemoglobin (15).

1.3.4.3 Distribution

Vitamin B₁₂ is stored mainly in the liver. The remainder of the body's cobalt is in inorganic form, mostly in the bones and soft tissues. In plasma, cobalt is mostly protein-bound to α_2 -macroglobulin and albumin, the latter probably serving as the major transport form (14). A total body burden of 1.1 mg cobalt was demonstrated by neutron activation analysis, 43% of which was in muscle and 14% in bone (16). When rats were given 40 mg/kg cobalt as CoCl₂ by 10 daily subcutaneous injections, liver accumulated 11% of the administered dose, bone about 2.5%, and kidney, pancreas, spleen and blood together about 0.9% (17).

1.3.4.4 Elimination

Early studies by Schroeder et al. (18) reported a daily dietary intake by healthy individuals of about 300 μ g cobalt, as inorganic cobalt plus Vitamin B₁₂. The associated outputs were 260 μ g/day in urine, 40 μ g/day in faeces, and 6 μ g/day in hair and sweat, maintaining body balance. These numbers seem high and should be re-examined by contemporary analytical methods, but they are widely quoted and are probably qualitatively useful. When ⁶⁰CoCl₂ was administered i.v. to healthy adults, 30% was recovered in the first 24 h urine collection while 9–16% was retained for elimination with a half-time ≥ 2 yrs (9). Sorbie et al. reported a comparable value of 18% excreted in the first 24 h urine following an oral dose (19). Initial binding to plasma proteins (chiefly albumin and α_2 -macroglobulin) appears to account for the slower phase. If balance was maintained in these studies, as indicated for dietary cobalt, then significant exchange with body cobalt stores occurs. After occupational exposure to inhaled inorganic cobalt, elimination is mostly urinary and again biphasic, with a rapid phase of about 2 days and a prolonged second phase (20).

1.3.5 Summary of toxic effects

1.3.5.1 Cobalt ingestion

Acute cobalt poisoning in humans is rare and fatalities have not been reported (2). The addition of cobalt salts to beer in the 1960s resulted in ingestion of up to 10 mg/day (less than 0.2 mg/kg/day) in some heavy drinkers, and urinary excretion increased up to 0.5 mg/day. A cardiomyopathy resulted with 20–50% mortality (21, 22). Increased absorption with a protein-deficient diet in the alcoholics may have contributed. Patients have received up to 37 mg Co/day as CoCl_2 for prolonged periods for treatment of refractory anemia, with few adverse effects (17), although hypothyroidism (23) and rare cardiac fatalities (24) have been reported. The cobalt content of the heart tissue of one girl was reported to be 8.9 $\mu\text{g/g}$ dry wt. post mortem (normal said to be 0.2 $\mu\text{g/g}$) (24).

1.3.5.2 Respiratory disease

Chronic occupational inhalation of cobalt dusts is associated with respiratory disease. In hard metal workers both obstructive disease (occupational asthma, work-related wheezing) and interstitial disease (fibrosing alveolitis) occur (4, 25). The same pattern of disease is seen in "cobalt lung" developed by diamond polishers (26, 27). Sensitization to cobalt probably underlies the occupational asthma (28) while non-immune mechanisms and multiple exposures probably give rise to interstitial lung disease in the small percentage of workers who develop it (29). Noting that most cases of interstitial lung disease are observed in the hard metal and diamond polishing industries, with little or no such disease reported with exposures to pure cobalt powder, Lison and Lauwerys (30) studied the toxicity of tungsten carbide and cobalt alone and together in macrophages *in vitro*. When both particles were present together the uptake of cobalt was increased several fold and toxicity was enhanced (30, 31). However, in the presence of cobalt powder alone, neither increasing the amount of solubilized cobalt nor stimulating phagocytosis reproduced the effect, leading to the conclusion that the specific toxicity of hard metal arises from biological interactions of tungsten carbide and cobalt that are as yet unknown (32). This conclusion is supported by a study of exposure to cobalt dusts (metal, oxides, and salts) in a refinery; while the prevalence of dyspnoea was related to the dustiness of the workplace air – as reflected in cobalt exposure indices – there was no evidence relating pulmonary fibrosis to exposure to cobalt alone (33).

1.3.5.3 Sensitization

In addition to occupational asthma, allergic dermatitis arises in cobalt-related industries, and has been observed in the hard metal, cobalt alloy, paint, cement, and rubber industries, and in Finnish potters handling cobalt clay (8, 34, 35). The dermatitis is an erythematous maculopapular type (5) and strongly associated with a history of eczema (34). Antibodies to Co-albumin complexes have been found in patients with occupational asthma (28). Rare instances of dermatitis overlying medical implants in patients testing sensitive to cobalt have been reported (36).

1.3.5.4 Carcinogenicity

Although the Deutsche Forschungsgemeinschaft (DFG) describes cobalt and its compounds as "carcinogenic working materials" (37), IARC has concluded that although there is sufficient evidence for carcinogenicity of cobalt metal powder in experimental animals and limited evidence for carcinogenicity of several cobalt compounds, there is inadequate evidence for the carcinogenicity of cobalt and cobalt compounds in humans (1). Cobalt and its compounds are therefore classified in Group 2B – possibly carcinogenic to humans (1).

1.3.6 Biological monitoring indices

Table 1.3.1 lists the biological indices that are likely to be useful for monitoring occupational exposure to cobalt. Reliable reference data for cobalt in biological fluids is limited; the low natural concentrations [e.g. probably 0.1–0.2 µg/L in plasma (38)] mean that control of contamination and of the analytical method must be rigorous. In individuals not occupationally exposed to cobalt, concentrations in blood and urine may be somewhat higher than in serum or plasma. Because of rapid elimination of cobalt in the urine and exchange with body stores, levels in all these fluids best reflect most recent exposures to soluble forms of cobalt (metal powders, hard metal, and cobalt salts). Inhalation of insoluble cobalt oxides also causes increased cobalt concentrations in urine and blood, but these increases do not correlate well with exposures (39). Non-invasive monitoring of body burden resulting from long-term exposures is not presently feasible. Cobalt concentrations in blood and urine are strongly correlated during occupational exposure (40–42). However, increases are much higher in urine than in blood and the detection limit for cobalt in urine is generally better than in blood. As a consequence, at exposures near the present occupational limits (see below) blood cobalt concentrations are 2–3 times the detection limit of atomic absorption methods while those in urine are several hundred times the detection limit (37). Therefore, only procedures for urinary cobalt are discussed here.

Table 1.3.1. Biological monitoring indices for cobalt and cobalt compounds

Indicator	Suitability	Ref.
Cobalt in urine	Best indicator of current exposure	(40–45)
Cobalt in blood	Correlates with urinary levels: limited reference data	(40–42)
Cobalt in serum/plasma	May be lower than in blood; limited reference data	(46, 47)

1.3.7 Cobalt in urine

1.3.7.1 Toxicokinetics

As noted above, biphasic urinary elimination occurs with the greatest amount of cobalt excreted in the first 24–48 h after exposure. While the data of Schroeder et al. indicate maintenance of body balance with excretion of 86% of daily dietary cobalt in urine (18),

administration of ^{60}Co by others showed only 20–30% 24 h urinary excretion (9, 19). Whether this reflects total elimination, or whether there is exchange of label with body stores seems unresolved. However, maximum excretion of 0.5–0.7 mg of total cobalt in urine in the first 24 h following ingestion of unlabelled CoCl_2 (48) suggests higher doses of cobalt from non-dietary sources are largely retained, although absorption might be decreased in this study by use of an enteric-coated tablet.

From the above discussion, it may be expected that end-of-shift spot urine collection will reflect the day's exposure, and that long-term exposure will lead to increased body stores and higher baseline excretion. This has been borne out in some studies and not others. For example, exposures to $2.5\text{--}105\text{ }\mu\text{g}/\text{m}^3$ of cobalt in the hard metal industry correlated well with the end-of-shift concentrations in urine of $1\text{--}35\text{ }\mu\text{g}/\text{L}$ on Monday (43). After a 4-week vacation, values returned to those of a control population. After the first week back at work, values returned to normal again by the following Monday morning, but did not do so in subsequent weeks, presumably reflecting cumulative exposure. Hard metal plant workers in a high-exposure group (mean $90\text{ }\mu\text{g}/\text{m}^3$) had urinary cobalt concentrations that increased at end-of-shift and decreased again by the following morning, though still remaining elevated even the following Monday morning (40). Both end-of-shift values on Friday and pre-shift values on Monday were strongly correlated with the mean exposure of the previous week. Similarly, porcelain painters using cobalt blue dye were studied after a 6-week vacation (42). Compared to controls ($0.94\pm 2.2\text{ }\mu\text{g}/\text{L}$), they had urinary concentrations of $4.8\pm 6.0\text{ }\mu\text{g}/\text{L}$ at the end of the vacation, that rose to $77\pm 177\text{ }\mu\text{g}/\text{L}$ after 4 weeks. Therefore, the rate at which urinary concentrations return to normal is not clearly defined, and may depend on the nature and level of exposure, as well as other factors in the exposed group.

1.3.7.2 Biological sampling

(a) *Sampling time and specimen*

Urine samples should be collected at the start of the first shift of the workweek as an indication of previous exposure, at the end of the first shift to assess daily exposure to soluble forms of cobalt, and after the last shift of the week to monitor weekly exposure. For example, if day shifts are worked Monday to Friday, the Monday morning sample reflects occupational exposure in comparison with a control population. The difference between Monday morning and Monday evening samples correlates with a time-weighted average exposure during the day (see below). The difference between Monday evening and Friday evening samples indicates cumulative exposure during the week, assuming time-weighted average exposures are the same on Monday and Friday.

(b) *Contamination possibilities*

If the industry is one in which exposure is to cobalt dusts, mixed dusts, or metal fumes or aerosols, contamination from the work environment is likely. Collection should be in a clean area removed from the atmosphere of the workplace. Outer clothing worn on the job should be changed and the hands washed thoroughly before collection.

(c) Sampling device and containers

Containers must be acid-cleaned and opened only for the minimum time required for collection. Several protocols for adequate cleaning have been described (49). Screw cap polypropylene containers are preferred for storage purposes.

(d) Preservatives, shipment, and stability

Specimen containers should be placed in plastic bags and shipped on ice in plastic containers (50). This author is unaware of studies on the stability of urine samples for specific analysis of cobalt. The best recommendation at present is to follow the procedures for Ni, i.e., acidification of urine with conc. HNO_3 (10 mL/L) and storage at 4°C for one week or -20°C if longer times are required (49, 51).

1.3.7.3 Recommended analytical method

The method of choice for analysis of cobalt in urine is electrothermal atomic absorption spectrometry (ET-AAS). The analyst is faced with a choice between measurement after chelation and extraction on the one hand and direct measurement with Zeeman-corrected (Z-) ET-AAS on the other. Improvements in detection limits can be achieved by chelation and concentration; a value of 10 ng/L has been reported (52), but is not always achieved (41, 53). However, performance adequate for measuring levels in a control population – and certainly for measuring the increases that are relevant in occupational exposure – has been achieved with direct Z-ET-AAS (13, 50, 53, 54). Therefore, Z-ET-AAS is considered the better choice based on relative simplicity, general applicability, and diminished chances for contamination from sample manipulation and reagent addition. Furthermore, chelation/extraction is problematic with blood samples (52).

(a) Principles of the method

Urine is acidified and centrifuged, and the clear supernatant is analysed directly by ET-AAS at 240.7 nm, with Zeeman background correction.

(b) Reagents required

High purity HNO_3 (Merck 'Suprapur', BDH 'Aristar', or equivalent) and Triton X-100 are the only reagents required.

(c) Equipment required

An electrothermal atomic absorption spectrometer with Zeeman background correction and equipped with an autosampler is required, as well as a centrifuge. Pyrolytically coated graphite tubes are used. Access to a class 100 air work area (e.g. a HEPA-filter hood) is strongly recommended.

(d) Procedures

i) Calibration

The instrumental response is calibrated with urine containing known added amounts of cobalt prepared from dilutions of a cobalt standard in 1% Triton X-100/1.3 N HNO₃ added at 1:10 (v/v). There are no certified reference materials for cobalt in human urine, although Nycomed (Seronom Trace Elements in Urine) and Bio-Rad (Lyphocheck urine) provide materials with preliminary recommended values and assigned values, respectively. Therefore, interlaboratory comparison or use of a second, independent method (see below) is important.

ii) Procedure

1 mL of urine (acidified at the time of collection) is transferred to a centrifuge tube and 100 µL of 1% Triton X-100 in 1.3 N HNO₃ is added. These procedures should be carried out in the clean-air environment. The tightly capped tube is centrifuged and a suitable volume of supernatant is transferred to a cup of the autosampler immediately before analysis. 1.5 mL plastic microcentrifuge tubes work well and are spun at top speed (ca. 10,000 x g) for 1 min. Only plastic pipette tips, centrifuge tube and sample cup come in contact with the sample, and all must be thoroughly acid-cleaned. The sample is analysed by atomic absorption at 240.7 nm, with Zeeman background correction. Furnace parameters should be optimized in each laboratory.

These suggestions presuppose that monitoring laboratories will not generally have access to clean rooms. By carrying out as much sample manipulation as possible in a class 100 hood, capping the tube to avoid contamination with metal particulates arising from the centrifuge rotor, and minimizing the time of exposure to room air before analysis, reasonable results should be achieved even at the concentrations expected in urine from people without occupational exposure.

(e) Criteria of analytical reliability

i) Trueness

Accuracy has been demonstrated by interlaboratory comparison (50). Recovery from urine spiked with 40 µg/L has been reported to be 101% (53).

ii) Precision

Long-term imprecision assessed by the relative standard deviation of 10 urine samples containing 11.2 µg Co/L was 6.7% (54). Bouman et al. reported a within-run and between-run relative standard deviation of 3.8% and 8.9% respectively, by direct Z-ET-AAS, at 33 µg/L (53).

iii) Detectability

Detection limits in the range 0.1–0.3 µg/L are achieved (13, 50, 53), well below the level caused by significant occupational exposures.

(f) Quality assurance

i) Special precautions

Pooled urine should be saved and analysed on an on-going basis to serve as an internal quality control. Performance should also be assessed against available reference materials with recommended concentrations of cobalt. The German Society of Occupational and Environmental Medicine provides an external quality control programme (55).

ii) Interferences

Zeeman background correction was found to overcome potential interferences from other metals, including Fe, Ni, Mn, Zn, and Cu in blood, and CaPO_4 in urine (54). General matrix effects should become apparent when the quality assurance procedures are adhered to.

(g) Sources of possible errors

i) Pre-analytical

The initial rapid phase of elimination of absorbed cobalt takes place over 24–48 h and so collection time around the end-of-the-shift should not be critical for obtaining a representative sample. As for any analyte in urine, the adequacy of the sample is best judged by saving an aliquot for the measurement of creatinine. Considerations regarding creatinine and the choice of expressing analyte concentration per urine volume or per creatinine mass are not specific to cobalt and are not discussed here. By far the greatest risk of error is contamination of the sample, both during collection and during sample preparation in the laboratory. This is especially true when the subjects are control populations or those with low levels of exposure, so that natural concentrations near the detection limit of the method will be measured. It must be stressed that analysis of body fluids for cobalt requires rigorous acid-cleaning of all materials coming into contact with the sample, and minimizing exposure to room air (46). Diluents must be checked for cobalt content by the use of appropriate method blanks.

ii) Analytical

Inadequate analytical sensitivity may be a problem, especially in control samples. This should become apparent with appropriate quality control.

(h) Reference to the most comprehensive description of the method and evaluation

Direct measurement of cobalt in urine by ET-AAS with Zeeman correction has been described in some analytical detail by Christensen et al. (54) and Bouman et al. (53). The latter authors compared the method to determination with deuterium arc correction, with and without prior chelate extraction. Detection limits were slightly better with extraction (0.2 $\mu\text{g/L}$ vs. 0.3 with Z-ET-AAS), as were within- and between-run imprecision. However, very similar results were obtained with urine from 25 healthy volunteers (53). Recently, Christensen and Apostoli reviewed the analytical literature on cobalt in human blood and urine, as part of the TRACY project (56) on reference values of trace elements in body fluids. Eleven publications from eight different laboratories were considered useful for the purposes of establishing reference values of cobalt in urine, and of

these seven (13, 42, 44, 45, 47, 50, 53) – representing five laboratories – used direct analysis with Z-ET-AAS.

1.3.7.4 Other analytical methods

When chelate extraction is used prior to ET-AAS, N,N-hexamethyleneammonium-hexamethylenedithiocarbamate (HMADTC) (43, 52, 53) or ammonium pyrrolidine dithiocarbamate (APDC) (41) are most common, with extraction into a suitable solvent, such as methylisobutyl ketone. Alexandersson (40) used ion exchange for pre-concentration. Electrochemical analysis is frequently used as a second method in order to assess accuracy. Methods include differential pulse adsorption voltammetry (DPAV) and anodic stripping voltammetry (DPASV) (52, 54). Neutron activation analysis is also applicable to cobalt measurement in body fluids (46).

1.3.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Although it is not yet possible to set reference values for urine cobalt in people not occupationally exposed, and many earlier published reports must be discounted because of inadequate contamination control or poor analytical sensitivity, values in the range 0.1–1 µg/L should be expected. Angerer et al. reported a range of 0.04–1.25 µg/L (95% confidence level 0.85 µg/L) in 79 healthy subjects (geometric mean 0.08 µg/L) (57), and in a later study of a larger population revised the mean down to 0.07 µg/L (95% confidence level 0.71 µg/L) (52). Alexandersson found an arithmetic mean of 0.60 ± 0.60 µg/L in office workers who smoked and 0.30 ± 0.10 µg/L in non-smokers (40). Minoia et al. (47) reported an arithmetic mean of 0.57 ± 0.26 µg/L (range 0.12–2 µg/L) in 468 members of the general Italian population, while Christensen and Poulsen (58) found arithmetic means of 0.76 ± 0.72 µg/L in Danish men and 0.77 ± 0.89 µg/L in women.

(b) Published exposure limits and biological action levels

The American Conference of Governmental Industrial Hygienists (ACGIH) defines threshold limit values (TLV) based on the time-weighted average (TWA) exposure during a 5-day, 40-h workweek, and short-term exposure limits (STEL) that should not be exceeded in any 15-min time-weighted average. For cobalt carbonyl and hydrocarbonyl, the TLV-TWA is 0.1 mg/m³ as cobalt. For cobalt metal, dusts, and fumes, the TLV-TWA is 0.05 mg/m³ and the TLV-STEL is 0.1 mg/m³ (59). The TLV-TWA was revised downwards from 0.1 mg/m³ recently, and now the ACGIH has added to the Adopted List of changes for 1994–1995 a lower TWA-TLV of 0.02 mg/m³ for elemental and inorganic forms of cobalt (61). Based on the TLV of 0.02 mg/m³ ACGIH has proposed a Biological Exposure Index (BEI) for all forms of cobalt, regardless of solubility. The value is 15 µg/L (255 nmol/L) in urine collected at the end of the last shift of the workweek. Switzerland has an occupational exposure limit for cobalt compounds and dusts of 0.01 mg/m³ (60).

The DFG does not list BAT and MAK values for cobalt compounds, considering it to be a potentially carcinogenic and mutagenic substance, precluding assessment of safe tolerance values (37). However, they define a technical exposure limit (TRK) based on attainability with current technologies to serve as a guideline for necessary protective procedures. The TRK for production of cobalt powder and catalysts, hard metal, and magnets is 0.5 mg/m^3 . For other uses of cobalt it is 0.1 mg/m^3 .

Exposure measurements and end-of-shift urine collection on individuals in a carbide tip saw blade production plant allowed Stebbins et al. to calculate a correlation between the TWA air cobalt content (in mg/m^3) and urine cobalt (in $\mu\text{g/L}$) (62). The relation $[\text{Co}]_{\text{urine}} = 766 \times \text{TWA} - 0.39$ ($r = 0.91$) predicts a concentration of $38 \mu\text{g/L}$ (646 nmol/L) at the TWA-TLV of 0.05 mg/m^3 . In keeping with this result, exposure to a variety of cobalt sources (reduction and electrolysis, grinding, hard metal, and cobalt salts) at 0.05 mg/m^3 was stated to be associated with a urine cobalt concentration of $30 \mu\text{g/L}$ (510 nmol/L) (52). A TWA of 0.05 mg/m^3 was also found to cause an end-of-shift urine cobalt of $34 \mu\text{g/L}$ (578 nmol/L) at mid-week that doubled at twice the exposure (41). The value was slightly lower after the first shift of the week. Scansetti et al. conclude that a TWA exposure of 0.1 mg/m^3 will lead to a urine cobalt of $30 \mu\text{g/L}$ (510 nmol/L) at the end of the week's first shift and $60 \mu\text{g/L}$ (1020 nmol/L) by the end of the week (43). Exposure to cobalt salts, oxides and metal powder at a TWA of 0.05 mg/m^3 gave rise to a value of $33 \mu\text{g/g}$ creatinine after the Monday shift that increased to $46 \mu\text{g/g}$ creatinine by Friday (12). Nemery et al. (63) found a correlation between urinary cobalt and exposure by personal monitoring in diamond polishers exposed below the TLV. Their data predict a mean urinary cobalt of $36 \mu\text{g/L}$ at an exposure of 0.05 mg/m^3 , independent of sampling time. There also appears to be a dose-related effect on pulmonary function tests (63). Consistent with these data, DFG reports "exposure equivalents for carcinogenic working materials" (EKA) values of 30, 60 and $300 \mu\text{g/L}$ in urine for exposures to 0.05, 0.10, and 0.50 mg/m^3 , respectively (37). Lauwerys and Hoet suggest a "tentative maximum permissible level" of $30 \mu\text{g/g}$ creatinine (12), or approximately 20–30 $\mu\text{g/L}$, therefore corresponding to an exposure at the TLV-TWA.

The general agreement of these values from different sources and the linearity of the response over a relevant range of exposure strengthens our confidence in urinary cobalt concentrations as a monitor of occupational exposure to cobalt metal, its soluble compounds, and hard metal dusts. However, recent data indicate that this correlation is poor when exposure is to insoluble cobalt oxide (39).

(c) Non-analytical interferences

i) Other exposures

Additional sources of cobalt exposure or factors which increase its absorption will potentially increase urinary excretion, but the latter may still reflect the effective exposure if systemic effects are important. With few exceptions, these additional sources are not expected to produce the levels of concern (i.e. $30 \mu\text{g/L}$). For instance, smokers have double the cobalt excretion of non-smokers on account of the cobalt content of cigarette smoke, but still only $0.6 \mu\text{g/L}$ (40). Mineral supplements and vitamin preparations con-

taining vitamin B₁₂, especially if abused, may be an exception. This means of ingestion may or may not contribute to, for example, the risk of lung disease from inhalational exposure in the workplace, but its contribution to urine cobalt should nevertheless be recognized. Sunderman Jr. et al. (50) have reported a slight increase in mean urine cobalt in patients with cobalt-alloy knee and hip prostheses up to two years after surgery that nevertheless reached values above 5 µg/L in two patients. Because dithiocarbamates are cobalt chelators, disulfiram therapy may increase cobalt excretion, but this appears not to have been studied.

ii) Diet and environment

Dietary intake of cobalt is variable, but insufficient to distort urinary cobalt measurements at levels of concern. However, dietary composition may influence the acquisition of ingested cobalt, since iron- and protein-deficient diets both increase absorption from the gastrointestinal tract. Residence in a contaminated environment of course represents additive exposure.

(d) *Sampling representative of recent or long-term exposure*

End-of-shift sampling represents exposure during the workday; a rapid phase of elimination of cobalt in urine occurs during the first 24 h after exposure (9, 19, 20) and the day's exposure correlates well with the cobalt content of the end-of-shift sample. Cumulative exposure during the workweek gives rise to higher end-of-shift values at the end of the week, however, and the difference between beginning and end-of-shift on a given day is therefore the best indicator of the exposure on a single day. Comparison of Monday morning and Friday evening samples may be important for assessing the week's exposure (12, 41, 43). The Monday morning urine reflects long-term exposure (12, 40, 42, 43). The rate at which urine cobalt returns towards normal with prolonged absence from work remains uncertain (42, 43) and probably reflects the nature, as well as the level of exposure.

(e) *Ethnic differences*

No ethnic differences in cobalt absorption, excretion, or susceptibility have been described.

1.3.8 Cobalt in blood and serum

Although cobalt in blood and serum generally shows good correlation with urinary cobalt, the latter is more than an order of magnitude higher at a given exposure. Thus, with exposure at current TLVs most laboratories will still be working near their determination limit if measuring blood or serum cobalt, whereas the higher levels arising in urine will be measured accurately if reasonable precautions are followed. The good correlation of urinary cobalt concentrations with exposure to soluble forms of the element, together with the limited reference data on blood, serum, and plasma levels, implies that urinary cobalt is adequate for biological monitoring and more invasive blood collection is not recommended for routine purposes.

1.3.9 Research needs

Urine cobalt is a sufficiently robust indicator of exposure except to cobalt oxides that there is no pressing need to evaluate other monitoring procedures. Reference values in non-occupationally exposed populations are not yet established with certainty. However, the concentrations that occur in urine of those exposed at current limits are sufficiently high that better reference levels or improvements in analytical methods are not essential for adequate monitoring. The genotoxic, teratogenic and carcinogenic potential of cobalt in humans, although low, has not been eliminated by existing epidemiological or laboratory studies. To rule out these effects completely will be difficult. Elucidating the role of co-exposures in producing the major health effects of cobalt – of carbides and other dusts in producing lung disease, and of Ni and Cr in skin sensitization – is perhaps the most important practical problem in the evaluation of cobalt in occupational health. More information is also desirable on the long-term fate of cobalt retained in the body beyond 24–48 h, and the extent to which recently absorbed cobalt exchanges with body pools.

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1.4 Nickel

1.4.1 Introduction

Exposure to nickel metal and its soluble and insoluble compounds occurs in a number of industrial settings, and contact with nickel-containing objects and its intake from natural sources is ubiquitous in the general public. The main health concerns for occupational exposure are skin sensitization and an increased risk of cancers of the respiratory tract. However, it must be remembered that i) skin sensitization affects as many as 10% of women (and fewer men) in the general population and excess occurrence in the workplace is not easily demonstrated, and ii) respiratory cancers have been linked with chronic inhalation of very high concentrations of nickel rarely encountered in the modern workplace. Soluble nickel compounds are rapidly absorbed in the lung and gastrointestinal tract and excreted over the next few days in the urine; urinary levels are adequate for monitoring such exposures, and correlate with serum levels. Insoluble compounds may become impacted in the airways and release nickel slowly over extended periods; serum and urine levels are indicative of the resulting "internal" source of exposure. While biomonitoring of nickel is useful for assessing exposure, it has not proven useful for biological risk assessment. Biological monitoring of nickel was reviewed extensively in 1986 (1) and the general conclusions of that examination were recently reaffirmed (2).

In this Chapter, metallic nickel and its soluble and insoluble compounds are considered. Unless specifically stated, "nickel" refers to any of these forms. Nickel carbonyl $[\text{Ni}(\text{CO})_4]$, CAS Registry Number 13463-39-3] is a low boiling, colourless liquid that decomposes in air at atmospheric pressure. Generated in the Mond process for production of high purity nickel, it is extremely toxic and has resulted in numerous industrial fatalities in the past (3). Urinary nickel concentrations correlate well with $\text{Ni}(\text{CO})_4$ inhalation and the severity of poisoning (4). However, $\text{Ni}(\text{CO})_4$ is not widely used today and is not considered further in this Chapter.

1.4.2 Physical-chemical properties

Nickel (Ni, atomic number 28, atomic wt. 58.693, CAS Registry Number 7440-02-0) is a grey powder or hard lustrous white metal of the first transition series. It is one of four ferromagnetic elements, along with Fe, Co, and Gd. Salts and compounds of biological significance are mainly in the oxidation state +2 (d^8). Other oxidation states occur transiently in enzymatic reactions in lower organisms, and possibly during harmful redox cycling. Major soluble forms encountered in industry [and their CAS Registry Numbers] include the acetate $\text{Ni}(\text{CH}_3\text{CO}_2)_2$ [373-02-4], chloride NiCl_2 [7718-54-9] and its hexahydrate [7791-20-0], nitrate $\text{Ni}(\text{NO}_3)_2$ [13138-45-9] and its hexahydrate [13478-00-7], and sulfate NiSO_4 [7786-81-4] and its hexahydrate [10101-97-0]. Major insoluble compounds include the sulfide NiS [16812-54-7] and its amorphous compounds [11113-75-0], subsulfide Ni_3S_2 [12035-72-2], various oxides including NiO [1313-99-1], hydroxide $\text{Ni}(\text{OH})_2$ [12054-48-7], and carbonate NiCO_3 [3333-67-3].

Conversion factors

1 $\mu\text{g/L}$ = 1 ppb = 17.04 nmol/L

1.4.3 Possible occupational and non-occupational exposures

Respiratory exposure is most important because of its role in nickel carcinogenesis (5). Respiratory and oral exposures are both important for acute toxicity, while dermal exposure is chiefly related to nickel dermatitis (6). Occupational exposures are widespread and occur in mining and refining of nickel ores and production of alloys, including stainless steel. Electroplating with nickel and stainless steel welding are also important sources of nickel exposure. Workers in glass bottle factories are frequently exposed to nickel from the production of molds which are commonly nickel. The electronics industries employ magnetic nickel alloys. Manufacture of nickel-cadmium batteries gives rise to exposure to both metals. Additional sources include the chemical, pigment (e.g. NiTiO_3) and ceramics industries, catalyst production (e.g. Raney nickel) for hydrogenation of soaps and oils, and making metal objects, such as jewelry, coins, and medical prostheses. Waste incineration and burning of fossil fuels also lead to increased inhalation of nickel. Uses of and exposures to nickel compounds have been reviewed thoroughly by IARC (5).

Dietary intake of nickel is at least 100 $\mu\text{g/day}$ (7). Nickel has not been proven essential for higher organisms, but is a component of several enzymes and enzyme cofactors in plants and microorganisms. Thus, vegetarian diets – particularly those rich in nuts and soy products – lead to a higher daily intake of nearly 1 mg in some cases (8). Urban atmospheres in the United States contain nickel at about 25 ng/m^3 but values of about 150 ng/m^3 have been recorded in polluted areas, particularly where fossil fuels are burned. Urban dwellers are reported to inhale 0.2–1.0 $\mu\text{g/day}$ (8, 9). Nickel in cigarette smoke may increase this value by as much as 4 $\mu\text{g/pack}$. Non-occupational exposures also arise from handling metal objects, such as jewelry and coins, and from implantation of medical prostheses made of nickel-containing alloys.

1.4.4 Summary of toxicokinetics

1.4.4.1 Absorption

(a) Inhalation

Inhaled nickel fumes and dusts, including welding fumes, result in particle deposition in the nasal sinuses, upper airways, and lungs, depending on the aerodynamic size distribution of particles. Nickel is poorly absorbed from these particles, which remain in the tissues and release nickel slowly. Therefore, significant increases in blood and urine nickel may not occur after exposure to these sources (10), although slow release means that long-term concentrations may reflect lung burden (11). Hypernickemia has been observed in former nickel refinery workers many years after retirement (12). In contrast, aerosolized soluble nickel compounds are better absorbed, and increases in blood nickel concentrations may be observed immediately after exposure (see detailed discussion below). Whereas forms of nickel soluble in aqueous media are readily absorbed, ligands

present in biological fluids may affect the bioavailability of less soluble forms. There is very little specific information on this matter.

(b) Dermal

Transdermal absorption of nickel is low, being chiefly through sweat glands and hair follicles and limited by the horny layer. However, binding of Ni^{2+} as hapten to ligands in the skin is involved in presentation of the allergen that triggers contact dermatitis (7). Corrosion by sweat plays a role in the release of nickel from alloys and coatings. Rates of release of nickel from certain alloys have been reviewed (13).

(c) Gastrointestinal

In a number of animal studies oral doses of nickel salts resulted in a rapid increase in blood nickel concentrations and a total absorption of 1–5% of the dose (7). Non-fasting human volunteers given a single oral dose of 5.6 mg nickel as NiSO_4 absorbed about 3% (14). Sunderman and coworkers have found similar low absorptions of NiSO_4 given to volunteers with food, but this value increased to $27 \pm 17\%$ when NiSO_4 was given in water after a fast (15). Using ^{61}Ni as a stable isotope tracer, we observed a fasting absorption of 30% at a dose of 20 mg of nickel per kg body weight as aqueous $\text{Ni}(\text{NO}_3)_2$ (16). Serum concentrations peaked at 2 h.

EDTA decreases the absorption of Ni^{2+} and disulfiram and dithiocarbamates enhance it (7).

1.4.4.2 Metabolic pathways and biochemical interactions

Nickel is transported in plasma bound to the N-terminal tripeptide of albumin and to other proteins such as α_2 -macroglobulin. In addition, a low molecular weight, ultrafilterable fraction comprises 27% of circulating nickel in the rat and between 20% (17) and 40% (18) in man. This fraction includes nickel bound to amino acids – especially histidine – and acidic peptides. In tissues, nickel is probably bound non-specifically to various peptides and low molecular weight ligands. In the kidney, ligands potentially important in excretion include acidic glycosaminoglycan fragments (19). The nature of the ligand strongly influences cellular uptake of ionic nickel (20, 21).

1.4.4.3 Distribution

In animals, nickel is distributed throughout the body and to the fetus (22, 23), generally independently of the route of administration. Although inhalation of particulates gives rise to major deposition in the lung, kidney – followed by lung and liver – is otherwise the major organ of deposition of soluble nickel administered parenterally to animals in short-term studies (24). In autopsies of ten humans without occupational exposure to nickel, however, concentrations decreased in the order lung > thyroid > adrenal > kidney > heart > liver > brain > spleen > pancreas (25). This probably better reflects long-term accumulation than does initial clearance and distribution in short-term studies. Clearance from plasma obeys a two-compartment model (26) dominated by glomerular filtration and a soft tissue 'sink'. Nickel presented to cells as soluble Ni^{2+} mainly enters

extranuclear organelles, whereas phagocytosed particulates concentrate near the nuclear envelope and are then solubilized after which nickel accumulates in the nucleus (27). This may account for the higher carcinogenic potential of particulates, such as NiS and Ni₂S₃.

1.4.4.4 Elimination

Elimination of plasma nickel is mainly via the urine; a few percent enters the bile and elimination in sweat, saliva, hair, and nails occurs but is hardly significant. Ultrafilterable nickel undergoes rapid glomerular filtration but up to 99% is reabsorbed in the tubule (28). Plasma half-times after oral intake by fasting volunteers are approximately 12–24 h (15, 16). The half-time of urinary excretion appears to depend on the source of inhalational exposure and varies from 17–39 h for soluble nickel compounds inhaled by nickel-plating workers to 53 h for nickel compounds inhaled by welders, with an intermediate value of 30–50 h for insoluble nickel compounds inhaled by mold makers in glass bottle factories (8). Elimination of orally ingested nickel is nearly complete by 4 days, with very little whole-body retention (15).

After mice were exposed to NiCl₂ aerosol the dose was cleared with first order kinetics and 72% of the deposited dose was gone after 4 days. In comparison, after breathing NiO, Syrian hamsters retained 30% of the deposited dose after 100 days (7). Tanaka et al. (29) have found that the half-time of deposited NiO depends on particle size expressed as mass median aerodynamic diameter (MMAD). At MMAD = 1.2 μm, the half-time of NiO is 11.5 months. This increases to 21 months at MMAD = 4 μm. Recently the elimination of ⁵⁷Ni given to mice was followed by whole-body counting (30). After oral administration of NiCl₂, 2–10% of the dose was absorbed and the whole-body retention was only 0.02–0.36% of the dose at 45–75 h.

1.4.5 Summary of toxic effects

Most absorbed nickel is rapidly eliminated in the urine with little or no effect on the kidney and its acute toxicity is low. Therefore, the long-term carcinogenic potential of nickel salts and compounds, and particulate nickel retained in the lungs and upper airways is the impetus for biomonitoring. Nickel toxicology has been extensively reviewed (8, 31, 32).

1.4.5.1 Acute toxicity of Ni²⁺

An accidental occurrence in a nickel electroplating factory afforded a unique opportunity to study the effects of acute ingestion of large quantities of soluble nickel compounds by men (33). Cooling water contaminated with nickel plating solution (NiSO₄ and NiCl₂) back-siphoned into a drinking fountain. Thirty-two workers drank water with 1.6 g nickel per liter. Twenty became symptomatic with doses estimated at between 0.5 and 2.5 g nickel. Symptoms included nausea, vomiting, diarrhea, headaches, giddiness, lassitude, cough, and shortness of breath. Similar symptoms were observed in 23 renal dialysis patients accidentally exposed to nickel-contaminated dialysis fluid (34). In both cases, symptoms lasted from one to several days with complete recovery in

all subjects. Transient homonymous hemianopsia occurred in one male volunteer after ingestion of only 50 µg of Ni/kg body wt after a fast (15). Although this might be related to the vasoactive properties of nickel, causation is unproven. Sunderman Jr. et al. (33) also mention a reported fatality of a 2.5-year old girl who ingested 2–3 g of nickel as NiSO₄ from a chemistry set.

1.4.5.2 Immunotoxicity and respiratory disease

Hypersensitivity to nickel has been well described in a monograph (35) and recent reviews (36, 37). Positive dermal patch tests to nickel occur in 7–10% of women and 1–3% of men, usually accompanying positive patch tests to Co and (or) Cr. The dermatitis usually begins as areas of erythema at the sites of contact with nickel (e.g. hands, ear lobes) and spreads to distant areas, usually in a symmetrical distribution, progressing to eczema. The dermatitis has been exacerbated by giving sensitive individuals diets naturally high in nickel (38). Sensitivity is also manifest as asthma, conjunctivitis, and inflammation around dental implants and orthopaedic prostheses. Rarely, anaphylactic reactions have occurred after parenteral injections of nickel-contaminated medications (6).

In addition to occupational asthma, some nickel platers, refiners and welders have developed chronic pulmonary disease, such as bronchitis and pneumoconiosis. Nickel may be an etiologic agent in these cases, although IARC (5) was unable to determine its causal significance. Occupational exposure to nickel is also associated with an increased incidence of nasal polyposis and septal perforation, hypertrophic rhinitis, and sinusitis, although epidemiological data are incomplete (6).

1.4.5.3 Carcinogenicity

Certain nickel compounds are potent carcinogens in animals and have been studied extensively (39). These studies will not be reviewed here because there is now sufficient evidence for carcinogenicity to humans. An International Committee on Nickel Carcinogenicity in Man (40) concluded that risks of respiratory cancers are primarily related to exposure to less soluble (oxidic and sulfidic) nickel compounds at concentrations above 10 mg/m³ and soluble nickel compounds at concentrations in excess of 1 mg/m³. IARC (5) reviewed this data in 1990 and concluded that nickel compounds are carcinogenic to humans (Group 1), whereas metallic nickel is possibly carcinogenic to humans (Group 2B). The major studies have been carried out at INCO, Ontario; MOND/INCO, South Wales; and Falconbridge, Norway. Among the Ontario workers, no nasal cancer was observed among those in sintering for more than 5 years with exposure mainly to sulfidic nickel, but the standard mortality ratio (SMR) for lung cancer was 492. In another group of sinter workers with additional exposures to nickel oxides and soluble nickel, this value rose to 789 and nasal cancers were also observed (SMR≈13,000). Comparable results were observed in leaching and calcining workers with similar exposures, whereas among electrolysis workers with relatively much lower exposures to all classes of nickel compounds, no excess cancers at either site occurred. In Wales, mixed exposures for ≥ 5 years to nickel oxides, sulfides, salts and metal in furnace operators and calcining, milling, grinding, and hydrometallurgy workers were

associated with SMRs for lung cancer of 300–1200 and for nasal cancer of 1,000–78,000. In the Norwegian study, workers in electrolysis (with low exposure to sulfidic nickel) who had never worked in other departments had an increased SMR for lung cancer (476) compared to those in calcining, roasting and smelting who had never worked in electrolysis and had negligible exposure to nickel salts (SMR = 254). All data are summarized from IARC (5). It must be pointed out that the data of these studies span a good part of this century and do not reflect current operational practices; nor should much of the exposure data be considered accurate in view of current analytical protocols. The proven associations with human lung and nasal cancers are for nickel sulfates, and the mixtures of nickel oxides and sulfides encountered in the nickel refining industry. Production of human tumours at other sites or by other nickel compounds remains uncertain.

1.4.6 Biological monitoring indices

Because absorbed nickel is cleared rapidly in the urine, serum and urine nickel show a correlation after exposure to soluble nickel compounds and both are indicative of recent exposure (table 1.4.1). Nickel does not accumulate in the body with common levels of non-occupational exposure. However, it must be remembered that insoluble and particulate forms of nickel can accumulate in the upper airways and lungs and serve as an internal source of exposure at a much later time. Therefore, whereas changes in urine and serum are good indicators of exposure to bioavailable nickel over the preceding 1–2 days, increases in the nickel content of these fluids will also reflect long-term exposure from these insoluble sources, and on-going, long-term monitoring programmes are important.

Table 1.4.1. Biological monitoring indices for nickel and nickel compounds

Indicator	Suitability	Ref.
Ni in urine	Indicator of current exposure to bioavailable nickel, useful for end-of-shift monitoring	(33, 41–43)
Ni in serum/plasma	Correlates with urine levels in acute absorption; more reliable in long-term monitoring for exposure to insoluble nickel compounds	(11, 33, 42–44)
Ni in nasal mucosal biopsy	Indicative of long-term exposure to insoluble compounds; not feasible for routine monitoring	(12)

1.4.7 Nickel in urine

1.4.7.1 Toxicokinetics

Because a significant part of the nickel in the circulation is in ultrafilterable form, urinary elimination is efficient and expected to follow absorption rapidly. Thus, most plasma nickel newly absorbed by volunteers was cleared in the urine by 1–2 days (15, 16). Kinetics of nickel in the urine in an occupational setting are therefore dominated by absorption; mixed exposures to soluble and insoluble forms will give rise to biphasic

elimination, the rapid phase reflecting exposure to soluble nickel and a variable, slower phase dependent on the rate of conversion of nickel to bioavailable forms. Nevertheless, a number of studies have reported correlations between nickel in urine and in the workplace air, through mutual correlation with blood nickel (11). Some of the earlier studies should be discounted because of failure of analytical methods available at the time to demonstrate accurate performance with samples from non-occupationally exposed individuals (discussed in (45)), but the principles appear to hold, and have been partially confirmed in more recent studies (42).

1.4.7.2 Biological sampling

(a) Sampling time and specimen

Spot urine samples should be collected at the beginning and end of a workday. Many scenarios will involve exposures to both soluble nickel compounds and to insoluble compounds and particulates. Therefore, even before the first shift following a weekend or vacation concentrations may be elevated due to long-term exposure. However, the incremental increase at the end of the shift should reflect exposure to soluble nickel compounds during the shift.

(b) Contamination possibilities

If the industry is one in which exposure is to nickel dusts, mixed dusts, metal fumes or aerosols, contamination from the work environment is likely. Collection should be in a clean area removed from the atmosphere of the workplace. Outer clothing worn on the job should be changed and the hands washed thoroughly before collection. Furthermore, contamination from the collection and sample processing apparatus is a major concern for nickel and careful acid washing of the sample container is mandatory.

(c) Sampling device and containers

Containers must be acid-cleaned and opened only for the minimum time required for collection. Several protocols for adequate cleaning have been described (45). Screw cap polypropylene containers are preferred for storage purposes to minimize water loss on storage (46).

(d) Preservatives, shipment, and stability

Urine is acidified at the time of collection with HNO_3 (10 ml/L). Specimen containers should be placed in plastic bags and shipped on ice in plastic containers (47). If the sample is to be analysed within one week, storage at 4°C is adequate. Otherwise the sample should be stored at -20°C (45, 48). It must also be noted that Stoeppeler (49) has shown by using radiotracer ^{63}Ni that Ni adsorbs to the precipitate that forms when urine is acidified. Thus some authors recommend storage at -20°C without acidification. Adsorptive losses were 5% at pH 6 and 1% at pH 1. (Addition of 10 ml/L conc. HNO_3 gives a pH of about 1).

1.4.7.3 Recommended analytical method

The recommended method of measurement of nickel in urine is electrothermal atomic absorption spectrometry (ET-AAS). The earlier IUPAC reference method for nickel in urine involved chelate extraction (50), but this has been superseded by direct analysis of diluted, acidified urine using Zeeman background correction (41, 45, 48).

(a) Principles of the method

Acidified urine is diluted with acid and, if cloudy, centrifuged prior to direct analysis by ET-AAS at 232.0 nm, with Zeeman background correction.

(b) Reagents required

High purity HNO_3 (Merck 'Suprapur', BDH 'Aristar', or equivalent) is the only reagent required.

(c) Equipment required

An electrothermal atomic absorption spectrometer with Zeeman background correction and equipped with an autosampler is required, as well as a centrifuge. Pyrolytically coated graphite tubes are used. Access to a class 100 air work area (e.g. a HEPA-filter hood) is strongly recommended.

(d) Procedures

i) Calibration

The instrumental response is calibrated with urine containing known amounts of nickel prepared by serial dilution of a nickel standard in 0.1 M HNO_3 . Urine is then diluted with 0.1 M HNO_3 to a final dilution of 1:1 (v/v). Bio-Rad Lyphocheck and Nycomed Seronorm reference urines are available for internal control. For measurement at lower levels encountered in control populations, interlaboratory comparison and the use of a second method are important.

ii) Procedure

0.5 mL of urine (acidified at the time of collection) is transferred to a plastic centrifuge tube and 0.5 mL 0.1 M HNO_3 is added. These procedures should be carried out in the clean-air environment. The tightly capped tube is centrifuged and a suitable volume of supernatant is transferred to a cup of the autosampler immediately before analysis. 1.5 mL plastic microcentrifuge tubes work well and are spun at top speed (ca. 10,000 x g) for 1 min. Only plastic pipette tips, centrifuge tube and sample cup come in contact with the sample, and all must be thoroughly acid-cleaned. The sample is analysed by atomic absorption at 232.0 nm, with Zeeman background correction. Furnace parameters should be optimized in each laboratory.

(e) Criteria of analytical reliability

i) Trueness

Accuracy at 4 µg/L has been reported with the Behring Institute reference material, and demonstrated in this range by interlaboratory comparison against D₂ arc-corrected ET-AAS with an interlaboratory relative standard deviation of 10% (51). The method was found to agree (41) with results of the earlier IUPAC reference method (50) at ca. 2 µg/L. A recovery of 99±5% at 20 µg/L has been reported (41). One study mentions without detail that some samples were validated by comparison with neutron activation analysis (52).

ii) Precision

Within-run relative standard deviations of 4.7% at 2 µg/L (41) and 1.5% at 32 µg/L (53) have been reported. Long-term imprecision is about 5% above 10 µg/L (41, 53).

iii) Detectability

Detection limits are about 0.5 µg/L (41, 47).

(f) Quality assurance

i) Special precautions

Pooled urine should be saved and analysed on an on-going basis to serve as an internal quality control. Performance should also be assessed against available reference materials with recommended concentrations of nickel. The German Society of Occupational and Environmental Medicine provides an external quality control programme.

ii) Interferences

Zeeman background correction is important for minimizing interferences. No interferences were found from As, Ba, Bi, Cd, Cr, Co, Cu, Au, Fe, Pb, Mn, Hg, V, Zn (all 50 µM), Ca, or Mg (25 mM) added to urine (41). Mg(NO₃)₂ has been used as a modifier (Ni(NO₃)₂ is reduced to NiO and then to metallic nickel in the furnace) but this is replaced by using a HNO₃ matrix. Use of pyrolytically coated tubes minimizes formation of refractory carbides. A cleaning step after atomization is generally used to decrease sample-to-sample interferences including carry over (45).

(g) Sources of possible errors

i) Pre-analytical

The first urine post-shift should be representative; elevations decline over 24–48 h after exposure. The adequacy of the urine sample should be assessed by saving a portion for determination of specific gravity or creatinine, but this is true for any analyte in urine and no specific recommendations are offered here. Specific gravity < 1.010 or creatinine < 0.3 g/L indicates an unacceptably dilute specimen. The greatest risk of pre-analytical error is contamination of the sample, both during collection and during sample preparation in the laboratory. This is especially true when the subjects are control populations or those with low levels of exposure, so that natural concentrations near the detection limit of the method will be measured. It must be stressed that analysis of body fluids for nickel requires rigorous acid-cleaning of all materials coming in contact with the sam-

ple, and minimizing exposure to room air (44, 48). Diluents must be checked for nickel content by the use of appropriate method blanks.

ii) Analytical

Inadequate analytical sensitivity may be a problem, especially in control samples. This should become apparent with appropriate quality control.

(h) Reference to the most comprehensive description of the method and evaluation

Direct measurement of nickel in urine by ET-AAS with Zeeman correction has been described in detail by Sunderman Jr. et al. (41) and reviewed by the same laboratory (48). A critical appraisal of sources of pre-analytical errors has recently appeared (45). Templeton et al. (54) reviewed the analytical literature on nickel in human blood and urine, as part of the TRACY project (55) on reference values of trace elements in body fluids. Six publications from five different laboratories were considered useful for the purposes of establishing reference values of nickel in urine. Of these five labs, one (56) used Zeeman-corrected ET-AAS after chelate extraction. The other four used direct measurement with variations on the method described here, two employing Zeeman correction (41, 47, 52) and two D₂-arc correction (51, 53).

1.4.7.4 Other analytical methods

No other method has been adequately validated for the direct determination of nickel in urine, at least at the levels expected in populations not occupationally exposed to the metal and its compounds (57). Neutron activation analysis was used as a confirmatory method in one large study (52), but no details were given and the sensitivity of the method for nickel is poor (58). Voltammetric methods promise great improvements in detection limits (59, 60), but the requisite sample preparation results in significant blanks (57). Detection limits of about 1 µg Ni/L are reported for analysis of urine by inductively coupled plasma-atomic emission (61) and inductively coupled plasma-mass spectrometry (62).

1.4.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Reported concentrations of nickel in urine of healthy individuals without known occupational exposure have recently been critically reviewed with assessment of the care exercised both in sampling and analysis (54). Sunderman Jr. et al. (41) reported a value of 2.0 ± 1.5 (0.5–6.0) µg/L [mean $\pm$ s.d. (range)] in Americans, and in a later study (47) 1.5 ± 0.2 (< 0.5–4.6) µg/g creatinine. A study of 878 Italians found a mean of 0.9 µg/L (range 0.1–3.9) (52). A French study reported 1.59 ± 1.67 µg/g creatinine (56). Slightly higher values were found in Finland (geometric mean 4.1 µg/L) (53) and China (3.2 ± 1.7 µg/L) (51). We conclude that the best estimate of the value of urine nickel expected in non-occupationally exposed people is < 1–4 µg/L.

(b) Published biological action levels

There are no published BATs or BEIs for nickel and its compounds. The Finnish Institute of Occupational Health has set a Biological Action Level (BAL) of 76 $\mu\text{g Ni/L}$ (1.3 $\mu\text{mol/L}$) in a urine sample collected after the shift at the end of the workweek (63). The Deutsche Forschungsgemeinschaft does not list BAT and MAK values for nickel compounds, considering them instead to be carcinogenic, precluding assessment of safe tolerance values (64). Rather, they define a technical exposure limit (TRK-) as an airborne level which is attainable with current technologies to serve as a guideline for necessary protective procedures. The TRK for metallic and insoluble forms of nickel is 0.5 mg/m^3 . For nickel compounds in inspirable droplets it is 0.05 mg/m^3 .

Angerer et al. reported on small groups of workers exposed to various nickel compounds or to Co and nickel salts together (42). The latter had a mean urine nickel of 58 $\mu\text{g/L}$ at a nickel exposure level of 0.03 mg/m^3 . The former had mean nickel exposures of 0.67 mg/m^3 that produced a mean urine nickel of 93 $\mu\text{g/L}$. In contrast, 103 welders exposed to insoluble nickel at 0.09 mg/m^3 in welding fumes had a mean urine nickel of only 15 $\mu\text{g/L}$ (42). The timing of collection was not described. The latter result is in keeping with the DFG "exposure equivalents for carcinogenic working materials" (EKA) values for nickel metal, oxides, sulfides and carbonate, of 15 $\mu\text{g/L}$ at an exposure of 0.10 mg/m^3 , rising to 30 and 45 $\mu\text{g/L}$ at 0.30 and 0.50 mg/m^3 , respectively (64).

Lauwerys and Hoet (11) have reviewed the earlier literature on nickel exposure in the electroplating and refining industries. There, exposures to soluble nickel compounds correlate well with end-of-shift urine and serum nickel, which also correlate with each other. A time-weighted average (TWA) exposure of 0.1 mg/m^3 soluble nickel produces a urinary nickel concentration of about 70 $\mu\text{g/L}$ (1.2 $\mu\text{mol/L}$). However, for welders Angerer and Lehnert (43) suggest that the limit value for nickel in urine should lie between 30 and 50 $\mu\text{g/L}$ (0.51 and 0.85 $\mu\text{mol/L}$), corresponding to an exposure of 0.5 mg/m^3 . The imprecision of this value reflects the variation in the parameter when exposure is not confined to soluble nickel compounds.

Exposure limits are generally in keeping with the above guidelines. The American Conference of Governmental Industrial Hygienists (ACGIH) defines threshold limit values (TLV) based on the TWA exposure during a 5-day, 40-h workweek. The ACGIH has proposed revising the TLV-TWA downwards to 0.05 mg/m^3 for nickel metal, soluble, and insoluble compounds (65). Switzerland has an occupational exposure limit for soluble nickel compounds of 0.05 mg/m^3 ; in Canada and the United Kingdom the value is 0.1 mg/m^3 . Values for insoluble compounds are generally higher, e.g. 0.5 to 1 mg/m^3 in the Netherlands, Finland, United States and the United Kingdom (66, 67).

(c) Non-analytical interferences

i) Other exposures

Exposures from medical and dental prostheses and cigarette smoke can both increase serum (and presumably urine) nickel, but are likely insignificant in relation to intake from diet and drinking water (45).

ii) Diet and environment

Dietary intake of nickel is variable, and can give rise to observable changes in urinary nickel measurements. Formulation of a diet naturally high in nickel gave rise to an approximately three-fold increase in 12 h urinary nickel excretion after 4 days (38). Residence in a contaminated environment of course represents additive exposure. In the USA, rural air typically contains 6 ng Ni/m^3 , while in urban air the concentration is about 25 ng/m^3 (9). Values up to 170 ng/m^3 occur in some industrial centres (8). Seasonal variation can occur due to increased burning of fossil fuels during winter months. Nickel in soil varies between about 5 and $50 \text{ } \mu\text{g/g}$ depending on geological factors, while various unpolluted water supplies generally have nickel concentrations in the range of 1 to $50 \text{ } \mu\text{g/L}$ (49). Water supplies in industrially polluted areas can have more than 1 mg Ni/L . Higher exposures in drinking water that give rise to higher serum levels (see below, section 1.4.8.5 c.ii) will also presumably increase urine levels.

(d) Sampling representative of recent or long-term exposure

Because nickel in the circulation is cleared in the urine over several days (15, 28), spot urines will reflect recent exposures to soluble nickel. A post-shift urine will reflect exposure during the workday, and pre-shift urinary nickel may increase during the work-week and decline again over a weekend. With exposure to insoluble nickel compounds, urine will again reflect absorption, but doses and rates are not predictable. Elevated concentrations can occur even years after occupational exposure in those with a significant lung burden of nickel.

(e) Ethnic differences

No ethnic differences in nickel absorption, excretion, or susceptibility have been described.

1.4.8 Nickel in serum

1.4.8.1 Toxicokinetics

After oral ingestion of soluble nickel compounds by fasting humans, serum levels peak at 2–3 h and return to baseline after 72 h (15, 28, 68). Sunderman Jr. et al. (68) have reported rate constants for transport of nickel from serum to urine and serum to tissues of $0.21 \pm 0.05 \text{ h}^{-1}$ and $0.38 \pm 0.17 \text{ h}^{-1}$, respectively, with a back rate constant from tissue to serum of $0.08 \pm 0.03 \text{ h}^{-1}$. Less is known about serum clearance after inhalation. After inhalation of soluble nickel by electroplaters, the half-time for plasma nickel was 20–34 h (69).

1.4.8.2 Biological sampling

(a) Sampling time and specimen

If concern is with exposure to soluble nickel during the preceeding shift, then urine will be sufficient. Serum is a useful adjunct to urine for long-term exposure and exposure to slowly released insoluble nickel. In this scenario, timing of collection is not important,

but may conveniently be done at the time of urine collection at the end of a shift. Whole blood should be allowed to clot and the serum transferred with a cleaned plastic pipette to a second tube. Plasma analysis is not recommended as the requisite use of anti-coagulants is a problematic source of nickel contamination. Collection with the subject reclining is preferred to standardize distribution of non-diffusible blood components, and use of a tourniquet is to be avoided in order to minimize haemolysis (70).

(b) Contamination possibilities

Contamination from the work environment is less likely than for urine since blood is collected directly into a syringe. However, immediate transfer to an acid washed (and therefore not evacuated) tube requires brief opening of the tube on location, and this should be done in a clean area removed from the atmosphere of the workplace. The skin must be washed thoroughly before collection; sweat is a major source of nickel, and nickel will concentrate on the skin as sweat evaporates during a workshift. Furthermore, contamination from the collection and sample processing apparatus is a major concern for serum nickel – even more so than for urine nickel. For example, significant contamination from plastic tubes, pipette tips, etc. has been carefully documented (44) and careful acid washing of both the syringe and the sample container is mandatory.

(c) Sampling device and containers

Blood should be collected into an acid-washed polypropylene syringe and transferred to an acid-washed polyethylene tube. Although use of a stainless steel needle is sometimes thought to preclude analysis of serum for nickel (8, 58), excellent results have been obtained in one study by flushing a stainless steel needle first with a pre-collection specimen (44). This question requires further evaluation (54). A needle of 19 gauge or greater is preferred to minimize haemolysis.

(d) Preservatives, shipment, and stability

Samples should be shipped as described for urine and stored at -20°C. No preservatives are added.

1.4.8.3 Recommended analytical method

The recommended method of measurement of nickel in serum is electrothermal atomic absorption spectrometry (ET-AAS). The earlier IUPAC reference method involved chelate extraction (50), but this has been superseded by direct methods. Protein precipitation has been advocated (48), but excellent sensitivity and results have been obtained by direct dilution (44) and this latter procedure is recommended here.

(a) Principles of the method

Serum is diluted with 1/3 volume of 1% Triton X-100 in 1 mM HNO₃ and subjected to direct analysis by ET-AAS at 232.0 nm, with Zeeman background correction.

(b) Reagents required

High purity HNO_3 (Merck 'Suprapur', BDH 'Aristar', or equivalent) and Triton X-100 are the only reagents required.

(c) Equipment required

An electrothermal atomic absorption spectrometer with Zeeman background correction and equipped with an autosampler is required. Pyrolytically coated graphite tubes are used. Access to a class 100 air work area (e.g. a HEPA-filter hood) is strongly recommended.

(d) Procedures

i) Calibration

The instrumental response is calibrated with diluted serum containing known amounts of nickel added in the Triton X-100/ HNO_3 diluent. Nycomed Seronorm and NIST SRM 8419 are reference sera with certified nickel concentrations of 3.2 and $1.8 \pm 0.6 \mu\text{g/L}$, respectively, but the NIST value is not found by all authors (44). The Second Generation Biological Reference Material (Freeze-dried Human Serum) available from Dr. J. Versieck has a more 'natural' level (recommended value $0.23 \mu\text{g/L}$) (71).

ii) Procedure

1.5 mL of serum is transferred to a plastic tube and 0.5 mL of diluent (1% Triton X-100 in 1 mM HNO_3) is added. These procedures should be carried out in the clean-air environment. The sample is analysed directly by atomic absorption at 232.0 nm, with Zeeman background correction. A total of 100 μL is injected into the furnace by auto-sampler in two 50 μL aliquots with drying in between. Furnace parameters should be optimized in each laboratory.

(e) Criteria of analytical reliability

i) Trueness

Accuracy at $0.23 \mu\text{g/L}$ was demonstrated with the Versieck reference material (44). Similar accuracy has been achieved after protein precipitation instead of simple dilution (47) with recovery of $96.9 \pm 2.7\%$ at $8 \mu\text{g/L}$ (72).

ii) Precision

Within-run relative standard deviations of about 3% can be expected at natural concentrations of nickel (44, 48). Between-batch imprecision of 8.1% was found at $3.5 \mu\text{g/L}$ (72).

iii) Detectability

A detection limit (defined as blank + 3s) of $0.06 \mu\text{g/L}$ was found for the specific protocol described here (44).

(f) Quality assurance

i) Special precautions

In the absence of proficiency testing programmes, pooled serum should be analysed as an internal quality control. Performance should also be assessed against the available reference materials.

ii) Interferences

As for urine, Zeeman background correction is important for minimizing interferences.

(g) Sources of possible errors

i) Pre-analytical

As with urine nickel, the greatest risk of pre-analytical error in serum is contamination of the sample, both during collection and during sample preparation in the laboratory. Collection tubes and diluents must be checked for nickel content by the use of appropriate method blanks. In addition, the adequacy of stainless steel needles for collection is still an open question (see above), and it would be prudent to check periodically a second post-flush sample for agreement with the first, if such needles are used.

Several authors (44, 48) have stressed the importance of carrying out all the procedures in a clean-room facility. However, collection under such circumstances is not feasible for monitoring purposes, and monitoring laboratories will generally not have access to such facilities. By carrying out as much sample manipulation as possible in a class 100 hood, capping the tube to avoid contamination with metal particulates arising from the centrifuge rotor, and minimizing the time of exposure to room air before analysis, reasonable results should be achieved even at the concentrations expected in serum from people without occupational exposure.

ii) Analytical

Inadequate analytical sensitivity may be a problem, especially in control samples. This should become apparent with appropriate quality control.

(h) Reference to the most comprehensive description of the method and evaluation

The protocol described here is based on the work of Nixon et al. (44) and is in general principles similar to the method reviewed by Sunderman Jr. et al. (48) except that dilution replaces deproteinization. A critical appraisal of sources of pre-analytical errors has recently appeared (45). As part of the TRACY project on reference values of trace elements in body fluids we have also reviewed the data on nickel in serum (54). Eight publications from five different laboratories were considered useful for the purposes of establishing reference values of nickel in serum. All used Zeeman-corrected ET-AAS (44, 47, 56, 73–77).

1.4.8.4 Other analytical methods

No other method has been adequately validated for the direct determination of nickel in serum at the levels expected in people not occupationally exposed to the metal and its

compounds (57). As noted above, the sensitivity of neutron activation analysis for nickel is poor (58). Voltammetric methods have not been employed for routine measurement of serum nickel. The detection limit by ICP-MS is above the serum nickel concentration expected in unexposed subjects (62).

1.4.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

The reported concentration of nickel in serum has declined in recent years as analytical sensitivity by AAS has improved and the importance of contamination has been recognized. Gammelgard and Veien (77) found 0.14 ± 0.15 $\mu\text{g Ni/L}$ (mean $\pm$ s.d., range 0.10–0.79) in Danish control subjects, consistent with the values of 0.14 ± 0.09 $\mu\text{g/L}$ (44) and 0.2 ± 0.1 (mean $\pm$ s.e., range < 0.05 –1.0) (47) found in Americans.

(b) Published biological action levels

Published TLV and TRK levels for nickel are given above (Section 1.4.7.5 b). EKA values have not been proposed for serum. However, serum or plasma nickel levels are felt by some authors to be less variable than urinary levels when exposures are to insoluble nickel compounds. Whereas serum and urine concentrations correlate well after exposure to soluble compounds (11), the correlation is poor after exposure to welding fumes (43). However, while exposures to soluble compounds produce higher serum levels than those to insoluble compounds, the differences are less than noted above for urine. For example, whereas exposures to 0.03 mg/m^3 nickel salts produced about four times the mean urinary nickel found following exposure to 0.09 mg/m^3 nickel in welding fumes, the corresponding plasma values were $3.3 \mu\text{g/L}$ for the salts and $4.8 \mu\text{g/L}$ for the fumes (42). In the absence of dose-response relationships for all nickel compounds, individual authors have made recommendations that serum or plasma nickel values be kept below $10 \mu\text{g/L}$ [170 nmol/L] (78) or even $5 \mu\text{g/L}$ [85 nmol/L] (11). A TWA exposure to 0.1 mg/m^3 of soluble nickel gives an end of shift plasma nickel of $7 \mu\text{g/L}$ [119 nmol/L] (79).

(c) Non-analytical interferences

i) Other exposures

As for urinary nickel, other non-occupational exposures are likely to be insignificant in relation to intake from diet and drinking water (45). Among several health-related factors that have been suggested to increase serum nickel, renal dialysis and acute myocardial infarction seem best documented (45).

ii) Diet and environment

After dietary intake, nickel concentrations in serum and urine are correlated; the comments made above for urine apply equally to serum. Hopfer et al. (76) have compared serum nickel concentrations in healthy hospital workers in the USA ($n=43$) and Canada ($n=22$). The Canadian cohort lives in a community of nickel mines and smelters, and is exposed to higher concentrations of nickel in tap water and soil. These people had

higher concentrations of nickel in their serum (0.6 ± 0.3 $\mu\text{g/L}$ in Sudbury, Canada versus 0.2 ± 0.2 $\mu\text{g/L}$ in Connecticut, USA, $p < 0.05$).

(d) Sampling representative of recent or long-term exposure

As with urine, end-of-shift serum nickel values will reflect exposures to soluble nickel during the workday, may rise during the week, and will be correlated with urine values. In the case of exposure to insoluble compounds, serum nickel levels may or may not rise, depending on absorption, and no strong correlation with urinary nickel can be expected. With insoluble compounds, serum values may be less variable than urinary nickel concentrations, and therefore more reliable an indicator of exposure, although dose-response information will not be obtained. Increased serum nickel concentrations have been observed many years after removal of occupational exposure in those with a significant lung burden of particulate nickel (12).

(e) Ethnic differences

No ethnic differences in nickel absorption, excretion, or susceptibility have been described.

1.4.9 Nasal mucosal biopsy

Long-term inhalation of insoluble nickel particulates can be reflected in the nickel content of nasal mucosal biopsies (12). However, there is insufficient information to establish reference levels or interpret exposures, and this invasive procedure should be considered only for research purposes.

1.4.10 Research needs

There is no clear relation between health effects and increased serum or urine nickel following chronic exposure to nickel and its compounds. Furthermore, because serum and urine nickel do not necessarily increase after exposure to insoluble compounds, another means of biological monitoring would be desirable. With both soluble and insoluble nickel compounds, threshold levels for increased carcinogenic risk are not known; the proposed reduction in the ACGIH TLV to 0.05 mg/m^3 for all nickel compounds was an act of caution rather than a response to clear scientific data. There seems to be little information on the potential risk to the fetus of maternal exposure, and it is not clear whether the same exposure limits should apply to women of child bearing years. Genotoxicity (80) has been assessed by measuring sister chromatid exchange in research protocols (81), but such investigations have not yet generated epidemiological data and are not presently available for monitoring.

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(see Chapter 1.3)

Chapter 2. Biological monitoring of selected solvents

2.1 Benzene

2.1.1 Introduction

Benzene is one of the most extensively studied solvents, and several comprehensive reviews have been published on its toxic effects, carcinogenicity, metabolism, and kinetics (1–3). Biological monitoring of occupational exposure to benzene is well established (4–6).

2.1.2 Physical-chemical properties (2)

The chemical abstract service (CAS) number of benzene is 71-43-2. At room temperature, benzene is a clear, colourless liquid. Benzene has a characteristic odour. Physical chemical properties of benzene have been listed in table 2.1.1.

Table 2.1.1. Identity and physical-chemical properties of benzene

Chemical structure	
Chemical formula	C ₆ H ₆
Relative molecular mass	78.11
Flash point	-11.1°C
Flammable limits	1.3–7.1%
Melting point	5.5°C
Boiling point	80.1°C (at 760 mmHg)
Relative density d ₄ ²⁰	0.8787
Relative vapour density	2.7
Odour threshold	4.8–15 mg/m ³
Solubility	Water: 1.8 g/L; miscible with acetic acid, acetone, chloroform, diethyl ether, ethanol
Log (n-octanol/water partition coefficient)	1.56–2.15
1 cm ³ /m ³ (20°C, 760 mmHg)	3.2 mg/m ³
1 mg/m ³ (20°C, 760 mmHg)	0.31 cm ³ /m ³

2.1.3 Possible occupational and non-occupational exposures

Benzene has been extensively used as a solvent in rubber and leather industries in the past. At present, however, it seems that its use is less extensive, and even the benzene

content of solvent mixtures has become lower although still in the 1980s high concentrations of benzene in workplaces have been reported (7). Occupational exposure still occurs in petroleum refining, transport and distribution of fuels notably gasoline, in the maintenance work of gasoline-powered engines, as well as in the manufacture of benzene (2, 8, 9).

Benzene is ubiquitous in the environment, the main sources being evaporation from gasoline, which contains some 1–5% benzene, the amount varying in different countries. Benzene is also present in exhausts from automobile engines, as well as cigarette smoke and smoke from other combustion processes (2).

2.1.4 Summary of toxicokinetics

Benzene – as a lipid soluble chemical – is distributed in the organism in different compartments, mainly depending on the lipid content of the organs. Its distribution has traditionally been described based on two or three compartments (2, 10); physiologically-based pharmacokinetic (PBPK) models have also been developed (11–13).

2.1.4.1 Absorption

(a) Inhalation

Inhaled benzene is readily absorbed; the pulmonary retention stays at approximately 50% for several hours at exposures between 2–100 cm³/m³ (10, 14, 15).

(b) Dermal

It was estimated from *in vitro* studies using human skin that dermal absorption from gaseous benzene contributed rather little to total absorption, but that absorption from liquid benzene could be a significant route of exposure (16). In confirmation of the latter finding, it was observed that the dermal route was the main route of absorption of benzene among garage workers with exposure to liquid gasoline (17).

(c) Gastrointestinal

Gastrointestinal absorption of benzene has led to acute intoxications. This suggests effective absorption, although quantitative data in humans are lacking (2).

2.1.4.2 Metabolic pathways and biochemical interactions

Benzene is oxidized primarily in the liver by the cytochrome P-450-dependent monooxygenase to benzene oxide. Although several cytochrome P-450 isozymes may catalyse this reaction, at low levels of exposure the ethanol-inducible isozyme CYP IIE1 seems to be mainly responsible (1). After this initial reaction, several secondary metabolites are formed enzymatically and non-enzymatically; benzene metabolism is illustrated in figure 2.1.1.

Glucuronide and sulphate conjugates of phenol are the major urinary metabolites of benzene. Others include conjugates of catechols and quinol, mercapturic acids, *trans*, *trans*-muconic acid, and the reaction product of benzene with guanine, N-7-phenyl-guanine (See figure 2.1.1.) (1, 2, 18).

Because several other chemicals are metabolized by the same enzyme systems, it is to be expected that simultaneous combined exposures may result in metabolic interactions; it has been reported that in workers exposed both to toluene and benzene, the metabolism of benzene to *t,t*-muconic acid, phenol, quinol (but not to catechol) was decreased (19, 20).

2.1.4.3 Distribution

The limited solubility in water and preferential partition in the lipid phase lead to accumulation of benzene in the fat and fatty tissues (2). After inhalation exposure to 1600 mg/m³ benzene for six hours, the concentration of benzene in bone marrow was 3.3, and that in adipose tissue, 14.2 times higher than in the blood in rats (21).

2.1.4.4 Elimination

Benzene is mainly excreted in the urine as metabolites, notably conjugates of phenol with glucuronic and sulphuric acids, and into exhaled air, in unchanged form. It was estimated that after occupational exposure to benzene at a level of 100 cm³/m³, 13.2, 10.2, 1.9, 1.6, and 0.5% of the absorbed amount was excreted in the urine after the working hours as phenol, quinol, *t,t*-muconic acid, catechol, and 1,2,4-benzenetriol, respectively (22). The proportion of benzene absorbed excreted via exhalation was 8–17% (10, 15, 23). Small amounts of unmetabolized benzene have also been detected in the urine (24, 25).

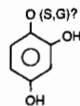
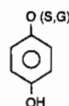
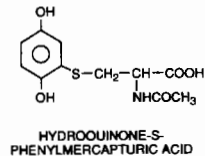
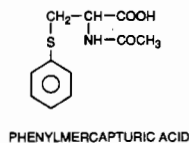
The elimination of benzene at levels of occupational exposure encountered at present, follows first order kinetics, with 2–3 consecutive half-times corresponding to the disposition of benzene in different body compartments (2). The shortest half-times reported are approximately 10–15 min, the intermediate 40–60 min, and the third 16–20 h (5, 23). In a more extensive follow-up study, even longer half-times have been observed (23, 26).

2.1.5 Summary of toxic effects

Exposure to very high concentrations of benzene by inhalation or large oral doses leads to central nervous system depression and eventually to death. In long-term occupational exposure depression of different bone marrow cell lines (thrombocytopenia, anaemia, granulocytopenia and aplastic anaemia) may be life-threatening. Benzene causes leukaemia; the risk seems to be highest for the myelogenous variety (2, 3, 27).



O (S,G)

CC(=O)N[C@@H](Cc1ccccc1O)C(=O)ONc1nc(O)c2nc(N3C=CC=CC=C3)n21
N-7-PHENYLGUANINE
$$\begin{array}{ccccccc} & & \text{H} & & \text{H} & & \\ & & | & & | & & \\ \text{HOOC} & - & \text{C} & = & \text{C} & - & \text{C} & = & \text{C} & - & \text{COOH} \\ & & | & & | & & \\ & & \text{H} & & \text{H} & & \end{array}$$

TRANS,TRANS-MUCONIC ACID

Figure 2.1.1. A. Metabolism of benzene (1, 2). Note that the pathways leading to the formation of catechol from phenol and of 1,2,4-trihydroxybenzene from catechol could not be verified in a study in rabbits (18). B. Urinary metabolites of benzene.

2.1.6 Biological monitoring indices

Table 2.1.2. *Biological monitoring of exposure to benzene*

Indicator	Suitability	Ref.
Benzene in blood	Specific, sensitive	10, 28–31
<i>trans, trans</i> -Muconic acid in urine	Reasonably specific, sensitive	19, 30–37
Phenylmercapturic acid in urine	Specific, sensitive, sophisticated methodology	31, 38–40
Benzene in urine	Specific, sensitive; limited experience	24, 25
Benzene in exhaled breath	Specific, sensitive; limited practicality	10, 28, 41–44
Catechol in urine	Limited experience	47
Quinol in urine	Limited experience	47
Benzenetriol in urine	Limited experience	22
Phenol in urine	Non-specific, insensitive	10, 19, 34, 45, 46
Protein adducts	Insensitive; sophisticated methodology	48–50
Chromosome aberrations in lymphocytes	Non-specific, insensitive	51, 52

Benzene in blood is perhaps the best approach available at present (table 2.1.2). However, the sampling is somewhat invasive, and the short half-time immediately after the exposure is problematic. *trans, trans*-Muconic acid in urine is sensitive, and reasonably well validated, but a recent study suggests that the present methods may not have adequate specificity. Urinary phenylmercapturic acid seems to be specific and sensitive, but at present requires sophisticated methodology. Perhaps for the future, the most promising approach is the analysis of benzene in the urine, although at present the experience is very limited.

2.1.7 Benzene in blood

2.1.7.1 Toxicokinetics

The elevated concentration of benzene in the blood decreases after the exposure first with a half-time of approximately 10–15 minutes, then approximately 40–60 minutes, and thereafter approximately 16–20 hours (5, 23).

2.1.7.2 Biological sampling

(a) Sampling time

Optimal timing of specimen collection is 16 h after the cessation of the exposure, i.e., in the morning after the exposure, and before the new exposure. When the exposure is low,

at or below $1 \text{ cm}^3/\text{m}^3$ 8-h TWA, the sensitivity of the method may not be sufficient, and the sample has to be collected at 60 ± 10 min after the exposure, e.g. after the lunch hour.

(b) Specimen

10 mL of whole blood.

(c) Contamination possibilities

Vacuum tubes (or their caps) may contain chemicals which give rise to a signal with the photoionization detector, and show similar chromatographic elution as benzene. Before using them, the absence of this contamination has to be verified.

When collecting morning specimens it is important to verify that the workers have not been exposed to benzene after the cessation of the previous workday, and especially in the morning of sample collection.

(d) Sampling device and container

Heparinized glass vial; take the vial as full as possible, and mix well.

An alternative, probably superior sample collection procedure has been published (29, 31, 46), in which approximately 5 mL of the blood sample is immediately after collection transferred into a 20 mL headspace vial. The sample is reportedly stable, and can be transferred as such to the automatic sampler of the head-space gas chromatograph. Before use for sampling, the vials and the Teflon-coated ribbon caps have to be heated at 100°C for at least a week.

(e) Anticoagulant

Litium heparin; citrate gives identical results.

(f) Preservative

None.

(g) Shipment

By mail at room temperature. Time in mailing not to exceed 2 days.

(h) Stability

Two days at room temperature.

2.1.7.3 Recommended analytical methods

(a) Principle of the method

Benzene is analysed gas chromatographically using the headspace technique and photoionization detection.

(b) Reagents required

Benzene (pro analysi or similar); fluorobenzene (pa), ethanol 94% [Not absolute ethanol which contains benzene].

Absence of interfering chromatographic peaks (coeluting with benzene or fluorobenzene) in the water and ethanol used must be checked for each batch before use.

(c) Equipment required

Gas chromatograph with an automatic head-space sampler and photoionisation detector and integrator. The separation is achieved using two successive capillary columns: methylsilicone 30 m x 0.53 mm, film thickness 2.65 μm combined through a glass-covered metal tube with a 5% phenylmethylsilicone column (30 m x 0.53 mm, film thickness 2.65 μm). Analytical balance (0.01 mg).

*(d) Procedures**Gas chromatographic conditions:*

Sampling: Cycle 30 min, thermostation 80°C, 23 min, pressurization time 1.5 min, injection time 0.1 min, syringe elevation time 0.2 min, sample transfer line temperature 120°C. Gas flow (nitrogen) 12.5 mL/min. Chromatography: 40°C (4 min) – 10°C/min – 100°C (1 min) – 30°C/min 120°C (3 min) 10°C/min 200°C/min. Detector temperature 120°C.

Preparation of standards

Internal standard. (Used for checking successful analytical procedure, not for quantitation). Stock standard, 1 μL of fluorobenzene in 20 mL of 94% ethanol, can be stored until used at -20°C. Working internal standard: 100 μL of the base standard in 500 mL water.

Benzene standard. For accurate delivery of volatile liquids, the exact amounts used in the preparation of the stock standards are determined by weighing. First stock standard (approximately 13 mmol/L): 1/1000 (v/v) benzene in 94% ethanol. Distributed in tightly sealed vials the standard may be stored at -20°C for 6 months. Second stock standard (approximately 0.3 $\mu\text{mol/L}$): 3 parts of stock standard I diluted in 100000 parts (v/v) of water. Working standards, covering the concentration range of approximately 4–300 nmol/L are prepared from the II stock standard by diluting in water and adding the internal standard solution (2 mL). The working standards can be stored in refrigerator for a week.

Analytical procedure

An analytical run comprises nine standards and the samples as duplicates and a reagent blank in triplicate.

Sample preparation. The head space vials are cleaned immediately before use by blowing clean compressed air through them. 2 mL of the sample is transferred into headspace vial (within 5 h after their arrival in the laboratory) and 2 mL of the internal standard solution is added. In the head space vials the samples can be stored for a week in the refrigerator.

Quantification. The results are calculated from the standard curve using linear regression. The average recovery of benzene from blood – as compared to the aqueous standards – is 75%; this is taken into account in the calculation.

(e) Analytical reliability

i) Trueness

No blood-borne gasoline component, which gives rise to a signal in photoionization detector and coelutes with benzene in this elution system has been observed. The different recovery from blood specimens, as compared to aqueous standards, is corrected for by using an average recovery factor.

ii) Precision

The relative standard deviation of the method is 30% at the concentration level 3–17 nmol/L, 8% at the level 50–100 nmol/L, and 6% at the level of 200–400 nmol/L.

iii) Detectability

1 nmol/L (signal-to-noise ratio 2).

(f) Quality assurance

The external quality assurance scheme of the German Society of Occupational Medicine has recently included blood benzene analysis in the programme.

i) Special precautions

No stable quality control specimens in blood matrix are available. New standard dilutions are checked using the old set before being taken into use. The response of the photoionisation detector is followed by recording the responses to standards. Benzene in the laboratory air may contaminate the analysis; use of benzene or benzene-containing solvents, as well as smoking cannot be allowed in the facilities.

(g) Sources of possible errors

i) Preanalytical

Benzene or compounds coeluting with benzene may leach from reduced-pressure blood-collection vials (53).

ii) Analytical

Although the photoionization detection and use of two different 30 m capillary columns in succession make interferences by other volatile hydrocarbons unlikely (and none have been detected so far), it is possible that complex mixtures may contain chemicals

that interfere with the analysis, and the identity of unexpected large peaks should be verified by mass spectrometry.

(h) Reference to the most comprehensive description of the method

The method has been described in the reference (28). The method is specific to benzene. It has a detectability that allows measurement of blood benzene after 8-h time weighted average exposure to approximately $1 \text{ cm}^3/\text{m}^3$ of benzene in a morning specimen. A similar detectability cannot be achieved by using the less specific but more commonly used flame ionisation detector.

2.1.7.4 Other analytical methods

Blood benzene has been analysed using dynamic head space sampling combined with cold trapping (purge & trap) and quantitation with capillary gas chromatography-mass spectrometry (54, 55), by purge and trap combined with gas chromatography-high resolution mass spectrometry (53), by purge and trap combined with capillary chromatography-flame ionisation detection (29, 31, 56).

2.1.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Data on reported values for the blood benzene in occupationally non-exposed people have been collected in table 2.1.3. They seem to be generally below 8 nmol/l among non-smokers, and below 15 nmol/L among smokers.

Table 2.1.3. Reported values for the blood benzene in occupationally non-exposed people

Non-smokers (nmol/L)			Smokers (nmol/L)			Ref.
N persons/samples	Median/ Mean	SD	N persons/samples	Median/ Mean	SD	
13/13	2.4/2.8	1.2	14/14	6.3/7.0	2.5	54
8/40	2.1/2.3	0.8	2/10	2.3/2.7	1.1	29
293/293	2.1/2.6	3.4	138/138	3.7/4.9	4.3	55
6/6	NR/0.8	0.8	3/3			10

NR = Not reported

(b) Basis for biological action levels

Eight-hour time-weighted exposure to $10 \text{ cm}^3/\text{m}^3$ of benzene was found to give a benzene concentration of 20 and 200 nmol/L in blood collected 16 h, or 1 h after exposure (10). In an after-shift specimen, blood benzene concentrations corresponding to 8-h TWA exposures of 0.3, 0.6, 1.0, 2, 4 cm^3/m^3 were estimated to be 0.9, 2.4, 5, 14, 38 $\mu\text{g/L}$ (0.01, 0.03, 0.06, 0.18, 0.49 $\mu\text{mol/L}$) (30).

(c) *Published biological action levels*

The Finnish Institute of Occupational Health gives a biomonitoring action level of 20 and 200 nmol/L for samples collected 16 h and 1 h after the exposure (6). The German exposure equivalent for carcinogenic chemicals (EKA) value is 5 µg benzene/L blood (64 nmol/L) in a specimen collected after the workday or workshift (30).

(d) *Non-analytical interferences*

i) Diet and environment

When morning samples are collected, exposure at the time of sample collection or immediately before it causes misinterpretation of the results. Cigarette smoke is an important source of benzene; the smokers should refrain from smoking on the day of sample collection.

ii) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication)

Benzene itself is measured and the analytical method is reasonably specific. Therefore chemicals other than benzene do not interfere with the interpretation. Because of the mutual inhibition of metabolism between benzene and toluene (20) people exposed simultaneously to both will have higher blood benzene levels than those exposed to benzene only at a similar level. Since the toxicity of benzene is thought to be caused by a metabolite (1), the health risks of people with combined exposure are not necessarily identical to those of persons with exclusive exposure to benzene but with same blood benzene concentration.

(e) *Sampling representative of recent or chronic exposure*

Concentrations of benzene in a specimen collected 1 h after the exposure reflect mainly the exposure during the 1–2 preceding hours (half-time in the order of 1 h), those in a specimen collected 16 h after the exposure mainly the exposure during the workday (half-time approximately 16–20 h).

(f) *Ethnic differences*

No ethnic differences in the disposition of benzene are known.

2.1.8 *trans, trans*-Muconic acid in urine

trans, trans-Muconic acid in urine was estimated to represent on average 1.9% of absorbed benzene (19). Several studies have indicated that there is a quantitative relationship between inhalation exposure to benzene, and the urinary excretion of *t,t*-muconic acid (19, 31–37, 46).

2.1.8.1 Toxicokinetics

Excretion of *t,t*-muconic acid in the urine showed a peak immediately after the exposure and decreased thereafter at a rate similar to that of urinary phenol, with a half-time of a few hours (32). A half-time of 6 h was reported in an abstract (57).

2.1.8.2 Biological sampling

(a) *Sampling time*

Optimal sampling time seems to be immediately after the workday.

(b) *Specimen*

Spot urine sample.

(c) *Contamination possibilities*

Since a metabolite is measured, contamination does not seem to be a problem.

(d) *Sampling device and container*

Polyethylene urine vials; no need for specific pretreatment.

(e) *Stability*

t,t-Muconic acid, at a level of 5 mg/L (35 μ mol/L), was stable in urine at room temperature for two weeks (32); samples with a low concentration were stable for one week but showed a 10–30% decrease after 2 weeks (35).

2.1.8.3 Recommended analytical methods

(a) *Principles of the method*

t,t-Muconic acid is extracted from the urine by an ion-exchange cartridge, and analysed by reversed phase liquid chromatography using ultraviolet detection.

(b) *Reagents required*

Anion-exchange extraction cartridges (e.g. Bond-Elut SAX), acetic acid (HPLC grade), methanol (HPLC grade), water (HPLC grade). The mobile phase in the chromatography is methanol in 0.1% phosphoric acid; it is filtered before use.

(c) *Equipment required*

HPLC with variable wavelength UV-detector and an integrator. The column used is a 200 x 4.6 mm 5 μ m C-8 reversed phase column (e.g. MOS Hypersil C8, Shandon Scientific, Ltd.)

(d) *Procedures*

The Bond Elut SAX cartridges are washed successively with 3 mL of methanol and 3 mL of water. 1 mL of the urine sample is applied on the cartridge, it is washed with 3 mL of 1% aqueous acetic acid, and *t,t*-muconic acid is eluted with 3 mL of 10% acetic acid and 1 mL of water. The volume is filled to 5 mL with water. 10 μ L is injected using loop injection. A flow rate of 1 mL/min, and detector wavelength of 259 nm are used.

The concentration of *t,t*-muconic acid in the specimen is calculated using peak heights from a standard curve of six standards, plus a blank, covering the concentration range 7–175 μ mol/L and prepared in urine from people not exposed to benzene.

No comprehensive studies are available on whether measured concentrations as such, or those corrected to a common relative density, or to creatinine excretion are preferable.

(e) *Analytical reliability*

i) *Trueness*

The recovery from urine is about 90%. Recently it was reported (37) that apparently elevated levels of *t,t*-muconic acid detected in the urine by using this method could not be verified by gas chromatography-mass spectrometry; no information was provided on the identity of the interfering chemical. This indicates that the method is not fully specific.

ii) *Precision*

Repeatability (relative standard deviation) as tested with samples with concentration levels < 7 μ mol/L is 25%, and at levels 7–70 μ mol/L, is 5.2%.

iii) *Detectability*

Detectability (signal to noise ratio of 2) is 0.7 μ mol/L.

(f) *Quality assurance*

No certified materials are available, but pooled samples from exposed people can effectively be used for internal quality control. *trans, trans*-Muconic acid is included in the external quality assurance scheme of the German Society of Occupational Medicine.

(g) *Sources of possible errors*

i) *Preanalytical*

Sorbic acid, which is used as a food additive, is in part metabolized to *t,t*-muconic acid.

ii) *Analytical*

As indicated above (See Trueness above), the method may not be fully specific. Unexpected high concentrations thus have to be verified by GC-MS.

(h) *Reference to the most comprehensive description of the method.*

Ducos P, Gaudin R, Robert A, et al. Improvement in HPLC analysis of urinary *trans, trans*-muconic acid, a promising substitute for phenol in the assessment of benzene exposure. *Int Arch Occup Environ Health* 1990;62:529–34 (slightly modified).

i) *Evaluation of the method*

The method has a good precision, and although the specificity may not be complete, interfering peaks seem to occur seldom in the urine of benzene-exposed workers. Although some non-exposed people exhibit measurable concentrations of *t,t*-muconic acid in the urine, the method is not sensitive enough for the quantitation of *t,t*-muconic acid in the urine of all non-exposed people.

2.1.8.4 Other analytical methods

The method of Inoue and co-workers (19) uses methanol precipitation before HPLC, and seems to be somewhat less sensitive. The method of Lee and co-workers (35) makes use of Dowex 1 anion exchange purification, and was reported to have superior sensitivity and repeatability at low concentrations. A GC-MS method employing pentafluorobenzylbromide as a derivatizing agent, has been described briefly (37). The isotope dilution gas chromatography-mass spectrometric method of Bechtold and co-workers (33, 58) has at least one order of magnitude better sensitivity than the liquid chromatographic methods and is capable of analysing reliably the *t,t*-muconic acid in the urine of non-exposed people. It could be a candidate for a reference method.

2.1.8.5 Guide to interpretation

(a) *Measured values in groups without occupational exposure*

The 97.5th percentile value for non-exposed factory workers in China was reported to be 1.4 mg/L (10 μ mol/L) ($n=213$) (19); the 95th percentile was reported to be 0.4 mg/L (2.8 μ mol/L) in a study in France (34), and 0.41 mg/gram creatinine in another study in Belgium (36). The mean (range) values for urinary *t,t*-muconic acid were 0.13 (0.03–0.33) mg/L [0.9 (0.2–2.3 μ mol/L)] in 23 non-smokers, and 0.25 (0.06–0.43) mg/L [1.8 (0.4–3.0 μ mol/L)] in 35 smokers (35). A German study reported a mean $\pm$ SD/median/95th percentile, and range for smokers to be 0.96 $\pm$ 1.84/0.29/1.81 and >0.5–8.70 mg/L [6.8 $\pm$ 12.9/2.0/12.7/<3.5–61.2 μ mol/L]. The corresponding figures for non-smokers were 0.44 $\pm$ 0.73/0.20/1.07 and <0.05–4.06 mg/L [3.1 $\pm$ 5.1/1.4/89.4/<0.4–28.6 μ mol/L] (37).

It is apparent that smoking has an effect on the concentration of *t,t*-muconic acid in the urine; in non-smokers the upper reference limit is approximately 3 μ mol/L, in smokers it is approximately 7 μ mol/L.

(b) *Basis for biological action levels*

From a linear regression line Inoue and co-workers (19) estimated that urinary *t,t*-muconic acid values corresponding to an eight-hour exposure to 1, 5, and 10 cm^3/m^3 ben-

zene are 4, 7.4 and 12 mg/L (28, 52, and 84 $\mu\text{mol/L}$). Corresponding values from the logarithmic regression line of Ducos and co-workers (34) are 1.0, 5.5 and 12 mg/L (7, 39, and 84 $\mu\text{mol/L}$). The most recent data (36) would indicate lower muconic acid concentrations in urine after exposure to benzene (0.8 and 1.4 mg/gram creatinine after an 8-h TWA exposure to 0.5 and 1.0 cm^3/m^3 , respectively).

(c) Published biological action levels

The German EKA (biological exposure equivalent) value corresponding to a TRK (technical guideline for occupational exposure to carcinogenic chemicals) of 1 cm^3/m^3 , is 2 mg/L (14 $\mu\text{mol/L}$) in after-shift specimen, and values corresponding 0.6, 2, 4, 6, 8 and 10 cm^3/m^3 are given as 1.6, 2, 3, 5, 7 mg/L *t,t*-muconic acid in the urine (11, 14, 21, 34, 48 $\mu\text{mol/L}$) (30).

(d) Non-analytical interferences

i) Diet and environment

Sorbic acid, which is used as a preservative in foods at levels between 0.01 and 0.5%, is metabolized to *t,t*-muconic acid. After an ingestion of 200 mg of sorbic acid, the peak urinary concentrations of *t,t*-muconic acid were approximately 0.6 mg/L (4.2 $\mu\text{mol/L}$) (32). In an abstract it was reported that change from one's normal to a (non-defined) standard diet decreased the urinary *t,t*-muconic acid concentration in volunteers (57).

ii) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

People exposed simultaneously to toluene and benzene had lower urinary *t,t*-muconic acid concentrations than those exposed to corresponding concentrations of benzene only (19).

(e) Sampling representative of recent or long-term exposure

Concentration of *t,t*-muconic acid in an after-shift urine specimen reflects the exposure to benzene during the preceding workday.

(f) Ethnic differences

None known.

2.1.9 Phenylmercapturic acid in urine

Phenylmercapturic acid excretion in the urine of benzene-exposed workers has been assessed using liquid chromatographic (39, 59) and mass spectrometric (31, 38, 40) methods. It was estimated that the proportion of benzene taken up by the lungs and excreted in the urine as phenylmercapturic acid was between 0.05 and 0.29% (40). Eight-hour exposure to a TWA concentration of benzene of 1 cm^3/m^3 was calculated to lead to an average concentration of 46 $\mu\text{g}/\text{gram}$ creatinine of phenylmercapturic acid in the urine in an after-shift specimen (40). A German group reported rather similar figures: after an average exposure to 0.8 cm^3/m^3 benzene, the average concentration of phenylmercaptu-

ric acid in an after-shift urine sample was 33 ± 56 $\mu\text{g}/\text{gram}$ creatinine (31). Analysis of phenylmercapturic acid in the urine is thus a promising approach for the biological monitoring of benzene exposure; however, GC-MS is still unavailable for many laboratories, and the HPLC method utilizing post column derivatization and fluorescence detection (39) – which reportedly has adequate sensitivity – has only been reported from one laboratory.

In Germany, and EKA-value, corresponding to the TRK of $1 \text{ cm}^3/\text{m}^3$ for phenylmercapturic acid concentration in an after-shift urine specimen is $45 \text{ } \mu\text{g}/\text{gram}$ creatinine (30).

2.1.10 Benzene in urine

The concentration of benzene in the urine voided after the workshift has been shown to be related to the concentration of benzene in breathing zone air (24, 25); the correlation was improved when smokers were excluded from the calculations. It is apparent that the method is quite promising: it is specific of benzene, and the physiological buffer function of the urine excretion alleviates the problems of the timing of sample collection that are quite important for blood sampling. However, at present the experience is quite limited, and the sample has to be collected in a glass vial in contrast to the traditional urine collections for the analysis of metabolites of chemicals in the urine.

2.1.11 Benzene in exhaled breath

Benzene in exhaled breath is in equilibrium with the blood benzene after the cessation of the exposure, and similar information may be derived from both analyses (10, 41–44). Similar analytical methods may also be applied (10). The sampling has to be standardized to catch either alveolar air only or mixed exhaled air. Because of problems in sample collection, stability, and standardization, the method has not been extensively used.

2.1.12 Catechol, quinol and benzenetriol in urine

Concentrations of catechol, quinol, and benzenetriol were elevated in workers exposed to benzene (22, 47). However, catechol and quinol were also observed in the urine of non-exposed people, and the analysis of the two could not identify persons exposed to benzene concentrations below $10 \text{ cm}^3/\text{m}^3$ (47). Urinary benzenetriol was calculated to represent approximately 0.5% of the amount of benzene inhaled; simultaneous exposure to toluene strongly decreased this proportion (22). The data so far are too scanty for the routine application of the analysis of benzenetriol in urine in the biological monitoring of benzene.

2.1.13 Phenol in urine

Phenol in urine has been used for the biological monitoring of benzene for decades. However, the highly variable urinary excretion of phenol without occupational exposure

– from presumably dietary constituents – makes this analysis highly insensitive. At exposure levels below approximately $10 \text{ cm}^3/\text{m}^3$, the exposed may no more be identified from among the non-exposed (10, 34, 45).

2.1.14 Protein adducts

S-Phenylcysteine adducts from benzene have been demonstrated in haemoglobin and albumin from experimental animals exposed to benzene (48, 49) using isotope dilution mass spectrometry. While such adducts could not be observed in haemoglobin in humans exposed to an average concentration of $28 \text{ cm}^3/\text{m}^3$ (48), the levels of albumin adducts were higher in humans exposed to an average of $23 \text{ cm}^3/\text{m}^3$ – but not to 4 or $8 \text{ cm}^3/\text{m}^3$. More recently, also N-phenylvaline adducts were studied using GC/MS among people exposed to benzene at an average level of $1 \text{ cm}^3/\text{m}^3$; no adducts could be detected (50). At present, it seems that these methods are not applicable for routine biological monitoring because of the sophisticated methodology involved, as well as limited sensitivity.

2.1.15 Chromosome aberrations

Benzene is clastogenic: it causes sister chromatid exchanges, micronuclei, and chromosomal aberrations in experimental animals, and several studies have reported chromosomal aberrations also in humans (1, 2, 60–63). However, no elevation in the frequency of chromosomal aberrations, in comparison to non-exposed referents, could be observed among workers, who were estimated to have, at some time, had a TWA exposure between 1 and $10 \text{ cm}^3/\text{m}^3$, and for whom short-time exposures were estimated to have exceeded 10, in part, $100 \text{ cm}^3/\text{m}^3$ (51). In the same population, there was an increase in the frequency of chromosome aberrations that was of borderline statistical significance. Most marked was the difference in gaps (52).

It seems that at the present levels of exposure, analysis of chromosomal aberrations – which is a very tedious task – is not applicable to routine biological monitoring of benzene exposure.

2.1.16 References

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2.2 Methanol

2.2.1 Introduction

Methanol is synthesized chemically, e.g. from methane or carbon monoxide. Because it is hydrophilic, its use has been increasing in various solvent preparations (1, 2), especially in water-based paints. Methanol has wide applications as an intermediate in various chemical synthesis, and also as a denaturant for ethanol. In some countries, methanol is used as automobile fuel. A number of cases of intoxication including blindness and even death were reported in the post-World War II period in association with the consumption of denatured alcohol, which was originally produced as fuel. A comprehensive review on toxicology is available (3).

2.2.2 Physical-chemical properties

Chemical formula	CH ₃ OH
Molecular weight	32.04
CAS No.	67-56-1
Vapour pressure (Pa)	21280 at 30°C
Conversion	1 ppm = 1.31 mg/m ³
Solubility	Miscible with water, alcohols, aromatics, chlorinated hydrocarbons, etc.
Log [P(o/w)]	-0.77

2.2.3 Possible occupational and non-occupational exposures

Occupational exposure to methanol takes place in association with its use, e.g. as a chemical intermediate and as a solvent. Because methyl esters, e.g. methyl acetate, can be readily hydrolysed *in vivo* (4), occupational exposure to such ethers will result in the formation of methanol in the body.

Methanol is formed physiologically *in vivo* (5), being present both in blood and in urine at low levels. Methanol is present in some home-use products, including antifreeze (6). Its use as a substitute for automobile gasoline has been increasing in some countries. Cases of methanol poisoning due to consumption of adulterated (methanol-containing) alcoholic beverages are well documented (7). Abuse (e.g. sniffing) of methanol-containing solvent preparations has been described (8–10).

2.2.4 Summary of toxicokinetics

2.2.4.1 Absorption

Experiences in clinical medicine and occupational health show that methanol is readily absorbed after inhalation or ingestion. In a volunteer exposure study, a lung retention of 58% was reported (11). Methanol in liquid form may be absorbed through intact skin

(12), and may increase urinary excretion of methanol to the level higher than the vapour concentration suggests.

2.2.4.2 Metabolic pathways and biochemical interactions

Methanol when absorbed will be enzymically oxidized stepwise to formaldehyde and then to formic acid (figure 2.2.1), the latter being considered to be responsible for metabolic acidosis and optic neuropathy (3, 13, 14). Formic acid thus formed will be further oxidized in part to carbon dioxide which will be subsequently eliminated in exhaled breath (14, 15). The oxidation of methanol *in vivo* is competitively inhibited by coadministered ethanol (16).

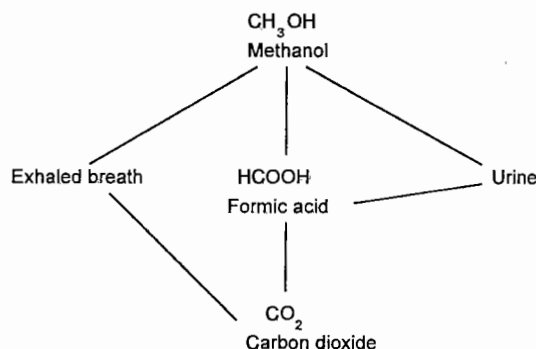


Figure 2.2.1. Schematic metabolism of methanol (simplified from 3)

2.2.4.3 Excretion

Urinary excretion of unmetabolized methanol is rapid with a biological half-time of 1.5 to 2.0 hrs (11). Formic acid is also excreted in urine but with a longer half-time; a half-time of 2.25 hrs was reported in one study (17), and a tendency to accumulate over a course of a workweek was observed in another study (18) which suggests even a longer half-time.

2.2.5 Summary of toxic effects

Methanol when ingested is narcotic like ethanol. After a latent period of 10 to 15 hours, the patients may have gastrointestinal (e.g. nausea and abdominal pain) and neurological symptoms (e.g. dizziness, headache and eyesight disturbance) depending on the dose. At a high dose, typical clinical signs are optic neuropathy and metabolic acidosis. Visual symptoms range from blurred vision to complete blindness. Metabolic acidosis may result in coma and then death (3, 19). Death was reported after an intake of "a few ounces" of methanol (3). Irritation of mucous membrane was noticed after vapour exposure in industrial settings (20).

2.2.6 Biological monitoring indices

Available indices are summarized in table 2.2.1.

Table 2.2.1. Available biological monitoring indices

Index	Remarks	Ref.
Methanol in urine	Non-invasive but not very specific	(20, 21)

2.2.7 Methanol in urine

2.2.7.1 Toxicokinetics

See section 2.2.4 Summary of toxicokinetics.

2.2.7.2 Biological sampling

(a) Sampling time

Because urinary excretion of methanol has a short half-time, i.e., 1.5 to 2 hrs (see section 2.2.4), selection of sampling time is of critical importance. In cases where the exposure intensity is expected to be rather constant throughout a workshift, an end-of-shift urine sample can be taken as a representative sample. When a wide variation in exposure intensity is possible during a shift, urine samples should be collected throughout the workshift.

(b) Contamination possibilities

See section 2.2.7.3.1g and section 2.2.7.3.2g.

(c) Stability

Samples should be refrigerated (but not frozen) when kept overnight or longer. Samples may be stable for a week when refrigerated.

2.2.7.3 Recommended analytical methods

Gas chromatography (GC) with headspace technique has been most commonly employed (11, 17, 22, 23, 24). Analysis by direct injection of urine samples into a GC column is also possible (21). An example of each method follows (20, 21).

2.2.7.3.1 Headspace GC

(a) Principle of the method

A methanol-containing urine sample is heated in a headspace vial so that methanol vapour concentration in air over liquid surface (headspace air) reaches an equilibrium with the methanol concentration in the urine. A part of the headspace air is introduced via a loop to a GC equipped with a flame ionization detector for chromatographic determination. Use of a DB-WAX wide-bore capillary column is recommended. The following is an example (20).

(b) Reagents required

- Methanol as a standard
- Helium as a carrier gas
- Hydrogen gas
- Nitrogen gas

(c) Equipment required

- A GC equipped with a flame ionization detector
- A headspace air sampler
- A heating bath for headspace GC
- 20-ml headspace vials
- A data processor

(d) Procedures

In a methanol vapour-free room, 5.0 mL of urine is taken in a vial for headspace GC, and the vial is immediately sealed with a Teflon-coated septum. The vial is kept at 80°C for 60 minutes in a heating bath. A 1 mL portion of the headspace air is introduced to the GC by means of an automatic sampler and analysed on a DB-WAX wide-bore capillary column (60 m in length, 0.53 mm in diameter and 1 μ m in film thickness) at 60°C. Helium as a carrier gas is allowed to flow at 5 mL/min. Splitless mode is employed. Air, hydrogen and nitrogen (make-up gas) are supplied to the detector at 2.8 kg/cm², 1.4 kg/cm² and 5.0 kg/cm², respectively. The injection port, the loop (connecting the air sampler and the GC) and the detector are heated at 150°C, 100°C and 250°C, respectively. The retention time of methanol is about 16 min, and the determination can be repeated after 20 min. A calibration curve is prepared by the addition of pure methanol (e.g., up to 500 mg/L) to either water or urine from non-exposed subjects, followed by the analysis under standard conditions. Whereas both water and urine from a non-exposed subject give essentially the same slope of the calibration curves, it should be noted that methanol is present in the urine from non-exposed subjects as to be discussed below (see section 2.2.7.4 – guide to interpretation).

(e) Criteria of analytical reliability

When samples containing 20 mg/L methanol are studied, the relative standard deviation after repeated determination is about 5%. The recovery is 99%. The detection limit is 0.1 mg methanol/L urine.

(f) Quality assurance

Quality assurance should be performed within the laboratory, as no quality control schemes are available. For this purpose, methanol diluted in water, e.g. at 20 mg/L, and kept refrigerated in tightly sealed glass containers can be used as a standard.

(g) Sources of possible errors

Methanol is hydrophilic, and urine samples when kept in contact with the methanol vapour-polluted room air may absorb methanol (leading to an overestimation of exposure). Sampling and further handling of the urine should be carried out in a methanol vapour-free environment for prevention of contamination. Methanol is very volatile. Thus, pos-

sible loss (leading to an underestimation of exposure) of methanol from urine samples should be avoided by keeping the urine samples in containers with little air space and under refrigerated (but unfrozen) conditions. On-site transfer of amount (i.e. 5 mL) of the urine to a headspace vial is recommended. The vial should allow contact of sample urine only with glass, Teflon and aluminium.

(h) Reference to the most comprehensive description of the method

Kawai T, et al. Methanol in urine as a biological indicator of occupational exposure to methanol vapour. *Int Arch Occup Environ Health* 1991;63:311–8.

The method is specific and sensitive enough to detect occupational exposure to methanol. Relatively high background level of methanol however makes it difficult to detect low level methanol exposure (see 2.2.7.4 – guide to interpretation).

2.2.7.3.2 Direct injection of urine to GC

(a) Principle of the method

After centrifugation, a portion of a methanol-containing urine sample is injected into a GC for chromatographic separation and determination. Use of a wide-bore capillary column is recommended for better separation, but a packed column can also be employed. The following is an example (21):

(b) Reagents required

- Methanol as a standard.
- For a wide-bore capillary column GC: helium as a carrier gas; and hydrogen gas.
- For a packed column GC: nitrogen gas as a carrier gas; and hydrogen gas.

(c) Equipment required

- A GC equipped with a flame ionization detector and a glass insert (packed with 4 mg of quartz wool for GC in a length of about 5 mm) placed between an injection port and a column.
- A data processor
- A clinical centrifuge.

(d) Procedure

A portion of a urine sample is spun at $1870 \times g$ for 15 min in a clinical centrifuge. A GC equipped either a wide-bore capillary column (DB-WAX; 60 m in length, 0.53 mm in diameter and 1 μm in film thickness) or a packed column (made of glass, 4 m in length and 3.2 mm in diameter, packed with 10% SBS-100 on Shimalite TPA, 60-80 mesh) is used. For analysis on the wide-bore capillary column, carrier helium is allowed to flow at 10 mL/min, and hydrogen and air are supplied to the detector both at 0.5 kg/cm². The injector, the column and the detector are heated at 150°C, 40°C and 250°C, respectively. One μL sample is introduced per injection. A peak from methanol appears in about 4 min, and the run time is 20 min. When using the packed column, carrier nitrogen is allowed to flow at 15 mL/min, and hydrogen and air are supplied to the detector at 0.6 kg/cm² and 0.5 kg/cm², respectively. The injector, the column and the detector are

heated at 150°C, 55°C and 150°C, respectively. Five μL of the sample are introduced per injection, and the methanol peak will appear in 15 min and the run time is 20 min. A calibration curve can be prepared by the addition of methanol (e.g. up to 500 mg/L) to either water or urine from non-exposed subjects, followed by the analysis under standard conditions. Whereas both water and urine from a non-exposed subject give essentially the same slope in the curves, it should be noted that methanol is present in the urine from non-exposed subjects as to be discussed below (see section 2.2.7.4 – guide to interpretation). The quartz glass in the insert should be renewed when it is heavily stained, e.g. after 50 determinations.

(e) Criteria of analytical reliability

For a wide-bore capillary column, the relative standard deviation after repeated determinations is about 4%, the recovery is 98%, and the detection limit is 0.1 mg methanol/L urine, when samples with 20 mg/L methanol are assayed.

For a packed column, the relative standard deviation after repeated determination is about 4%, the recovery is 90%, and the detection limit is 0.2 mg methanol/L urine, when samples with 20 mg/L methanol are assayed.

(f) Quality assurance

See 2.2.7.3.1.f.

(g) Sources of possible errors

See 2.2.7.3.1.g.

(h) Reference of the most comprehensive description of the method

Kawai T, et al. Simple method for determination of methanol in blood and its application in occupational health. *Bull Environ Contam Toxicol* 1991;47:797–803.

The method is specific and sensitive enough to detect occupational exposure to methanol. Relatively high background level of methanol, however, makes it difficult to detect low level methanol exposure (see 2.2.7.4 – guide to interpretation).

2.2.7.4 Guide to interpretation

Human volunteer exposure experiments (11) as well as occupational health surveys (11, 20) have established a linear correlation between the intensity of exposure to methanol vapour and resulting methanol concentration in urine.

(a) *Measured values in groups without occupational exposure*

Methanol is present in urine from non-exposed subjects at the levels of up to 2.6 mg/L (11) or even higher.

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (25) has adopted 15 mg methanol/L in urine collected at the end of a workshift as a “Biological Exposure Index (BEI)”. The value is set as a group mean which corresponds to the time-weighted average concentration of occupational exposure to methanol at 200 ppm when exposure will not fluctuate widely. In case the fluctuation of exposure is unknown or likely to be wide during a shift, sampling over the whole shift is recommended.

Deutsche Forschungsgemeinschaft (26) has adopted 30 mg methanol/L in urine collected at the end of a workshift as a “Biological Tolerance Value (BAT-value)”.

(c) Non-analytical interference

Methyl esters will be readily hydrolysed *in vivo* to give rise to methanol. Thus, exposure to methyl esters, e.g. methyl acetate (4) and methyl methacrylate (27), may induce elevation in urinary methanol level. The presence of methanol in some home-use products, e.g. an antifreeze (6) precludes the use of methanol in urine for biological monitoring at low level exposure, e.g. below 50 ppm (28). Methanol was present in adulterated alcoholic beverages (7). Cases of intentional inhalation of methanol (or methyl acetate) vapour have been reported (8–10). The social habit of drinking ethanolic beverages may affect methanol metabolism (17, 18).

2.2.8 Other indices

Methanol in blood can be readily measured both by headspace GC and by direct GC injection, and correlates significantly with methanol exposure (22). Blood sampling, however, is inevitably invasive. In obtaining blood samples for methanol determination, care should be taken not to use methanol-containing skin disinfectant to avoid any methanol contamination. Methanol in exhaled breath has also been proposed as an indicator of exposure (29, 30).

Formic acid in urine has been long studied as a candidate for the indicator of occupational exposure to methanol, and fully automated headspace GC system (28) and hand-saving enzymic assay methods (31, 32) have been established. The level of formic acid in urine from non-exposed subjects is relatively high, e.g. 2.6 mg/L (11) or even higher (22). Furthermore it was found that formic acid in urine is a less sensitive exposure indicator than methanol in urine (28, 33). Accordingly biological monitoring by means of urinary formic acid is applicable only when methanol exposure is in excess of 200 ppm (28).

2.2.9 Research needs

Available indicators of biological monitoring of occupational exposure to methanol, i.e., methanol in urine and formic acid in urine, are not sensitive enough to detect low level methanol exposure, e.g. below 50 ppm. Further studies for more sensitive indicators are required.

The effects of co-exposure to other chemicals (including solvents) on methanol metabolism is yet to be investigated. Whether correction for co-exposure in evaluation is necessary, and if it is, how it can be done, should be studied. Quantitative evaluation of the effects of ethanol intake as a social habit on methanol metabolism is of practical importance in occupational health.

2.2.10 References

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2.3 Methylene chloride (Dichloromethane)

2.3.1 Introduction

Methylene chloride is a colourless solvent that is widely used in industry as a solvent and in the home as a paint stripper. In an occupational environment, methylene chloride is absorbed through inhalation, and through dermal contact of the liquid. It is metabolized to carbon monoxide and thus presents an additive hazard to workers who also are exposed to carbon monoxide directly or through smoking. Some, but not all, toxicity in humans is due to the metabolism to carbon monoxide and the formation of carboxyhemoglobin. IARC has concluded that methylene chloride is possibly carcinogenic to humans. Numerous reviews of methylene chloride have been published (1–5).

Published biological action limits for methylene chloride in blood and carboxyhemoglobin are health-based and are based on human data. Available specific gas chromatography methods are well described. Alternative spectrophotometric methods are available and in common use for carboxyhemoglobin.

2.3.2 Physical-chemical properties (1)

Methylene chloride (CAS 75-09-02) is a non-flammable solvent present in the air of the workplace as a vapour. Its physical properties are listed as follows.

Molecular weight	84.93
Molecular formula	CH_2Cl_2
Specific gravity	1.3266
Boiling point	40°C
Vapour pressure	Saturated vapour pressure is 46.5 kPa at room temperature.
Solubility	Methylene chloride is soluble in water (20 g/L), and soluble in alcohol and ether. The partition coefficients are: octanol: water 20.0; blood:gas 6.0; blood:fat 85.0 (6).

Conversion factors

Methylene chloride	$1 \text{ ppm} = 3.53 \text{ mg/m}^3$; $1 \text{ mg/m}^3 = 0.28 \text{ ppm}$
CO	(at 25°C, 1 atm) $1.0 \text{ mg/m}^3 = 0.87 \text{ ppm}$; $1.0 \text{ ppm} = 1.1 \text{ mg/m}^3$

2.3.3 Possible occupational and non-occupational exposures

Occupational exposures occur during the use of methylene chloride as a solvent, a degreasing agent, and a paint stripper.

Non-occupational exposure to methylene chloride is common. It is widely used as a heavy-duty paint stripper and has been used as an aerosol propellant. The concerns about its toxicity have limited its use among the general public in recent years. Methylene chloride is a common solvent released into the atmosphere by industry and is present at many hazardous waste sites (1).

2.3.4 Summary of toxicokinetics

2.3.4.1 Absorption

In the workplace, the principal route of absorption is inhalation. Dermal contact with liquid methylene chloride also leads to absorption. Gastrointestinal absorption is not likely in the workplace.

(a) *Inhalation*

Methylene chloride is readily absorbed by inhalation with blood levels and alveolar air levels reaching an equilibrium, or steady state, after 2–4 hours of exposure (7). Retention in the lungs has been reported to be as high as 70%, with other authors having reported lower retention (7–10). Exercise enhances uptake, but the ratio of inhaled over exhaled concentration decreases (7, 8). Increased body fat enhances retention (11–12).

(b) *Dermal*

Dermal absorption of liquid methylene chloride was demonstrated in humans experimentally and has been reported in furniture strippers (13–14).

(c) *Gastrointestinal*

Gastrointestinal absorption is not common in the workplace, but has been reported in poisoning case reports (2).

2.3.4.2 Metabolic pathways and biochemical interactions

Methylene chloride is metabolized in humans by two distinct pathways. One pathway involves oxidation through the cytochrome P450 system to carbon monoxide and HCl through the reactive intermediate, formyl chloride. The other pathway involves metabolism by enzymes in the cytosol and conjugation with glutathione, forming formaldehyde, formic acid, and carbon dioxide. Metabolites of the latter pathway enter the one carbon metabolic pool (7, 8, 11, 15–21). Between 25–34% of the methylene chloride retained following inhalation exposure was metabolized to carbon monoxide. The remaining absorbed methylene chloride was metabolized to formic acid and carbon dioxide which entered the one-carbon pool (7). Figure 2.3.1 shows the metabolic pathways of methylene chloride.

2.3.4.3 Distribution

Methylene chloride initially distributes to blood and vascular compartments followed by accumulation in adipose tissue (12, 22).

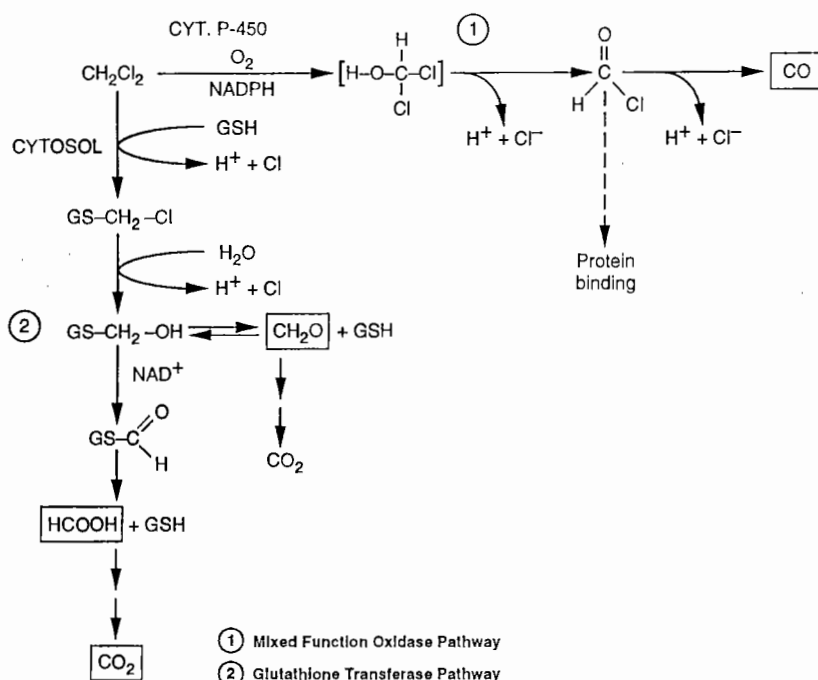


Figure 2.3.1. Metabolic pathways of methylene chloride

2.3.4.4 Elimination

Carbon monoxide and carboxyhemoglobin are released more slowly following exposure to methylene chloride than from exposure to carbon monoxide; this is probably due to the continued metabolism of methylene chloride. The elimination half-time is likely to be multiphasic, with an average of 7 to 10 hours compared to a half-time of 4 to 5 hours from exposure to carbon monoxide (7, 17). Following an 8-hour exposure to methylene chloride, the level of HbCO continues to increase up to two hours after the end of exposure, reflecting the continuing metabolism of deposited methylene chloride (7). Methylene chloride is rapidly eliminated in expired air and from the blood with multiphasic elimination half-times of 30 minutes and 15 to 20 hours (7).

2.3.5 Summary of toxic effects

Some, but not all, toxicity in humans is due to the metabolism to carbon monoxide and the formation of carboxyhemoglobin. IARC has concluded that there is sufficient evidence of the carcinogenicity of methylene chloride in animals; in humans the evidence was considered inadequate: the overall evaluation was that methylene chloride is possibly carcinogenic to humans (5). In addition to its suspect human carcinogenicity, other toxic effects of exposure in humans include central nervous system depression, eye, skin, pulmonary irritation, and anoxia (1).

2.3.6 Biological monitoring indices

Table 2.3.1 lists biological monitoring indices found for methylene chloride reported for assessment of human exposure. Methylene chloride in blood and carboxyhemoglobin are recommended. Carboxyhemoglobin is not specific since other sources of carbon monoxide, such as exposure to cigarette smoke, and combustion products also produce carboxyhemoglobin. Although carbon monoxide in expired air can be measured, it does not provide additional information compared to measurement of carboxyhemoglobin in blood. Volume 1 of this series describes methods for measurement of carbon monoxide in expired air as an index of exposure to carbon monoxide.

Methylene chloride in expired air also could be used, but expired air analysis shows much more individual variability and is not commonly used. No other biological monitoring indices have been recommended.

Table 2.3.1. Biological monitoring indices for methylene chloride

Indicator	Suitability for use	Ref
Methylene chloride in blood	Best indicator of exposure	23–25
Carboxyhemoglobin	Best indicator of 8-hour TWA exposures in blood collected 2–4 hours after exposure in the absence of smoking	23–25
Methylene chloride in expired air	Reflects rapid washout from lung; large inter-individual variability	14, 24–25

2.3.7 Methylene chloride in blood

2.3.7.1 Toxicokinetics

Methylene chloride is rapidly eliminated in expired air and from the blood with multi-phasic elimination half-times of 30 minutes and 15 to 20 hours (7). Concentrations in blood collected immediately at the end of the workshift reflect exposures during the last 2 to 3 hours. Specimens collected at later times reflect washout from fat compartments and thus are an indication of integrated dose over a longer period of time (7).

2.3.7.2 Biological sampling

(a) Specimen and sampling time

Venous whole blood specimens should be collected at the end of the shift. Specimens collected at the end of the shift reflect exposures during the last 2 to 3 hours.

(b) Contamination possibilities

The arm where blood is taken should be washed with soap and water to avoid contamination of the site with disinfectants, which may contain chlorinated hydrocarbons. If at

all possible, blood specimens should be collected outside of the work area away from airborne exposures to methylene chloride.

(c) Sampling device and container

Approximately 5 mL of whole blood should be collected using a disposable syringe pre-wetted with anticoagulant. After mixing to dissolve the anticoagulant, 2.0 mL whole blood is immediately transferred into a 20 mL crimp-top vial containing a TeflonTM-coated butyl rubber septum. The crimp-top vials and septa must be prepared as described in detail in "equipment".

(d) Anticoagulant

Dipotassium EDTA is the anticoagulant used for blood collection and should be present in the syringe. Ammonium oxalate is the anticoagulant used in the crimp-top vials. Other anticoagulants can be used.

(e) Preservatives

No other preservatives are necessary.

(f) Shipment

Specimens placed in crimp-top vials are shipped to the laboratory either frozen or in cold packs.

(g) Stability

Blood collected as described and placed in properly prepared crimp-top vials is stable frozen at -16 to -20°C for at least 6 months. Specimens must be brought to room temperature before analysis.

2.3.7.3 Recommended analytical methods

(a) Principle of the method

Methylene chloride and seven other halogenated hydrocarbons are determined by gas chromatography using the headspace technique. Blood samples are placed in sealed crimp-top vials immediately after collection. The headspace vials are heated and injected into a capillary column equipped gas chromatograph with electron capture detector (ECD). Calibration is conducted using blood samples with known amounts of methylene chloride added. Peak areas are plotted against concentration. An aqueous solution of halogenated hydrocarbons is used for external standards. The description which follows is focused on the determination of methylene chloride.

(b) Reagents required

Chemicals of the highest purity must be used. Methylene chloride; 2-Ethoxyethanol; defibrinated sheep's blood; ultrapure water (ASTM Type 1) or double distilled water; physiological saline (154.0 mmol/L); K₂-EDTA (or other suitable anticoagulant); am-

monium oxalate, p.a. (a pharmaceutical grade). Take 10 g of ammonium oxalate and dissolve up to 1000 mL with ultrapure water for a final concentration of 10 g/L; purified nitrogen (99.999%).

(c) *Equipment required*

Gas chromatograph with split-splitless injector, electron capture detector, analog recorder, and integrator/plotter; column: 60 m fused silica (0.33 mm id) coated with 100% dimethylpolysiloxane, chemically bonded and cross-linked, DB-1, 1 μ m film thickness; dry block heater, thermostatically controlled, with molds to fit crimp-top vials and air-tight syringe for sample injection; alternatively, an automated head-space gas chromatograph may be used in place of the dry block heater and syringe for large quantities of samples; microliter pipets, transfer pipets and volumetric flasks, various sizes; magnetic stirrer with stir bar; 20 mL crimp-top vials with TeflonTM-lined butyl rubber septa and aluminum caps, as well as crimping tools for sealing and opening. The crimp-top vials and septa are heated at 110°C in a drying oven for at least two days. After cooling, 20 mg of ammonium oxalate (2 mL of 10 g/L solution) is added and the solution is evaporated to dryness at 40°C in a drying oven. After cooling, the anticoagulant is present as a finely dispersed particulate on the walls of the vials. TeflonTM-lined septa are then placed on the vials and crimped closed with aluminum caps. The seals should not be able to be rotated by hand.

(d) *Procedure including calibration*

i) Calibration

Several calibration standards are prepared in different matrices. Many of these standards are stable over extended periods of time and only have to be prepared once every 6 months. Table 2.3.2 summarizes the preparation of standards in 2-ethoxyethanol (EE) and water.

Table 2.3.2. Preparation of methylene chloride (MeCl₂) standards in 2-Ethoxyethanol (2-EE), and water

	Standard A, MeCl ₂ in 2-EE	Standard B, stock MeCl ₂ solution in water	Standard C, aqueous MeCl ₂ external standard
Source and directions	Put 100.0 μ L MeCl ₂ (105.7 mg), into 20 mL vol. flask with 15 mL 2-EE. Dilute to mark with 2-EE.	Pipet 1.00 mL Standard A into a 1 L vol. flask. Add 5 mL of 2-EE. Di- lute to mark with ul- trapure water.	Pipet 100.0 μ L Standard B into 1 L vol. flask. Dilute to mark with ul- trapure water.
Concentra- tion	5280 mg/L	5280 μ g/L	528 μ g/L

Two mL of Standard C (aqueous external standard) is pipetted into each of many 20 mL crimp-top vials using an automatic pipettor. The vials are sealed using the TeflonTM-lined septa and crimped with aluminum caps. These standards are kept frozen at -16 to

-20°C. Each analytical run should include one or two external standards. Table 2.3.3 describes the preparation of saline and blood-based standards.

Table 2.3.3. Preparation of saline and blood-based standards

	Standard D, MeCl ₂ in physiological saline	Standard E, starting so- lution of MeCl ₂ in ani- mal blood	Standard F, stock MeCl ₂ in animal blood
Source and directions	Pipet 10.0 mL of Standard A into 50 mL volumetric flask. Dilute to mark with physi- ological saline.	Pipet 5.00 mL Standard D into 250 mL volumet- ric flask containing 20 mL animal blood. Dilute to mark with animal blood. Stir well.	Pipet 10.0 mL Standard E into 100 mL volumetric flask. Dilute to mark, with animal blood. Stir well.
Concentra- tion	1060 mg/L	21.1 mg/L	2.11 mg/L

A series of calibration standard solutions of methylene chloride in blood (Standard Solutions G) are prepared from various dilutions of the stock blood calibration standard (Standard F). Two mL of animal blood are added to each of many 10 mL volumetric flasks. Volumes of blood calibration stock solutions (Standard F) and the corresponding final concentrations of methylene chloride (Standards G) are added as shown in table 2.3.4. The flasks are filled to the mark with animal blood. In addition, a reagent blank that contains 10 mL of animal blood is prepared. After the calibration standards are thoroughly mixed, they are dispersed into crimp-top vials (2 mL each). The vials are immediately sealed with TeflonTM-lined butyl rubber septa and crimped with aluminum seals. The calibration standards are frozen at -16 to -20°C and are stable for at least 6 months.

Table 2.3.4. Pipetting scheme for preparation of calibration standards in blood

Standard no.	Volume stock so- lutions in blood (Standard F), mL	Final volume of Standard solutions G, mL	Concentration of methylene chloride in calibration standard (Standards G), µg/L
G-0 (reagent blank)	-	10	-
G-1	0.1	10	21.1
G-2	0.4	10	84.6
G-3	1.0	10	211
G-4	2.0	10	423
G-5	4.0	10	846

Calibration standards (Standard Solutions G), along with the reagent blank, are run through the procedure as described below. Peak areas are plotted against concentrations of methylene chloride, with the calibration linear over the range of standards. The use of the aqueous external standard (Standard C) can alleviate the need to plot a calibration

curve each time if one or more of Standard C is included in each run. A correction factor is calculated based on the response of the external standard at the time of preparation and the calibration curve run at the same time versus the response of the external standard at each run compared with the initial calibration curve. Each concentration obtained from the calibration curve is corrected by this factor as described below.

Evaluation with the aid of the calibration curve

When peak areas have been determined that correspond to the calibration curve peak areas performed at the same time, concentrations in µg/L can be read directly from the calibration curve.

Evaluation with the aid of an aqueous external standard (Standard C):

When the external standard is used, the concentration obtained for methylene chloride from the calibration curve, which was previously established, is corrected by multiplying by the quotient of the peak areas measured for the aqueous external standard, according to the following equation:

$$C_p^i = C_{cal}^i * S_a^i / S_{a+n}^i$$

- C_p^i is the concentration of methylene chloride in the sample.
- C_{cal}^i is the concentration of methylene chloride read off the calibration curve.
- S_a^i is the value for methylene chloride from the aqueous external standard (Standard C) on the day the calibration curve was prepared (day a).
- S_{a+n}^i is the value for methylene chloride from the aqueous external standard (Standard C) on the day of the sample analysis (day a+n)

ii) Procedure

Blood samples in crimp-top vials are placed into the incubator block of the automated headspace gas chromatograph or in the thermostated heating block for 90 minutes at 50°C to equilibrate methylene chloride between the vapour and liquid phases. 100 µL of vapour phase is injected into the gas chromatograph with a pre-warmed airtight syringe or injected using an autosampler-equipped headspace gas chromatograph. The gas chromatograph is set to the following conditions:

Electron capture detector using ^{63}Ni source

Temperatures Column – 60°C isothermal; injector – 230°C; detector – 300°C

Gases Carrier, purified nitrogen at 16 psi.

Makeup, purified nitrogen, 40 mL/min.

Split 1:30

Injection volume 100 µL

Retention time for methylene chloride 5.18 min.

(e) Criteria of analytical reliability

i) Trueness

Recovery experiments were carried out using animal blood spiked with 253 and 405 µg/L methylene chloride. Recovery at the lower range exceeded 100% due to a high

blank; recovery was 94.4% at 405 $\mu\text{g/L}$. The guidance level for methylene chloride in blood is 1 mg/L , a value considerably higher than either recovery experiment (26).

ii) Precision

The within-day precision, expressed as standard deviation and relative standard deviation, of spiked animal blood for methylene chloride at 742 $\mu\text{g/L}$ was 51.5 $\mu\text{g/L}$ and 6.9%, respectively. When samples were injected by autosampler, relative standard deviations of less than 5% were obtained (26).

iii) Detectability

The lower limit of detection for methylene chloride in blood, based on three times the signal-to-noise ratio was 25 $\mu\text{g/L}$ (26).

(f) *Quality assurance*

i) Special precautions

Blood should be treated as a biohazard, using universal precautions to avoid the transmission of HIV or HBV viruses.¹ As stated above, blood samples must be immediately placed in properly prepared crimp-top vials with ammonium oxalate anticoagulant. Internal controls, prepared in blood as described under calibration using a different source of methylene chloride, can be prepared at critical concentrations and stored in crimp-top vials. These are stable at least 6 months at freezer temperatures and can be used as internal quality control samples by inclusion of at least one per analytical run. Plotting of these quality control samples will provide some evidence of internal quality control. There are no external proficiency testing schemes for methylene chloride in blood.

ii) Interferences

Solvent-based materials must not be used to wash the arm prior to venipuncture. The method is specific for methylene chloride, with adequate separation of other common halogenated hydrocarbons. The authors report that solvent "R 113" interferes with methylene chloride (26).

(g) *Sources of possible errors*

i) Pre-analytical

There are several pre-analytical sources of error including the following:

Significant errors can be introduced regarding interpretation of the results if blood samples are not collected immediately after exposure. The short initial half-time results in rapidly falling blood concentrations.

Blood must be immediately placed in crimp-top vials and mixed well by swirling to avoid coagulation. Crimp-top vials must have tight seals that cannot be turned by hand.

¹ Centers for Disease Control, "Guidelines for Prevention of Transmission of Human Immunodeficiency Virus and Hepatitis B Virus to Health-Care and Public Safety Workers," *Morbidity and Mortality Weekly Reports* (1989), 36:(S-6), iii-vi, 1-37.

Vacutainer or other vacuum collection tubes cannot be used for blood collection because of the rapid loss of methylene chloride when the septa are removed for withdrawal of blood for transfer to crimp-top vials.

The highly sensitive electron capture detector will pick up contamination of methylene chloride in laboratory air or workplace air. Therefore, blood samples must be collected in an atmosphere free of methylene chloride. Blood should be handled in a laboratory area away from solvent extraction activities. It is essential that crimp-top vials and TeflonTM-coated butyl rubber septa be heated as described to remove volatile solvents. It is desirable to heat septa for a week. Seals on crimp-top vials must be checked by inclusion of a blank for each batch of prepared vials. If seals can be turned by hand, they are unsuitable.

ii) Analytical

Several steps must be controlled during analysis to avoid errors.

Blood samples must be equilibrated at 50°C for 90 minutes. Users should verify that this time interval is sufficient to reach equilibrium under conditions in the laboratory.

If an autosampler is used, it is important to allow at least 6 minutes for pressurization before injection. Carryover must be avoided by use of a nitrogen flush of the carrier lines at 20°C higher than the injection temperature.

If a manual injection is used, temperature control and use of a heated, airtight syringe is essential to avoid condensation in the syringe and large losses of solvent vapour. Carryover must be avoided by use of a nitrogen flush of the syringe after every injection.

At the end of the day, high-temperature carrier gas should be flushed through the column to remove volatile residuals. However, this process may create active absorptive sites on the column. Thus, aqueous standards must be injected onto the column following a bake out and prior to the next day, analytical runs to saturate absorptive sites.

The preparation of calibration standards is of great importance. The sequence described permits homogeneous dispersion of sparingly soluble methylene chloride into aqueous media. Because of the sensitivity of the ECD detector, Standards A and B must be prepared in a room separated from other analytical steps. The final dilutions of these standards and blood-based standards must be performed in a room with undetected levels of methylene chloride.

(h) Reference to the most comprehensive description of the method

The method outlined above was taken from reference (26).

(i) *Evaluation of the method*

The authors report that the method has been in common use for several years. The method was independently examined by H. Muttfler and R. Eisenmann, and found to be acceptable (26). Performance standards were previously stated above. No field evaluation of the method or methods comparison studies were identified.

2.3.7.4 Other analytical methods

There are a variety of methods for analysis of methylene chloride in blood. Most of these methods are based on headspace analysis using gas chromatography with flame ionization (27).

2.3.7.5 Guide to interpretation

(a) *Measured values in groups without occupational exposure*

No reports of background levels of methylene chloride using the described method were found. Reports of "not detected" have been found using methods with FID detection, with detection limits in the order of 0.1 mg/L.

(b) *Published biological action levels*

Table 2.3.5 shows biological action levels published by organizations and recommendations by individual authors.

Table 2.3.5. Published biological action levels for methylene chloride in blood

Biological action level, basis and collection time	Reference
1.0 mg/L whole blood collected at end of shift based on prevention of health effects	(23)
0.5 mg/L whole blood collected at end of shift based on prevention of health effects	(25)
1.0 mg/L whole blood collected at end of shift based on prevention of health effects	(24)

(c) *Non-analytical interference*

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, and medication

The procedure described is specific for methylene chloride. The effects of ethanol and medication on blood levels have not been reported in humans. Although methylene chloride in blood is specific, the toxic effects of methylene chloride are partially due to its metabolism to carbon monoxide. Thus, populations who smoke or who are exposed to carbon monoxide and methylene chloride are at greater risk for the additive effects of carbon monoxide from methylene chloride metabolism. Individuals who are obese will store more methylene chloride in fat (12, 22).

ii) Diet and environment

Methylene chloride was used in the decaffeination of coffee, although its use in recent years has been reduced. Sensitive methods, such as that described, may measure residual levels from consumption of large amounts of coffee decaffeinated using methylene chloride. Methylene chloride may be detected in individuals from environmental sources with this method, but no reports were found.

(d) Sampling representative of recent or long-term exposure, or biological effect

Sampling is representative of recent exposure during the last 2 to 3 hours. If blood samples are collected 2 to 4 hours after the end of exposure, then measurements of blood methylene chloride are representative of exposures over the preceding 8 hours. However, published guidelines are based on collection of blood at the end of the shift.

(e) Ethnic differences (e.g. enzyme deficiency, environment, diet)

No effects of ethnic differences have been reported.

2.3.8 Carboxyhemoglobin in blood (HbCO)

2.3.8.1 Toxicokinetics

Carbon monoxide and carboxyhemoglobin are released more slowly following exposure to methylene chloride than from exposure to carbon monoxide, probably due to the continued metabolism of methylene chloride. The elimination half-time is likely to be multiphasic, with an average of 7 to 10 hours compared to a half-time of 4 to 5 hours from exposure to carbon monoxide (7, 17). Following an 8-hour exposure to methylene chloride, the level of HbCO continues to increase up to two hours after the end of exposure, reflecting the continuing metabolism of absorbed methylene chloride (7).

2.3.8.2 Biological sampling

(a) Sampling time and specimen

To evaluate daily time-weighted average (TWA) exposure, blood samples should be taken after at least 3 hours of exposure, preferably at the end of the shift in order to provide data that is consistent with published biological monitoring guidelines. Whole blood samples should be collected by venipuncture.

(b) Contamination possibilities

Contamination of blood is not likely.

(c) Sampling device and container

Blood should be collected in a vacuum tube system to avoid losses of carbon monoxide that could occur in an open syringe collection system.

(d) Anticoagulant

Whole blood specimens obtained by venipuncture should be collected with ammonium oxalate anticoagulant.

(e) Preservative, shipment, and stability

Whole oxalated blood should be stored in the dark at 4°C in order to avoid bacterial action, which can result in both the production of carbon monoxide and the denaturation of hemoglobin. Blood samples to be analysed by direct reading spectrophotometric methods depend on the integrity of the erythrocytes and must be thoroughly mixed with anticoagulant immediately after collection. These specimens should be shipped and stored at 4°C and analysed within 48 hours of collection. Blood specimens for analysis by gas chromatographic methods do not depend on the integrity of the erythrocyte and can be kept at least 5 days at room temperature. Samples can be frozen for longer storage.

2.3.8.3 Recommended analytical methods

There are a wide variety of spectrophotometric methods available and commonly used for measurement of HbCO. However, most of the methods do not have sufficient trueness and precision to accurately determine concentrations of HbCO of less than 3%. However, these methods have considerable value for screening populations with exposures above 5% HbCO. They all require fresh, unclotted whole blood. References for some of these spectrophotometric methods plus a brief description of one method are given in Section 2.3.8.4.

To analyse blood specimens with HbCO levels between 0.5% to 5%, gas chromatographic methods are recommended, even though these methods are more elaborate and generally require more equipment and time to perform analyses (28–32). Blood specimens, including clotted blood, can be analysed by gas chromatographic methods. A separate determination of hemoglobin is also required. The selection of methods must be based on the expected range of HbCO. The gas chromatographic method described below was selected because it is well documented (33).

(a) Principle of the method

Carbon monoxide is released from hemoglobin by potassium ferricyanide (III), separated from other sample constituents on a molecular sieve gas chromatography column, reduced to methane by hydrogen with a nickel catalyst, and determined with a flame ionization detector. Hemoglobin is determined separately using the cyanomethemoglobin spectrophotometric method, using Drabkins reagent.

(b) Reagents required

- Potassium ferricyanide (III)
- Pre-column: Chromosorb G/AW coated with methanolic KOH
- Analytical column: Molecular sieve 5 A, 30–80 mesh

- Catalytic column: Chromosorb G/AW coated with nickel (II) nitrate
- Nitrogen, hydrogen, and purified air (80% nitrogen, 20% oxygen)
- 0.01% Carbon monoxide standard in nitrogen, carbon monoxide (> 98.6%)
- Cyanomethemoglobin reagent (Drabkins reagent) containing potassium ferricyanide (III), potassium cyanide, and sodium bicarbonate (commercially available)
- Cyanomethemoglobin standards (commercially available)

(c) Equipment required

- Gas chromatograph with flame ionization detector adapted for use of a 0.64 cm (ID) 20 cm pre-column, 0.64 cm (ID) 2 m molecular sieve separation column and a 0.64 cm (ID) 20 cm catalytic reduction column.
- Spectrophotometer or colorimeter equipped with a 540 nm filter, for determination of hemoglobin
- Tonometer for equilibrating standards, "headspace vials," gas-tight syringe, and other miscellaneous laboratory equipment

(d) Procedures and calibration

i) Calibration

Calibration standards for carbon monoxide are made up in whole blood taken from a non-smoking, unexposed person. A stock solution of 100% carbon monoxide in blood is prepared by passing carbon monoxide through the blood. Since carbon monoxide also dissolves in blood, excess unbound carbon monoxide is removed by flushing the container with nitrogen producing "nearly 100%" HbCO. The exact concentration of this high standard can be determined using a spectrophotometric method described later, since spectrophotometric methods are insensitive to dissolved carbon monoxide. Stock solutions can then be made over the range of 0.2–40% HbCO by mixing the "nearly 100% HbCO with blood from non-smokers, using a tonometer to mix the solutions. Diluted standards are analysed, along with specimens using whole untreated blood, as blanks. Blank corrected standards are plotted against HbCO concentration.

ii) Procedure

A venous blood sample containing oxalate anticoagulant is split between two headspace vials. After the vials are flushed with nitrogen, one vial receives a solution of potassium ferricyanide; the other serves as a blank. After equilibration, an aliquot of headspace is removed with a gas-tight syringe and injected into the gas chromatograph. After separation of the other components, carbon monoxide is catalytically reduced to methane and determined by flame ionization detection. Gas chromatographic conditions are: injector and pre-column (20 cm): 100°C; analytical column (2 meter): 100°C; catalytic column (20 cm) and detector: 300°C; and nitrogen carrier: 25 mL/min.

A separate determination of hemoglobin is required to correct for the hemoglobin content present in the blood used for calibration and in the blood from the exposed worker. The determination is performed by mixing 0.02 mL blood with 5 mL of the cyanomethemoglobin reagent, allowing the mixture to stand for 20 minutes. The absor-

bance is read at 540 nm against a calibration curve prepared by dilutions of the cyanomethemoglobin standard with the cyanomethemoglobin reagent (34).

Calibration for hemoglobin determinations are usually provided by commercially available hemoglobin determination kits based on the Drabkins reagent. Hemoglobin quality control materials are commercially available. The corrected HbCO percent is then calculated by multiplying the observed HbCO% by the ratio of the hemoglobin content of the specimen to hemoglobin content of the blood used for standard preparation.

(e) Criteria of analytical reliability

i) Trueness

Trueness, based on recovery studies at 5% HbCO, was 101%.

ii) Precision

The within-day precision, based on analysis of 10 samples at 5.07% HbCO, was 2.5% relative standard deviation. The day-to-day precision, based on 20 days of analyses at a 5% HbCO, was 8.8% relative standard deviation.

iii) Detectability

The estimated detection limit, based on three times the standard deviation of the blank, was 0.17% HbCO.

(f) Quality assurance

i) Special precautions

Blood should be treated as a biohazard, using universal precautions to avoid the transmission of HIV or HBV viruses (See footnote 1). The blood used as a stock solution must be from a non-smoker who is not exposed occupationally or environmentally to carbon monoxide or methylene chloride. If this blood specimen has appreciable HbCO, then results may be higher due to higher background blanks.

ii) Interferences

The procedure is specific and is not subject to interference.

(g) Sources of possible errors

i) Pre-analytical

Bacterial action, which may result in carbon monoxide formation and denaturation of hemoglobin, must be avoided. EDTA was reported to increase the level of HbCO and should be avoided as an anticoagulant (31). Atmospheric contamination of the sample with environmental carbon monoxide is minimized by flushing vials with nitrogen prior to releasing carbon monoxide with potassium ferricyanide.

ii) Analytical

The molecular sieve analytical column must be kept dry and free of excess carbon dioxide. The use of a pre-column is effective in maintaining a dry, efficient analytical column. Should the column become overloaded, it can be baked out overnight.

(h) Reference to the most comprehensive description of the method

The method described above is reference 33.

(i) Evaluation of the method

The method described above (33) included a laboratory validation study of precision, trueness, and estimates of the limit of detection. Precision, assessed by analysis of a blood specimen containing 5% HbCO, was 2.5% relative standard deviation. Day-to-day precision, over a 3-month period was 8.8%. Trueness, as assessed by recovery studies containing 5% HbCO, was 101%. The detection limit was 0.17% HbCO based on three times the standard deviation of the blank. No interlaboratory studies were reported.

2.3.8.4 Other analytical methods

Specific spectrophotometric methods for measurement of HbCO utilize two or more wavelengths in the visible region. These include automated visible spectrophotometry (35) and manual spectrophotometry at two or more wavelengths (36–40). A number of automated instruments are available to determine fractions of hemoglobin present in blood as HbCO, oxyhemoglobin, reduced hemoglobin, and methemoglobin. The CO-oximeter, described by Dubowski and Luke (35) has been widely used to assess occupational exposures. This is a good method if blood samples are fresh and if HbCO levels exceed 3%.

Measurements using oximeters were compared with measurements using gas chromatography (28). The difference between measured values was only $\pm 0.2\%$ HbCO. However, the trueness of $\pm 1\%$ HbCO, claimed by manufacturers of oximeters, is unacceptable for determination of background levels ($< 2\%$ HbCO) and is questionable for measurements of levels approaching 3.5% HbCO.

A representative manual spectrophotometric method suitable for assessment of HbCO at concentrations exceeding 2% is described by Commins and Lawther (38), a method that was evaluated in the 1970s in Europe and found to be acceptable (41). The German working group "Analysis of Hazardous Substances in Biological Material" recommended this method as an alternative to the gas chromatographic method. This method must be used on fresh, unhemolized, unclotted blood. A summary of the method follows.

0.01 mL of whole blood taken from a finger prick is dissolved in 10 mL of a 0.04% ammonia solution. The solution is divided into two halves. Carbon monoxide is displaced from one half by bubbling oxygen through the sample. The sample containing HbCO is placed in a spectrophotometer and read against the oxyhemoglobin sample as a blank. Readings are taken at 414, 420, and 426 nm to estimate HbCO. Readings are taken at 575 and 559 nm to estimate total hemoglobin. The percent HbCO is then calculated from the two results according to a formula described by the authors.

Calibration is done by determining the difference in absorption of blood treated with 100% oxygen and blood treated with 100% carbon monoxide. The performance characteristics, as described by the author, included determination of precision at HbCO concentrations of 1.4% HbCO (3.5% RSD), 6% HbCO (2.2% RSD), and 9.7% HbCO (1.2% RSD). Precision at 0.66% HbCO was reported as 10.6%. As previously discussed, the method was evaluated in 1977 by the European Commission and found to be acceptable.

2.3.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

HbCO in individuals not occupationally exposed to carbon monoxide or methylene chloride varies, depending on endogenous production of carbon monoxide, smoking habits, and environmental exposures. Such variation can affect the interpretation of HbCO measurements below 5%. Workers commuting via congested roadways may arrive at work with HbCO levels of 5% or more. Table 2.3.6 shows typical levels of HbCO in populations without occupational exposure to carbon monoxide or methylene chloride (42–44).

Table 2.3.6. Background carboxyhemoglobin levels

Group/Source of exposure	Average HbCO levels
Endogenous production	0.4% to 0.7% increasing up to 2.6% during pregnancy and up to 4%–6% in patients with hemolytic anemia
Urban population	1% to 2%
Commuters on urban highways	5% or more (CO levels on highways average 27.5 mg/m ³ rising up to 110 during temperature inversions)
Tobacco smokers	Cigarettes: One pack per day 5% to 6%, two to three packs per day 7% to 9%; Cigars: up to 20% HbCO

(b) Published biological action levels

Table 2.3.7 shows published biological action levels by organizations and recommendations by individual authors.

Table 2.3.7. Published biological action levels, HbCO in blood as an indicator of methylene chloride exposure

Biological action level, basis and collection time	Reference
5% collected at end of shift or end of exposure based on the prevention of adverse health effects	(23)
2.0% tentative maximum permissible concentration in non-smokers, based on prevention of adverse health effects	(25)

(c) Non-analytical interferences

- i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, and medications

Exposure to carbon monoxide, either through smoking, inhalation of side stream smoke, or environmental sources, will increase the level of HbCO in blood. Any population with decreased oxygen-carrying capacity or decreased oxygen availability may be at increased risk of carbon monoxide toxicity. Workers with respiratory disease that impairs oxygen delivery and pregnant workers and their fetuses may also be at an increased risk. Populations not adapted to living at high elevations (over 1,500 meters above sea level) are more sensitive to carbon monoxide because of reduced atmospheric oxygen. Native populations may not be more sensitive because of adaptation to lowered atmospheric oxygen (45). Heavy labour, high temperature, or high altitude contribute to the health risk of workers exposed to carbon monoxide and methylene chloride.

- ii) Diet and environment

Smoking habits and exposure to carbon monoxide should be noted as a source of error. However, since HbCO is a measure of exposure by all routes and from all sources, monitoring of HbCO may be of value. Samples taken from current tobacco smokers or workers exposed to carbon monoxide should not be used for evaluation of occupational exposure to methylene chloride because of the other sources of carbon monoxide assessed by HbCO measurements.

(d) Sampling representative of recent or long-term exposure, or biological effect

HbCO measurements represent exposure during the last few hours prior to sample collection. Measurements in samples taken during the first 3 hours of exposure or later than 15 to 30 minutes after the end of exposure cannot be used for the evaluation of 8-hour TWA exposures. Unlike exposure to carbon monoxide, the elimination of HbCO continues after exposure to methylene chloride ceases, due to continued metabolism of methylene chloride.

(e) Ethnic differences (e.g. enzyme deficiency, environment, diet)

No ethnic differences that affect HbCO production were found in the literature.

2.3.9 Research needs

Documentation of background levels of methylene chloride in blood and the relationship between methylene chloride exposure and levels of methylene chloride and carbon monoxide in blood need further clarification. The non-linear kinetics observed in rats has toxicological significance, if seen in humans. The relative effectiveness of expired methylene chloride, methylene chloride in blood, and carboxyhemoglobin as indicators of exposure need evaluation.

2.3.10 References

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Chapter 3. Biological monitoring of selected pesticides

3.1 Dithiocarbamates¹

3.1.1 Introduction

The dithiocarbamate family of chemicals is mainly used in agriculture and forms part of a large group of synthetic organic compounds that have been developed and used worldwide over the last 50 years.

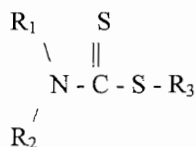
Dithiocarbamates are used as fungicides, a few have been used as herbicides and soil insecticides/nematocides. In industry, they are used as slimicides in water-cooling systems, in paper manufacturing, and as vulcanization accelerators and antioxidants in rubber.

Exposure occurs mainly by inhalation and percutaneous absorption under occupational conditions. The general population can be orally exposed via ingestion of residues from treated food.

There are reviews of experimental and human toxicology of dithiocarbamates (1–8).

Structure

The general formula of dithiocarbamates is characterized by the presence of the basic structure:



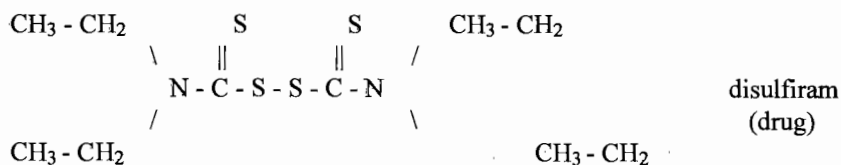
¹ Although biomonitoring of exposure has been performed for sometime by employers of factory workers for industrial health reasons, the data are regarded by industry as confidential medical records and are therefore not made publicly available.

Similarly, a few exposure studies have been performed on crop sprayers and the results submitted to several national registration authorities. Citations for these submissions to registration authorities or regulatory agencies are provided when available. Details of these studies also remain confidential and are not reported in the open literature.

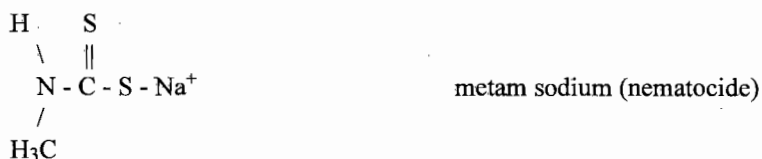
Depending on the types of mono-amines used in the synthesis, mono- or dialkyldithiocarbamates are formed. Reaction with diamines results in the formation of two terminal dithiocarbamate groups linked by an alkylene (ethylene) bridge. Both alkyl and ethylene dithiocarbamates form salts with metals.

More than 15 dithiocarbamates are known. The following are some of the most widely used.

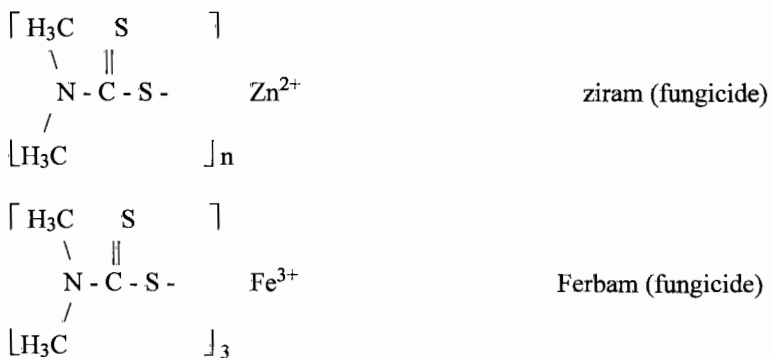
Tetraethyl thiuram disulfide



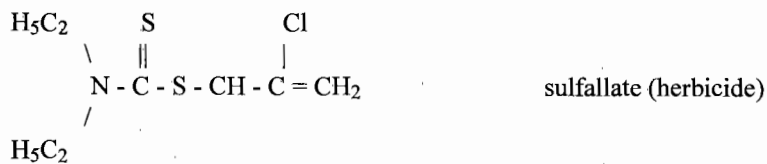
Methyldithiocarbamates



Dimethyldithiocarbamates



Diethyldithiocarbamates

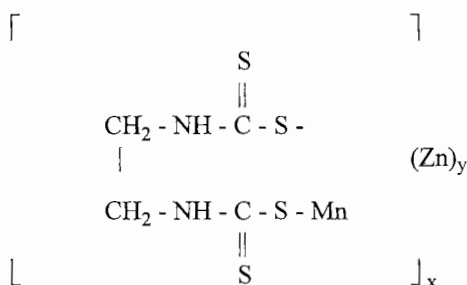


Ethylenebisdithiocarbamates

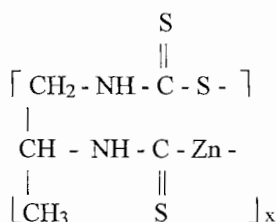
Maneb (fungicide)

- Zn

Zineb (fungicide)



Mancozeb (fungicide)

Propylenebisdithiocarbamate

Propineb (fungicide)

The division into the above subgroups is based on the character of the substitution for one or both hydrogens on each nitrogen. This classification has the advantage of distinguishing compounds being metabolized to ethylenethiourea (ETU) or propylenethiourea (PTU) from those incapable of forming this metabolite but characterized by their degradation into carbon disulfide (CS₂). This different metabolic pathway has a direct bearing on the biomonitoring of these compounds (see metabolic pathways – figure 3.1.1 and figure 3.1.2).

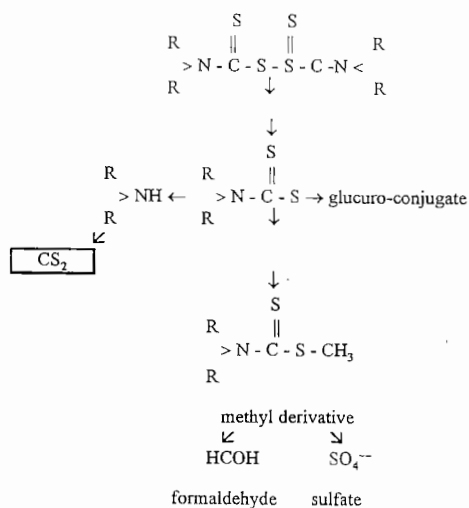


Figure 3.1.1. Schematic metabolic pathway for the decomposition of dialkyl dithiocarbamates in the rat

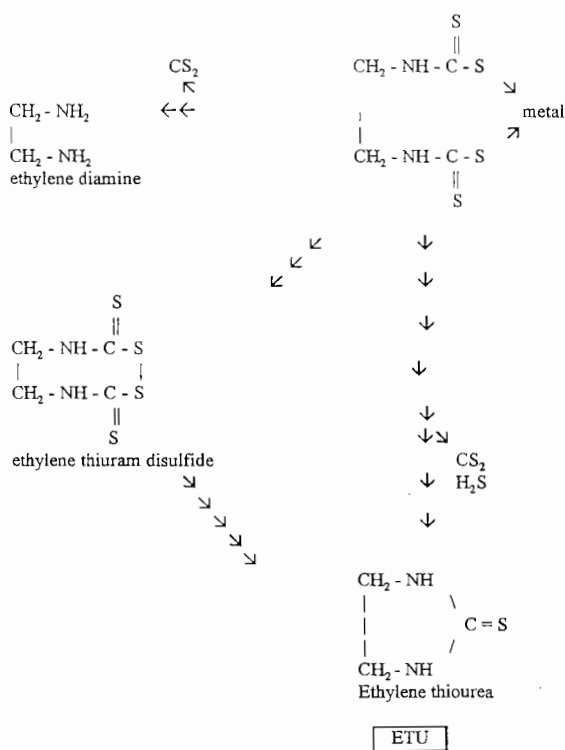


Figure 3.1.2. Schematic metabolic pathway for the decomposition of ethylenebisdithiocarbamates in the rat

3.1.2 Physical-chemical properties

Dithiocarbamates with hydrophylic groups, such as OH^- and COOH form water soluble heavy metal complexes. However, dithiocarbamate metal complexes used as fungicides are all insoluble in water, but are soluble in non-polar solvents. Alkylene bisdithiocarbamates containing two donor CS_2^- groups, which form polymeric chelates (ethylenebisdithiocarbamates) are insoluble in both water and non-polar solvents. Dithiocarbamates are unstable in acidic conditions and readily convert to the amine and carbon disulfide.

The main physical-chemical properties of a selected list of compounds are summarized in table 3.1.1.

3.1.3 Possible occupational and non-occupational exposures

Occupational exposure to dithiocarbamates occurs essentially during the manufacture and application of pesticides and in the rubber vulcanization industry.

Occupational exposure to ETU occurs in the rubber industry where the product as such is used as an accelerator of polymerization. It also occurs in the manufacturing/formulating pesticide industry and in agricultural uses through exposure to ETU as a by-product or degradation product.

Non-occupational exposure of the general population results from the occasional residues in the diet. However, dithiocarbamates degrade rapidly after application to crops.

A particular case of "voluntary" exposure is that of disulfiram, the anti-alcoholic Antabuse[®] which is therapeutically administered to induce intolerance to alcohol. Other chemically related compounds have been shown to have the same interaction with alcohol, among which thiram seems to be the most effective.

3.1.4 Summary of toxicokinetics

Dithiocarbamates may penetrate the organism through the respiratory tract (aerosol, dust), the skin and mucous membranes and the digestive tract.

3.1.4.1 Absorption

(a) Inhalation

This route of exposure occurs during the manufacturing of dithiocarbamate active ingredients and their formulation and during the spraying of crops with solutions of fungicide or herbicide dithiocarbamates. However, the improvement of formulation and spraying technologies, as well as the increased awareness of the agricultural profession over the last two decades has significantly decreased the potential for such route of exposure in agricultural workers. Respiratory exposure to ETU occurred also during the handling of rubber vulcanization accelerator activities.

Table 3.1.1. Physical-chemical properties of selected compounds

Common name	Molecular formula	Mol. weight	CAS number	Melting point	Vapour pressure	Stability	Solubility
Metham sodium	$C_2H_4NNaS_2$	129.2	137.42.8	decomposes without melting	non volatile	unstable in diluted aqueous solutions. decomposes in acids.	water at 20°C 722 g/L mod. sol. in methanol ethanol. Practically insoluble in other org. solv.
Ziram	$C_6H_{12}N_2S_4Zn$	305.8	137.30.4	240°C	negligible	decomposes in acids and by UV	water at 25°C 65 mg/L sol. in chloroform carbone disulfide insoluble in ethanol
Ferbam	$C_9H_{18}FeN_3S_6$	416.5	14484-64-1	decomposes above 180°C	negligible	decomposes on exposure to moisture and heat	water 20°C 130 mg/L soluble in org. solvents
Maneb	$(C_4H_6MnN_2S_4)_x$	$(265.3)_x$	12427-38-2	decomposes without melting at 192-204°C	negligible	decomposes on prolonged exposure to air or moisture	practically insoluble in water and organic solvents
Zineb	$(C_4H_6N_2S_4Zn)_x$	$(275.7)_x$	12122-67-7	decomposes without melting at 157°C	< 0.01 mPa at 20°C	unstable to light moisture and heat Auto ignition 149°C	water at 20°C 10 mg/L insoluble in org. solvents
Thiram	$C_6H_{12}N_2S_4$	240.4	137-26-8	146°C	negligible	decomposes in acids	water at 20°C 30 mg/L
Propineb	$(C_5H_8N_2S_4Zn)_x$	$(289.7)_x$	12071-83-9	decomposes at 160°C	< 1 mPa at 20°C	decomposes by moisture	< 1 mg/L water at 20°C

(b) Dermal

These compounds are poorly absorbed through the skin allowing for a low bioavailability through this route of exposure. Skin contact occurs during occupational exposure (manufacturing and agricultural handling, spraying and cleaning of equipment).

(c) Gastro-intestinal

Gastro-intestinal absorption of dithiocarbamates is fairly rapid as judged from experimental work in laboratory animals (1, 2). However, it would seem that metal-complexed alkylene compounds are much less absorbed. Ziram has been shown to cross the placental barrier and to accumulate in fetal issues (2).

3.1.4.2 Metabolic pathways and biochemical interactions

Dialkyldithiocarbamates such as thiram and disulfiram and ethylene/propylene bis-dithiocarbamates such as mancozeb, maneb, zineb and propineb are metabolized via different mechanisms (1).

The degradation of ethylene or propylene bis-dithiocarbamates leads to the formation of ethylenethiourea (ETU) or propylenethiourea (PTU), respectively.

Other compounds do not produce these metabolites.

The initial metabolites of ethylenebisdithiocarbamates (EBDCs) are different from the initial metabolites of dimethyldithiocarbamates. It appears that carbon disulphide and its metabolites are the only compounds common to the metabolism of all dithiocarbamate fungicides (see figures 3.1.1 and 3.1.2).

The similarity of the toxic properties of different kinds of dithiocarbamates suggests the relative unimportance of the metals used in the synthetic substitutions and of the other initial metabolites involved. Conversely, this similarity suggests the importance of the common metabolite, carbon disulphide and its metabolites. However, in those compounds, the degradation of which leads to ETU, this metabolite is important, as ETU is the metabolite of toxicological concern.

In fact, the similarity of poisoning by various dithiocarbamates and poisoning by carbon disulphide has been noted. This applies to the entire syndrome of acute poisoning by dithiocarbamates, to the results of their interaction with alcohol and to most of the clinically evident effects of repeated exposure to dithiocarbamates. It does not apply, however, to the anti-thyroid effects which have never been observed in humans or animals exposed to carbon disulphide (1).

The following compounds have been demonstrated to produce carbon disulphide both in laboratory animals and in humans: disulfiram – disodium ethylenebisdithiocarbamate (nabam) – ziram – thiram. Those generating ETU are mancozeb, maneb, zineb; propineb generates propylene thiourea (PTU).

One of the most important enzymatic process in the metabolism of dialkyldithiocarbamates is glucuronidation (9). Methylation of diethyldithiocarbamates by S-adenosyl methionine transmethylase in the liver and kidney can occur and leads to sulfate excretion (10).

3.1.4.3 Distribution

Dithiocarbamates and ethylenedithiocarbamates and their metabolites (especially carbon disulphide and ETU) are distributed through the blood following absorption to certain tissues and organs, such as the liver, kidneys, brain, and especially thyroid gland (ETU), but accumulation of these compounds does not occur because of their rapid metabolism. ETU is rapidly absorbed through the gastro-intestinal tract and rapidly cleared. ETU has been found to have a half-time of about 28 hrs in monkeys, 9–10 hrs in rats and 5 hrs in mice (11).

Absorption, distribution, excretion and biotransformation of mancozeb and maneb in experimental animals (mouse, rat and monkey) have been studied and the results analysed and reported by the FAO/WHO Joint Meeting on Pesticide Residues (3).

In the mouse, metabolites found in the urine of both sexes are, in decreasing order, ETU, ethylenethiuram monosulfide, ethylenethiourea-N-thiocarbamide.

In the rat, ETU was rapidly eliminated from the plasma of both males and females (half-time 4.0–4.7 hrs) and decreased below detectable levels within 48 hrs post-dosing. ETU was a major metabolite found in the urine, bile and faeces.

The estimated bioavailability of ETU in rats was about 6.8% on a weight/weight basis and 20% on a mole/mole basis (1).

3.1.4.4 Elimination

Dithiocarbamates and their metabolites are rapidly eliminated through the faeces and urine (2). Carbon disulphide and its metabolites are found in expired air following dermal or pulmonary exposure to dithiocarbamates, essentially thiram (See chapter on CS₂ in Vol. I of these guidelines).

However, no data are available to support the selection of carbon disulphide or one of its main metabolites (2-thiothiazolidine-4-carboxylic acid) as a biological marker of exposure to dithiocarbamates.

3.1.5 Summary of toxic effects

The toxicological database on several ethylenebisdithiocarbamates and on ethylenethiourea and propylenethiourea has been extensively reviewed by the FAO/WHO Joint Meeting on Pesticide Residues (3) and by the International Agency for Research on Cancer (IARC) (4–8).

In general, the acute toxicity of dithiocarbamates for mammals is relatively low (WHO classification) (12). Usually dithiocarbamates are classified as eye and skin irritants. Some may induce skin sensitization in susceptible individuals (1).

In subchronic and chronic experimental exposure to ETU and PTU-generating compounds, the target organ is the thyroid. The lesions observed range from hyperplasia to nodular goiter, adenoma and adenocarcinoma. Neurotoxic effects have also been reported in animals administered high doses of dithiocarbamates for prolonged periods of time. Whether this effect is resulting from "internal" exposure to carbon disulphide as a metabolite of dithiocarbamates circulating in the blood and a known neurotoxicant (See chapter on CS₂ in Volume I of these guidelines), has not been experimentally demonstrated. Some dithiocarbamates, including maneb, zineb and propineb have been reported to be teratogenic at high dosages, probably through the metabolites ethylenethiourea and propylenethiourea which are themselves teratogenic in laboratory animals. Long-term exposure to ETU and PTU at relatively high dietary concentrations has been shown to induce thyroid tumours in experimental animals (1–8). ETU is not genotoxic (3).

However, in human exposure situations, no effects on the thyroid or neurotoxicity have been reported with any clear or unquestionable information to establish a causal relationship (1, 2). Only skin reactions have been clearly established in exposed workers (1, 2).

3.1.6 Biological monitoring indices

Biological indices that could potentially be used for monitoring exposure to dithiocarbamates are listed below (table 3.1.2). This table has been reproduced in part from the chapter on CS₂ in Volume I of these guidelines.

Table 3.1.2. Biological monitoring indices of exposure to dithiocarbamates

Indicator	Suitability
CS ₂ in expired air	poor, limited occupational exposure data
CS ₂ in blood	limited data
CS ₂ in urine	poor, limited data
ETU in blood	poor, limited data, invasive
ETU in urine	best indicator
TTCA in urine	no data available

ETU generating compounds

Ethylenebisdithiocarbamates generate ETU as the main metabolite. It is excreted in urine where it can be detected and measured (13–16).

3.1.7 ETU in urine

The structure and physical-chemical properties of ETU are available (2).

3.1.7.1 Toxicokinetics

ETU is rapidly absorbed from the gastro-intestinal tract and is cleared from the body. Its main route of excretion is via the urine (2, 3).

No published data on human metabolism of ETU is available. However, several bio-monitoring studies of agricultural workers exposed to EBDCs (mancozeb and maneb) as well as to some ETU in the air from either product degradation or contamination during chemical synthesis are available. The half-time of ETU in human has been calculated to be close to 100 hrs. However, this half-time seems to be abnormally long and it is probably resulting from the effects of confounding factors (13). In the rat, ETU appears in the blood only 5 minutes after administration of an oral dose. Within 48 hrs, approximately 90% of the administered dose is eliminated via the urine and about 3% via the faeces. In monkeys, 55% was eliminated via the urine within 48 hrs and 1.5% via the faeces (17).

ETU and its metabolites have been found to have a half-time of about 28 hrs in monkeys, 9–10 hrs in rats and 5 hrs in mice (11).

Unpublished experimental data have shown that less than 8% of an orally administered dose of maneb or mancozeb is degraded to ETU in rats and about 2% in mice. In addition, it has been shown (unpublished) dermal penetration of these two EBDC compounds in laboratory animals is quite low (less than 1% for maneb in rats) and would not lead to a significant amount crossing the skin. Consequently, it is likely that the degradation of EBDCs on the skin of the exposed workers with subsequent absorption of ETU represents the major source of exposure to ETU.

3.1.7.2 Biological sampling

(a) Sampling time and specimen

Urine samples should be collected at the end of the workshift or at the end of a work-week in situations where there is a daily exposure, such as in the rubber industry or in professional contract agricultural spraying. Otherwise, urine samples should be collected at the end of the last day of the exposure period.

(b) Contamination possibilities

Care should be taken during the sample collection to avoid contamination by the parent product that might be deposited on the hands and clothing of the workers, as it may contain ETU as a contaminant of the agricultural product itself, or ETU could be generated by the degradation of the parent compound. Therefore, it would seem advisable to collect the urine sample after showering and changing to street clothes.

(c) Sampling device and container

Urine can be collected in any clean, dark, glass container.

(d) Preservative, shipment and stability

It is better to store and ship the samples at low temperature since ETU is easily oxidized to ethylene urea (EU) in biological systems and by photolytic reaction.

The stability of ETU in water or urine is poor during prolonged storage. Samples should be analysed as soon as possible after collection (18).

The US EPA normally advises that crops samples to be analysed for residues and other biological samples, such as urine be stored frozen at approximately - 20°C.

3.1.7.3 Recommended analytical method

An official method for ETU (19) has been developed for measuring residues of ETU in crops and has been demonstrated to give low and erratic recovery of ETU (20).

A method has been developed specifically to analyse, with reliable and consistent results, ETU in urine (and air) (18). This method is described below.

ETU in urine is determined by reversed-phase high performance liquid chromatography (HPLC) with ultraviolet detection at 230 nm. The detection limit for ETU is 0.1 ng per injection and linear response is found for the range 0.3–110 ng.

(a) Principle of the method and procedure

The urine samples (10 mL) are evaporated to dryness and thereafter 2mL of methanol and 150 mg of silica gel are added. Methanol is then evaporated and the sample is transferred to an aluminum oxide column. The elution is made by 2% methanol in dichloromethane. The eluate is then evaporated and 0.5 mL of water is added.

The HPLC analysis is carried out with Hewlett-Packard 1090 liquid chromatography equipped with a diode array detector. The HPLC is done with isocratic elution using methanol/ammonium acetate solution at 1.0 mL min⁻¹. The injection volume is 25 µL.

(b) Reagents required

ETU standard, purity 98% (Fluka AG Switzerland); methanol and dichloromethane of HPLC grade; silica gel Kieselgel 60 (70–230 mesh); aluminum oxide; for the mobile phase: 5% (v/v) methanol in 0.05 M ammonium acetate.

(c) Equipment required

Hewlett-Packard 1090 liquid chromatography with Nucleosil C18 reversed-phase column (column length 25 mm, internal diameter 4.6 mm Chrompack) thermostated at 40°C; rotary evaporator.

(d) Calibration

ETU in urine samples is determined by comparing with ETU standard dissolved in water to give concentration of 0.25 µg/mL.

(e) Criteria of analytical reliability

i) Trueness

Trueness, as assessed by recovery studies done with blank urine spiked with known amounts of ETU. The authors of the method report a mean recovery of 87–90 and 96% depending upon the level of ETU added to the urine samples (20).

ii) Precision

The precision has not been specifically reported. However, in the report (18), the standard deviation values, namely 87 ± 21 , 90 ± 2 and 96 ± 2 provides a fair idea of the precision which can be considered as adequate.

iii) Detectability

Levels of 0.2 µg/L ETU can be detected in urine (18).

(f) Quality assurance

i) Special precautions

Quality assurance must be performed within the laboratory, using portions of pooled urine from exposed workers, as no external proficiency testing programmes are currently available.

ii) Interferences

Interferences from the presence of EBDC's or breakdown products are possible. These can result from contamination of the samples during urine collection.

(g) Sources of possible errors

i) Pre-analytical

Care should be taken not to contaminate with EBDC parent compounds urine samples at the time of collection as they may contain small amounts of ETU as manufacturing or degradation contaminants or they may degrade into ETU during storage.

Inappropriate storage of urine samples (exposition to light or room temperature for prolonged periods of time) will result in loss of ETU content (18, 20).

Similarly, the effect of storage time and conditions on ETU stability in the final solution of purified extract has been investigated and it was shown that storage of the final solution overnight at 5°C resulted in an ETU loss. Therefore, ETU in final solutions of purified extracts must be determined without delay.

ii) Analytical

No particular source of error has been mentioned by the authors of the method.

(h) Reference to the most comprehensive description of the method

Kurtio P, Vartiainen T, Savolainen K. A high performance liquid chromatographic method for the determination of ethylene thiourea in urine and on filters. *Anal Chim Acta* 1988;212:297–301 (18).

(i) Evaluation of the method

This published method has been used under several exposure conditions and the results have been published (13–16). However, no indication of an inter-laboratory validation scheme is available.

3.1.7.4 Other analytical methods

An official method for ETU has been published (19). This method contains a procedure in which ETU is derivatized before GC determination. There is also an IUPAC published method (21). A review of all published chromatographic analytical methods published until 1985 indicates that a number of different methods are available (22).

However, most if not all of these methods, have been developed with the objective of measuring residues of ETU in crops or in the environment (soil, water). According to Kurtio et al. (18), the detection limit of the GC methods for ETU is not sensitive enough for detecting ETU in biological samples.

HPLC methods can be used for the analysis of ETU in several types of samples but none of the previously described methods with enough sensitivity provides totally reliable identification of ETU.

Recently Kurtio et al. (23) have developed and reported a method which is sensitive enough to be used for the detection of ETU in urine (and air) during and after exposure to ethylenedithiocarbamates and/or ETU. However, the drawbacks of this method are that it is laborious to be used routinely and that it requires sophisticated, expensive equipment. ETU is separated, identified and measured in urine samples by applying reversed-phase HPLC followed by thermospray mass spectrometry.

3.1.7.5 Guide to interpretation

The measurement of ethylenethiourea in urine has been shown to be suitable for biological monitoring of exposure to ethylenedithiocarbamates (13–16).

For dithiocarbamates that do not generate ETU as one of their major metabolites, such as ziram, thiram, disulfiram, it is not known whether carbon disulphide could be considered as a suitable indicator of exposure in human. It is interesting to note, when thiram is intraperitoneally administered to rats, the formation of carbon disulphide from thiram appears to be dosage-dependent: increasing the dosage from 15 to 30 mg/kg results in a 10-fold increase in the amount of carbon disulphide in expired air. When the dosage is increased 4 times to 60 mg/kg, carbon disulphide production is increased 40 times (1).

(a) Measured values of ETU in groups without occupational exposure

ETU has been measured in non-occupationally exposed individuals (administrative workers) included as "negative" controls in monitoring studies of agricultural workers exposed during spraying of crops. No ETU has been detected in the unexposed persons (unpublished).

(b) Published biological action levels

The only published biological action level for ETU is the acceptable daily intake (ADI) value of 0.004 mg/kg body weight. However, ADI is not an index for occupational exposure. It indicates the daily dose that can be ingested by an individual for a life-time through residues in food without any risk. This value can nevertheless be used to provide an idea of the level of a safe exposure in human. The ADI for EBDCs as a group (mancozeb, maneb, zineb, and metiram) is 0.03 mg/kg body weight (3).

The US National Institute for Occupational Safety and Health has published exposure limits of 5mg/m³ for thiram and of 1.5 mg/m³ for Ferbam (24).

The American Industrial Hygiene Association has developed Workplace Environmental Exposure Level Guides representing the workplace exposure levels to which nearly all workers could be exposed repeatedly without adverse effects. They are expressed as time-weighted average (TWA) concentrations or as ceiling values. At the moment, the only value of interest here is that set for mancozeb: 1 mg/m³ 8-hour TWA (27).

(c) Non-analytical interference

i) Exposure to other chemicals, co-exposure, ethanol intake, medication

Disulfiram is the only dithiocarbamate that is used therapeutically to produce intolerance to alcohol. However, it has been reported that exposure to several other dithiocarbamates, such as thiram and ziram, could also result in illness when combined with alcohol consumption (26).

ii) Diet and environment

ETU has known patent anti-thyroid effects in experimental animals (1). Epidemiological surveys have not confirmed that such effects existed in human occupationally exposed to ETU (3). It is known that sulfur-based compounds with anti-thyroid effects in human are naturally occurring in some edible vegetables, such as cabbages, turnip, and rutabaga. Data that would permit a meaningful comparison between exposure from this source and exposure from dithiocarbamates appears to be lacking.

Therefore, it is not known at this point whether interference from compounds present in the diet could occur during biomonitoring of workers. Another potential source of interference might result from therapeutic administration of thiourea-based anti-thyroid drugs. Again, no data is presently available that would indicate a possible interference with measurement of ETU in urine of workers.

(d) *Populations of special concern*

No information was located regarding populations of special concern. However, one case of sulfhemoglobinemia and acute hemolytic anemia caused by zineb in a glucose-6-phosphate dehydrogenase deficient individual has been reported (29). This effect can presumably result from any one of the EBDCs.

(e) *Ethnic differences*

There are no data on possible ethnic difference in metabolism of EBDCs.

3.1.8 Research needs

There are several published and unpublished epidemiological studies of workers occupationally exposed to dithiocarbamates and/or ETU in the rubber and pesticide manufacturing industry. Some studies on exposed agricultural workers have been done and a few have been published (13–16).

Further research would seem necessary to adequately evaluate the impact of the exposure to EBDCs and ETU on the exposed workers' thyroid functions.

For those dithiocarbamates that do not generate ETU it would seem very useful to investigate:

- 1) whether carbon disulphide in expired air could be regarded as a suitable index of exposure, as it is considered at the moment as a poor indicator of occupational exposure to carbon disulphide because of the limited human database available (See chapter on CS₂ in Volume 1 of these guidelines).
- 2) whether carbon disulphide in urine could be developed as a suitable indicator of exposure (the presently existing database is limited).
- 3) the possibility of using 2-thiothiazolidine-4-carboxylic acid (TTCA) in urine. TTCA is the most widely used biological indicator of exposure to carbon disulphide (Vol. I). Since carbon disulphide is one of the metabolites of dithiocarbamates in laboratory animals, it would be useful to explore whether this is also the case in humans and whether it generates enough TTCA to be practically measured.

3.1.9 References

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3.2 Pyrethroids

3.2.1 Introduction

To date no biological action levels have been set for biological monitoring of occupational exposure to some synthetic pyrethroids. To give readers an indication of what biomarker levels may be observed data obtained in the authors' laboratory has been included. Additional information on atmospheric pyrethroid levels has been included where possible.

Pyrethrins obtained from flowers of pyrethrum plants (*Chrysanthemum* spp.) have been used for many years as insecticides. Synthetic pyrethroids were developed in the early 1970s to overcome the photostability problems of the naturally occurring pyrethrins. Several hundred synthetic pyrethroids have been produced of which a few are widely used. The pyrethroids discussed in this chapter are permethrin, cypermethrin, deltamethrin and fenvalerate.

(a) Permethrin

Permethrin [3-phenoxybenzyl-(1R,1S)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate] was described in 1973. It has four isomeric forms and commercially available permethrin consists of a mixture of the *cis* and *trans* isomers with *cis:trans* ratios of 40:60 and 25:75 being commonly available. Permethrin has been used to treat lice and scabies on humans in shampoos, lotions and powders (1).

(b) Cypermethrin

Cypermethrin [(R,S)- α -cyano-3-phenoxybenzyl-(1R,1S)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] was first used commercially in 1977. It has eight isomers and the commercial form consists of a racemic mixture with *cis:trans* ratios varying from 50:50 to 40:60 (2). In addition alpha-cypermethrin (a racemic mixture of two *cis*-cypermethrin stereoisomers) is also commercially available (3).

(c) Deltamethrin

Deltamethrin [(S)- α -cyano-3-phenoxybenzyl-(1R,3R)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] was synthesized in 1974 and first marketed as an insecticide in 1977. Unlike the other synthetic pyrethroids discussed here deltamethrin occurs as a single isomer. It is used for pest control in the growing of cotton and other crops, on stored agricultural produce and is also used for vector control in public health (4).

(d) Fenvalerate

Fenvalerate [(R,S)- α -cyano-3-phenoxybenzyl (1R,1S)-2-(4-chlorophenyl)-3-methyl-1butyrate] was introduced in 1976 and has been widely used in agriculture but is also

used for insect control in public health. Fenvalerate has four isomeric forms and the technical product is a racemic mixture (5).

3.2.2 Physical-chemical properties

The structures and physical-chemical properties of permethrin, cypermethrin, deltamethrin and fenvalerate are summarized in table 3.2.1 (1, 2, 4–7). Synthetic pyrethroid pesticides are available in a variety of formulations, including emulsifiable concentrates, ultra-low volume formulations, wettable powders and dusts (1, 2, 4, 5).

3.2.3 Possible occupational and non-occupational exposures

Permethrin, cypermethrin, deltamethrin and fenvalerate are all used to control a wide variety of agricultural pests. In addition, several of these compounds may be used to treat conifer saplings prior to planting, wood for remedial timber treatment, buildings for control of insect vectors and domestic pests and humans for control of lice or scabies (1, 2, 4, 5).

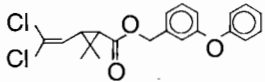
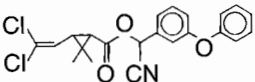
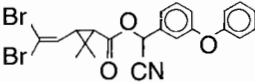
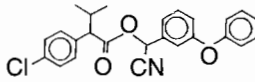
3.2.3.1 Occupational settings

Potential exposure to these pyrethroids may occur during manufacture and formulation (8), application to crops (9), the treatment of timber in situ (10), or treatment of buildings. Depending upon the formulation of the particular pesticide these occupational exposures may be via ingestion, inhalation or dermal absorption. In a study of 229 cases of acute poisoning following occupational use of pyrethroids the majority were due to inappropriate handling of the pesticides. Examples included spraying with higher than recommended concentrations, long exposure duration, spraying against the wind, clearing of blockages in sprayers by mouth and hands, and lack of personal protective equipment (11).

3.2.3.2 Non-occupational exposures

Non-occupational exposure to the synthetic pyrethroids occurs mainly via dietary residues, but may also occur from their use in public health. Absorption of permethrin has been reported in those treated with shampoos and dusts, although the proportion of the dose absorbed was generally low (1). In addition, the presence of permethrin in over the counter insecticidal repellents may lead to dermal exposure when applied to skin. Residue levels in crops treated according to good agricultural practice are generally very low. Some cases of poisoning following ingestion either by accident or as suicide attempts have been reported (11).

Table 3.2.1. The structures and physical-chemical properties of permethrin, cypermethrin, deltamethrin and fenvalerate

Property	Permethrin	Cypermethrin	Deltamethrin	Fenvalerate
Structure				
CAS Registry Number	52645-53-1	52315-07-8	52918-63-5	51630-58-1
Description of technical product	Viscous pale brown liquid	Viscous yellow liquid, more than 93% cypermethrin	White solid more than 98% deltamethrin	Viscous yellow to brown liquid contains 90-94% fenvalerate
Molecular weight	391.28	416.31	505.21	419.91
Molecular formula	C ₂₁ H ₂₀ Cl ₂ O ₃	C ₂₂ H ₁₉ Cl ₂ NO ₃	C ₂₂ H ₁₉ Br ₂ NO ₃	C ₂₅ H ₂₂ ClNO ₃
Melting point	34-35°C	60-80°C	98-101°C	
Boiling point	Ca. 200°C at 0.01 mm Hg		Decomposes above 300°C	Decomposes on distillation
Vapour pressure	0.045 mPa at 25°C	0.51 nPa at 70°C	0.002 mPa at 25°C	0.037 mPa at 25°C
Relative density	1.19-1.27 at 20°C	1.25 at 20°C		1.15 at 25°C
Water solubility	Ca. 0.2 mg/L at 20°C	Ca. 0.01mg/L at 20°C	Less than 0.002 mg/L at 20°C	Less than 1 mg/L at 20°C
Solubility in organic solvents	Soluble in majority of organic solvents e.g. hexane, xylene methanol, except ethylene glycol	Soluble in acetone, chloroform, xylene, cyclohexanone and hexane	Soluble in dioxane, cyclohexanone, acetone, benzene, xylene and dichloromethane	Readily soluble in acetone, ethanol, xylene, cyclohexanone and chloroform
Stability	Relatively stable to heat, moisture and light. More stable in acidic than alkali media.	Stable to heat, relatively stable to light. More stable in acidic media but hydrolysed in alkali media.	Stable to heat, light and air. More stable in acid than alkali media.	Stable to heat and moisture, relatively stable to light. Relatively stable in acid media, rapidly hydrolysed in alkali media.
References	1, 6, 7	2, 6, 7	4, 6, 7	5,6,7

3.2.4 Summary of toxicokinetics

3.2.4.1 Absorption

(a) Inhalation

Spray application of pesticides produces aerosols that can be absorbed by the lungs. A study of workers spraying tomato plants in greenhouses with mixtures of pirimiphos-methyl, dimethoate and permethrin concluded that respiratory exposure was generally 0.1% to 0.01% that of dermal exposure (12).

(b) Dermal

Dermal contamination is an important route of occupational exposure, especially for workers handling concentrated solutions of these pesticides. Pyrethroids are lipophilic compounds that are viscous, have relatively high boiling points and low vapour pressures. Therefore, unless they are removed by washing they will stay on the skin once contamination has occurred.

Rapid penetration of skin by pyrethroids has been reported with metabolites being detected in urine within a few hours of exposure (13, 14). However, estimates of the extent of absorption in man *in vivo* suggest that generally less than 5% of the applied dose is absorbed (1, 14). Studies *in vivo* in rats and monkeys found that after application of ^{14}C -permethrin to skin for 24 hr radioactivity could be detected in urine for up to 14 days after exposure. In rats 43–46% of the dose was recovered in urine after application to the back and in monkeys 14–21% of the dose was recovered after application of the pesticide to the forehead and 5–12% recovered after application to the forearm (15). *In vitro* studies of dermal absorption of ^{14}C -cypermethrin through excised rat (16) and human skin (17) supported in flow through diffusion cells have been reported. A maximum of 5% (rat skin) and 2% (human skin) of the applied dose was recovered in receptor fluid after 3 days.

(c) Gastrointestinal

Gastrointestinal absorption has only been reported in individuals with accidental or intentional ingestion of synthetic pyrethroids (11). However, gastrointestinal absorption is possible when workers put objects contaminated with pyrethroids in their mouths (11). In addition, gastrointestinal exposure may potentially occur when cooking surfaces or implements become contaminated with pesticide following treatment of food preparation areas for cockroaches, etc.

3.2.4.2 Metabolic pathways and biochemical interactions

Pyrethroid esters contain an 'acid' moiety and an 'alcohol' moiety and metabolic studies in laboratory animals have investigated the fate of both parts of the parent molecule (18–27). Metabolic pathways in mammals for several pyrethroids have been reviewed (1–5, 28). Limited data on the metabolism and excretion of pyrethroids in man is available (1, 4, 14, 29, 30).

The principal routes of metabolism for permethrin, cypermethrin, deltamethrin and fenvalerate are hydrolysis of the ester linkage to form an 'acid' metabolite and 3-phenoxybenzyl alcohol. The 'acid' moiety formed varies with the particular pyrethroid, for permethrin and cypermethrin the metabolites are *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylic acid (Cl₂CA). For deltamethrin the 'acid' moiety is 3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropanecarboxylic acid (Br₂CA) and for fenvalerate 2-(4-chlorophenyl)-3-methyl-1-butyric acid (CPBA). In mammals conjugates of the acid metabolites with glucuronic, sulfuric and amino acids may be found in urine.

The "alcohol" moiety of permethrin, cypermethrin, deltamethrin and fenvalerate is 3-phenoxybenzyl alcohol which undergoes further metabolism to form 3-phenoxybenzoic acid (3PBA). In addition, oxidation at the 4' position of the phenoxy group can lead to formation of 4'-hydroxy-3-phenoxybenzoic acid (4OH3PBA). Amino acid conjugation of 3-PBA and 4OH3PBA moieties show great variability with the mammalian species studied. The structures of metabolites that may be potential indicators for biological monitoring of exposure to permethrin, cypermethrin, deltamethrin and fenvalerate are shown in table 3.2.2.

Data from human volunteer experiments and field studies suggest that metabolism of pyrethroids in man is qualitatively similar to that seen in animals but there are important quantitative differences (1, 4, 14, 29, 30). Generally, only a small proportion of the dose (>0.5%) is excreted as parent pyrethroid.

3.2.4.3 Distribution

Animal studies have shown that following exposure the pyrethroids are widely distributed but are rapidly and readily excreted (1-5). However, the small proportion of the dose that enters tissues, such as fat and brain, may persist for several days after exposure (19).

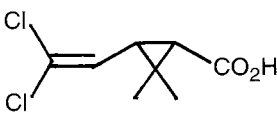
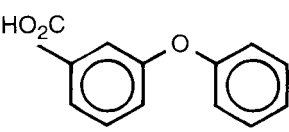
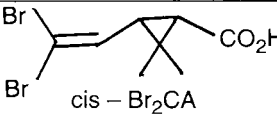
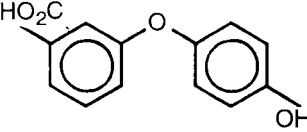
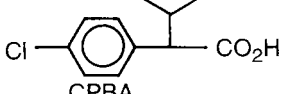
3.2.4.4 Elimination

(a) Permethrin

Studies of ¹⁴C-permethrin in rats have shown that the majority of the dose is rapidly excreted, however half-times of *cis* and *trans* isomers in fat of 7-10 days and 4-5 days, respectively, have been found (19).

A human volunteer study has been reported (1) in which two volunteers who consumed 2 and 4 mg of permethrin (25:75, *cis:trans*) excreted 18-37% and 32-39% of the administered dose as Cl₂CA in 24 h urine samples. The majority of this was excreted within the first 12 hrs, but half-times of elimination were not reported.

Table 3.2.2. Major metabolites of permethrin, cypermethrin, deltamethrin and fenvalerate

Pyrethroid	Metabolite derived from 'acid' moiety	Metabolite derived from 'alcohol' moiety
Permethrin	 cis/trans - Cl ₂ CA	 3-PBA
Cypermethrin		
Deltamethrin	 cis - Br ₂ CA	 4OH 3PBA
Fenvalerate	 CPBA	

(b) Cypermethrin

In an *in vivo* study of ¹⁴C-cypermethrin in the rat, this pyrethroid was largely metabolized and excreted from body tissues although elimination of cypermethrin isomers from fat was much slower with half-times of 7–10 days (19).

Four volunteers were administered single oral doses of a 1:1 *cis/trans* mixture of cypermethrin ranging from 0.25 to 1.5 mg (29). Within 24 h they had excreted, on average, 49% of *cis*-cypermethrin and 78% of *trans*-cypermethrin as *cis*- and *trans*-Cl₂CA respectively, this proportion being independent of dose. Half-times of elimination were not obtained from these experiments. A follow-up study looking at repeated oral administration of cypermethrin and urinary excretion of Cl₂CA in humans has been reported (30). The results suggest that cypermethrin does not accumulate as there was no increase in urinary Cl₂CA levels as dosing progressed.

A more recent human volunteer study investigating absorption of cypermethrin has been reported (14). Urinary excretion of the metabolites *cis*- and *trans*-Cl₂CA, 3PBA and 4OH3PBA was measured for 5 days after administration of either a 3.3 mg oral or a 31 mg dermal dose. Half-times of elimination were approximately the same for the four metabolites but varied between dose route having a range of 11–27 h for oral dosing and 8–22 h following dermal application (14). Differences in the proportion of the dose excreted as the metabolites of interest and in urinary metabolite profiles were seen be-

tween the dose routes. It was suggested that this may be a useful way of differentiating between the route of exposure to cypermethrin (14, 31).

(c) Deltamethrin

In an *in vivo* study (32) in rats, about 50% of the intravenously administered ^{14}C -deltamethrin was hydrolysed in blood within 0.7–0.8 min. Deltamethrin levels in the liver peaked at 5 min, but peaked in the central nervous system within 1 min. In rats deltamethrin was shown to have a half-time in fat of 5–6 days (19).

A human volunteer study using ^{14}C -deltamethrin showed that urinary excretion accounted for 51–59% of the initial radioactivity. Approximately 90% of urinary radioactivity was excreted during the first 24 h following administration. The apparent half-time of urinary excretion was 10.0–13.5 h. Faecal elimination at the end of the observation period represented 10–26% of the dose. Total recovery of radioactivity in urine and faeces amounted to 64–77% of the initial dose after 96 h (4).

(d) Fenvalerate

Rapid elimination of fenvalerate and its metabolites has been observed in cows dosed with radiolabelled fenvalerate (24). Unmetabolized fenvalerate was the major product in the faeces but only trace amounts were found in urine. In a study where rats were dosed with fenvalerate trace levels remained in fat tissue and had a half-time of elimination of 7–10 days (19). Miyamoto (27) has reported the metabolic fate of ^{14}C -labelled fenvalerate. Excretion of radioactivity was rapid and complete with greater than 97% being recovered in excreta and the highest residues in fat. However, when ^{14}CN -labelled fenvalerate was used, recoveries were reduced to 76–89% and radioactivity was found to be distributed in the stomach contents and skin, as well as in fat.

3.2.5 Summary of toxic effects

Pyrethroids are neurotoxic, causing certain biochemical and histological changes in the nerve tissues, but they are not known to cause delayed neuropathy. The major site of action for synthetic pyrethroids is the sodium channels in nerve membranes (33). Available evidence indicates that these pesticides produce a prolongation of the transient increase in sodium permeability of the nerve during the excitatory phase of an action potential. This effect is considered to be directly responsible for repetitive nerve activity which can cause myoneural junction acetylcholine depletion leading to muscular weakness and ataxia (33–36). The effects of acute exposure to pyrethroids in man can be graded as to the severity of exposure (11). After dermal exposure to pyrethroids people may experience abnormal facial sensations, such as burning, itching or tingling. These symptoms are collectively described as paraesthesia (37) and have been observed primarily with the α -cyano pyrethroids, such as cypermethrin, deltamethrin and fenvalerate (8, 13, 38–43). Paraesthesia is not seen following ingestion of pyrethroids (11) whilst permethrin has rarely been found to cause such sensations (43, 44). Pyrethroids do not appear to cause contact sensitization (45).

Systemic effects following less severe exposure include dizziness, headache, anorexia, irritability and fatigue (11, 46). Nausea is also seen but is more usual after ingestion of the pyrethroid. Following more severe exposures systemic effects include coarse muscular fasciculations, disturbances of consciousness, and convulsive attacks (11).

The International Agency for Research on Cancer has recently evaluated the carcinogenicity of deltamethrin, permethrin and fenvalerate (and also summarized toxicity, metabolism and exposure data) (47).

3.2.6 Biological monitoring indices

Table 3.2.3. Biological monitoring of exposure to pyrethroids

Indicator	Suitability for use	References
Intact pyrethroid in urine	Not widely reported might be suitable for heavy exposures	48, 50, 51
'Acid' moiety in urine	Recommended indicator for assessing exposures	14, 29, 30, 49, 52, 53
3-PBA and 4OH3PBA in urine	Recommended indicator for assessing exposures	14, 49

(a) Permethrin

Measurement of unchanged permethrin in urine is only suitable for assessing heavy occupational exposures or following accidental or intentional poisoning. An analytical method involving solid phase extraction of permethrin from urine has been reported (48). For permethrin and cypermethrin the major metabolic products are the same (1, 2). Therefore, measurement of Cl_2CA in urine is recommended as the best indicator of exposure (14, 29, 30, 49). In addition, analytical methods for 3PBA and 4OH3PBA have been reported (14, 49) (table 3.2.3).

(b) Cypermethrin

Human exposure to cypermethrin has been widely studied (14, 29–31, 47). An analytical method for measurement of unchanged cypermethrin in urine has been reported but may only be suitable for assessing heavy occupational exposure or following poisoning with cypermethrin (48). In the majority of literature reports concerning assessment of exposure to cypermethrin urinary Cl_2CA levels have been analysed as a biological indicator (14, 29–31, 49). Measurement of urinary 3PBA and 4OH3PBA has been suggested as an additional indicator which may provide a better overall assessment of exposure than the use of Cl_2CA alone (14, 49). Information concerning route of exposure may be derived from metabolite ratios (14, 49).

(c) Deltamethrin

The indicators proposed for biological monitoring of workers exposed to deltamethrin are unchanged deltamethrin and its metabolite Br_2CA in urine (50–52). The 'alcohol'

moiety of deltamethrin is also metabolized to 3PBA and 4OH3PBA; however, there do not appear to be any reports of these metabolites being used to assess exposure to this pesticide.

(d) Fenvalerate

Unchanged fenvalerate can be detected in the urine of workers and sprayers who are heavily exposed to this pesticide (50, 51). An analytical method for CPBA has been reported (53) but does not appear to have been widely used for monitoring for exposure to fenvalerate. Although 3PBA and 4OH3PBA are metabolites of fenvalerate there are no reports of these compounds being used as indicators of exposure to fenvalerate.

3.2.7 Assessing exposure to permethrin and cypermethrin

This method involves analysis of urine samples for *cis*- and *trans*-Cl₂CA, 3PBA and 4OH3PBA.

3.2.7.1 Toxicokinetics

Six volunteers given oral (3.3 mg) and dermal (31 mg) doses on separate occasions excreted detectable levels of Cl₂CA, 3PBA and 4OH3PBA for 5 days after exposure. Dermal absorption of cypermethrin was estimated to be approximately 1% of the applied dose. A similar proportion (mean 1.2%, range 0.3–2.1%) of an applied dose of 14C-permethrin was recovered in 0–5-day urines from treated volunteers (reference quoted in 14). For cypermethrin average half-times of elimination were estimated to be 16.5 h (range 11–27) for oral dosing and 13 h (range 8–22) for dermal application (14).

3.2.7.2 Biological sampling

(a) Sampling time and specimen

If the purpose of biological monitoring of permethrin or cypermethrin is to estimate the dose that may have been absorbed, then 24-h urine collections should be obtained as this gives a better estimate of total exposure. Otherwise urine samples should be collected at the end of the shift towards the end of the workweek.

(b) Contamination possibilities

Care should be taken to ensure that samples do not become contaminated from clothing or hands when urine is voided.

(c) Sampling device and container

Clean screw top plastic containers are suitable.

d) Preservative, shipment and stability

No preservative is required, samples should be transported to the laboratory as quickly as possible, preferably frozen. Prior to analysis samples should be stored frozen (-20°C), whenever possible.

3.2.7.3 Recommended analytical method

The analytes Cl₂CA, 3PBA and 4OH3PBA are quantified by gas chromatography mass spectrometry (GC-MS) after derivatization. Creatinine determination is recommended as reference values may be quoted after correction for urinary creatinine levels (10). If urine samples have creatinine levels below 0.3 g/L (3 mmol/L) an additional sample should be collected.

(a) Principles of the method

Urine samples are mixed with internal standards, heated with acid to hydrolyse conjugated metabolites, subjected to solvent extraction and the dried extract derivatized prior to gas chromatography mass spectrometric analysis.

(b) Reagents required

Certified reference materials for *cis*- and *trans*-Cl₂CA or 4OH3PBA are not available; however, 3PBA, 4PBA and 4OH4PBA are available commercially. Solvents required are analytical grade peroxide-free diethyl ether, methanol and ethyl acetate. Chemicals required include analytical grade anhydrous sodium sulphate, concentrated sulphuric acid (S.G. 1.18), pentafluoropropionic anhydride, pentafluoropropanol and trifluoroacetic acid. A supply of nitrogen is required for solvent evaporation and helium as a carrier gas for the gas chromatograph.

(c) Equipment required

A gas chromatograph attached to a mass spectrometer able to operate in electron impact multiple ion monitoring mode with automated chromatographic data handling. A 20 m x 0.32 mm fused silica capillary column coated with a 0.5µm thick RTX 1701 film (B. Woollen – personal communication) and a 2 m x 0.53 mm deactivated fused silica column to act as a retention gap should be installed in the gas chromatograph. Additional equipment includes a water bath or heat block able to operate from 40°C to 100°C, a rotary mixer, and a centrifuge. Procedures involving the use of solvents or derivatizing agents should be carried out in a fume cupboard.

(d) Procedures

i) Calibration

A seven point calibration curve was prepared in urine from an individual with no exposure to pyrethroids. An example of the metabolite concentration range covered would be 0–1000 µg/L of urine for each metabolite (i.e., *cis*- and *trans*-Cl₂CA, 3PBA and 4OH3PBA). The calibrants are taken through the sample treatment procedure with the samples to be analysed.

ii) Sample treatment

Urine samples (5 mL) in screw capped tubes are mixed with internal standard solution containing 4-(4-hydroxyphenoxy)benzoic acid (4OH4PBA) and 4-phenoxybenzoic acid (4PBA) in methanol (e.g. 50 μ L of 5 mg/L solution). After addition of concentrated sulphuric acid (1 mL) the tubes are sealed and heated for 2 h at 100°C. After heating tubes are cooled and diethylether (4 mL) added, the tubes resealed and mixed to extract for 0.5 h. The tubes are centrifuged and the ether layer transferred to clean tubes and mixed with anhydrous sodium sulphate, the tubes are centrifuged and the ether layer transferred to clean screw capped tubes. The anhydrous sodium sulphate is washed with diethyl ether (4 mL), centrifuged and the ether washings added to the first extract. The combined ether extracts are evaporated to dryness using a stream of nitrogen.

Derivatization was a two-stage process, the first stage (esterification of carboxylic acid groups) was effected by addition of pentafluoropropionic anhydride (200 μ L) and pentafluoropropanol (50 μ L) and heating the sealed tubes at 90°C for 0.5 h. After cooling trifluoroacetic acid (100 μ L) was added, the tubes resealed and heated at 90°C for another 0.5 h; this completed the derivatization (acylation of phenolic groups). After cooling the reagents were gently evaporated under nitrogen and the residue dissolved in ethyl acetate (150 μ L) prior to injection into the GC-MS.

iii) Analytical conditions

The carrier gas used was helium with a flow rate of 1 mL/min and splitless injections were used to introduce samples into the gas chromatograph. Using the capillary column described the gas chromatographic conditions were: injector temperature 200°C, initial oven temperature 75°C held for 0.2 min, increased at 40°C/min to 140°C held for 5 min then a second ramp of 25°C/min to 220°C and finally 15°C/min to 250°C held for 7 min.

For multiple ion monitoring mode the following ions were used Cl_2CA m/z 163 and 305, 3PBA and 4PBA m/z 197 and 346, 4OH3PBA and 4OH4PBA m/z 361 and 508. In each case the higher mass ions were used for quantitation. Metabolite concentrations were determined from calibration curves obtained from linear regression analysis of peak area ratios for the analytes and internal standards against concentration of metabolite in calibration samples.

(e) Criteria of analytical reliability

i) Trueness

Extraction efficiency was not determined as calibration samples were prepared with each batch of urine samples to be analysed.

ii) Precision

At 50 μ g/L intra-assay variation was typically 5% relative standard deviation.

iii) Detectability

The limit of detection for the analysis was 0.5 μ g/L for all four analytes.

(f) Quality assurance

i) Special precautions

Quality control should be performed within the laboratory as no external quality control schemes are available.

ii) Interferences

No interferences have been reported using this method. However, contamination of the urine with parent permethrin or cypermethrin could lead to interferences in measurement of levels of Cl₂CA.

(g) Sources of possible error

i) Pre-analytical

Contamination of samples with intact pyrethroid should be avoided, the hydrolysis conditions used during sample preparation are sufficient to hydrolyse permethrin and cypermethrin to produce Cl₂CA.

ii) Analytical

Normal standards of care within the laboratory are required.

(h) The reference to the most comprehensive description of the method

Woollen BH, et al. The metabolism of cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration. *Xenobiotica* 1992;8:983–91.

(i) Evaluation of the method

Human volunteer (14) and field studies with cypermethrin (10, 31) have been performed using this method or with slight modifications. These studies have shown that the analytical method is able to detect metabolites following low (less than 3 mg total internal dose) exposure to cypermethrin. In addition, field studies have shown its viability in measuring occupational exposure to permethrin (B.P. Nutley – unpublished results).

3.2.7.4 Other analytical methods

A gas chromatographic method with electron capture detection (GC-ECD) of methyl esters of Cl₂CA has been reported (29, 30). This required urine (5 mL) which was refluxed with sulphuric acid (5% v/v) in methanol (25 mL) for 1.5 h. The methylated metabolites were extracted, subjected to silica gel column chromatography and the eluted methylated derivatives analysed by GC-ECD. The method had a detection limit of 5–10 µg/L urine. One paper describes an analytical method for Cl₂CA with a separate, but similar, method for 3PBA and 4OH3PBA; these were used to study operator exposure to cypermethrin (49). These methods required 50 mL of urine for each assay and involve acid hydrolysis, solvent extraction with dichloromethane, derivatization with pentafluorobenzyl bromide, sample clean up with Florisil columns and positive ion electron impact GCMS detection. Detection limit was 1–2 µg/L urine.

3.2.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Metabolites of interest are not usually detected in urine from individuals with no known occupational exposure to permethrin or cypermethrin. In addition, urine samples obtained from workers on a Monday morning prior to starting work dipping conifer saplings in permethrin solution did not usually contain detectable levels of Cl_2CA or 3PBA, however 4OH3PBA was not monitored (B.P. Nutley – unpublished results).

(b) Published biological action levels

There are no published biological action levels for permethrin or cypermethrin. A summary of results from workers exposed to permethrin and cypermethrin, (B.P. Nutley – unpublished results) are given in table 3.2.4. The results are from end-of-shift samples and have been corrected for urinary creatinine content.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

Exposure to synthetic pyrethroids other than permethrin and cypermethrin that contain the 3-phenoxybenzyl moiety may lead to excretion of 3PBA and 4OH3PBA in urine. It is possible that excretion of 3PBA and/or 4OH3PBA may be useful as a screening technique for exposure to a variety of pyrethroids; a list of these is given in table 3.2.5. Measurement of Cl_2CA is suitable for permethrin, cypermethrin, alpha-cypermethrin or zeta-cypermethrin exposure. No literature reports were found concerning the effects of co-exposure of other chemicals (e.g. components of pesticide formulations), medication or alcohol on urinary excretion of pyrethroid metabolites.

Table 3.2.4. Urinary metabolite levels following exposure to permethrin or cypermethrin

Occupational group	Urinary metabolite levels (nmol/mmol creatinine) mean (range)		
	<i>Cis + Trans</i> Cl_2CA	3PBA	4OH3PBA
Pest control operatives treating premises with Permethrin (n=30 urine samples)	3.0 (0–24)	5.0 (0–46)	N/A
Dipping conifer saplings in solution of Permethrin (n=88 urine samples)	16.2 (0–67)	37.8 (0–246)	N/A
Planting conifer saplings dipped in solution of Permethrin (n=38 urine samples)	7.4 (0–37)	3.3 (0–12.1)	N/A
Treatment of forearms with commercially available insect repellents containing 1% Permethrin (urine sample equivalent to the end of the shift)	22	33	N/A
Remedial timber treatment with Cypermethrin (10) (n=12 urine samples)	1.0 (0–3.2)	0.8 (0–2.0)	0.7 (0–3.0)

Table 3.2.5. List of pyrethroids that contain 3-phenoxybenzyl or α -cyano-3-phenoxybenzyl alcohol that may be metabolized to 3-phenoxybenzoic acid (from 6)

Acrinathrin	Esfenvalerate
Cycloprothrin	Fenpropathrin
Lambda-cyhalothrin	Fenvalerate
Cypermethrin	Flucythrinate
Alpha-cypermethrin	Tau-fluvalinate
Zeta-cypermethrin	Permethrin
Cyphenothrin	Phenothrin
Deltamethrin	Tralomethrin

ii) Diet and environment

No reports were found concerning the effects of diet or environment on excretion of Cl_2CA , 3PBA or 4OH3PBA.

(d) Sampling representative of recent or long-term exposure

Half-times of excretion for Cl_2CA , 3PBA and 4OH3PBA range from 8–27 hrs after exposure to cypermethrin (14). Urine collection post-shift at the end of the week may give an indication of pesticide exposure over the week (54). Alternatively 24-h urine collections may be used to estimate the dose of permethrin or cypermethrin (31) received.

(e) Populations of special concern

No populations of special concern have been identified.

(f) Ethnic differences (enzyme deficiency, environment, diet)

No reports of ethnic differences in metabolite excretion have been found.

3.2.8 Assessing exposure to deltamethrin

This method involves analysis of urine samples for 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (Br_2CA).

3.2.8.1 Toxicokinetics

Studies in animals have shown that 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (Br_2CA) and its glucuronide are the major metabolites of the “acid” moiety of deltamethrin. Levels of Br_2CA in urine have been used for biological monitoring of exposure to deltamethrin. Results have shown that urinary excretion of free and conjugated Br_2CA is rapid and may persist for up to 2 days following exposure (34, 52).

3.2.8.2 Biological sampling

(a) Sampling time and specimen

Urine samples should be collected at the end of the workshift at the end of the work-week.

(b) Contamination possibilities

Care should be taken to ensure that samples do not become contaminated from clothing or hands when urine is voided.

(c) Sampling device and container

Urine samples should be collected in clean plastic containers.

(d) Preservative, shipment and stability

Urine samples should be acidified with hydrochloric acid (1 mL to 100 mL urine) and should be stored at a low temperature (1–4°C) or deep frozen until analysed.

3.2.8.3 Recommended analytical method

High performance liquid chromatography (HPLC) with ultraviolet detection at 254 nm is suitable for determination of Br₂CA in urine.

(a) Principles of the method

Urine samples are treated with β -glucuronidase/sulphatase to hydrolyse conjugated Br₂CA which is then extracted with hexane. The organic phase is concentrated and Br₂CA determined by HPLC.

(b) Reagents required

Methanol of HPLC grade and hexane of analytical grade. Commercially available, β -glucuronidase/sulphatase solution (5.2 $\pm$ 2.6 U/mL, Boehringer Mannheim, GmbH, FRG) is also required.

(c) Equipment required

An HPLC equipped with a UV detector capable of monitoring 254 nm is required. A C18 ultrasphere HPLC column (4.6 x 150 mm, 5 μ packing) is used for the separation. A supply of nitrogen, centrifuge and water bath are required for sample preparation. Thin layer chromatography plates (Sil G/UV₂₅₄, Polygram) are sometimes needed for sample pretreatment. Procedures involving solvents should be performed in a fume cupboard.

*(d) Procedures**i) Calibration*

No certified reference standards are available and synthesis of Br₂CA may be required. The purity of synthesized standard (molecular weight 298) must be verified by mass spectrometry. The calibration range is 0.3–13 µg/mL (methanol).

ii) Sample pretreatment and extraction

Free Br₂CA can be extracted initially, samples are subjected to enzyme treatment and conjugated Br₂CA analysed subsequently. Urine (2 mL) was adjusted to pH 6.5 and extracted with hexane (2 x 2 mL). The combined hexane layers were evaporated under nitrogen at 40°C in a water bath. For conjugated metabolite the extracted urine sample was adjusted to pH 5.5 with 5M and 1M acetic acid. An aliquot of β-glucuronidase/sulphatase solution (1 part per 100 parts of urine v/v) was added and the samples incubated at 20°C for 20 h. After incubation the pH of the sample was adjusted to 6.5 and the extraction, evaporation and reconstitution procedures described earlier repeated. Methanol (200 µL) was added to the dried extracts and the sample mixed and centrifuged.

ii) HPLC determination

A sample (5–20 µL) of reconstituted extract was injected into the HPLC with UV detection at 254 nm. Prepared pure Br₂CA in methanol was used as the external standard. The isocratic mobile phase consisted of methanol:water (90:10 v/v) with a flow rate of 1 mL/min. The analytical column used was a C₁₈ ultrasphere, 4.6 x 150 mm, 5µ packing.

*(e) Criteria of analytical reliability**i) Trueness*

Recoveries of urine samples spiked with Br₂CA averaged 95%.

ii) Precision

Within-day precision was 3% relative standard deviation at 26 µg/L and 65 µg/L.

iii) Detectability

The lower limit of detection for Br₂CA in urine, based on three times the signal to noise ratio was 10 µg/L. The detection limit can be improved by taking a larger volume of urine (20–100 mL).

*(f) Quality assurance**i) Special precautions*

No external proficiency testing schemes are available, therefore internal quality control must be conducted by using spiked samples. Blanks are necessary to ensure the quality of the results.

When large quantities of urine are to be used for higher detection sensitivity urine samples must be pretreated by thin layer chromatography using Silg/UV₂₅₄ plates prior to HPLC.

ii) Interferences

No interferences have been reported.

(g) Sources of possible error

i) Pre-analytical

Initial elimination of Br₂CA is rapid; therefore urine samples should be collected at the end of a workshift.

ii) Analytical

Urine samples should be analysed as soon after collection as possible.

(h) The reference to the most comprehensive description of the method

Yao P, et al. Biological monitoring of deltamethrin in sprayers by HPLC method. J Hyg Epidem Microbiol Immunol 1992;36:31-6.

(i) Evaluation of the method

The above method has been used to determine Br₂CA in urine of sprayers (34, 52) and cases of suicide (52). The results showed that Br₂CA was detected in urine confirming absorption of deltamethrin. From 3 to 48 h after the start of spraying, Br₂CA was detected in urine samples in the range 9 to 133 µg/L of urine. The metabolite could be detected at a relatively low level in the urine of a sprayer and at a high level in the urine from a suicide patient. Levels of Br₂CA were about 250 times higher than that of parent deltamethrin in the urine of patients with acute deltamethrin poisoning (52).

3.2.8.4 Other analytical methods

No other analytical method with the required sensitivity could be found.

3.2.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Deltamethrin metabolites are unlikely to be present in urine from individuals without occupational exposure to this pesticide.

(b) Published biological action levels

No reference values are available.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

No other chemicals are known to produce Br₂CA.

ii) Diet and environment

No information is available regarding effects of diet and environment on Br₂CA excretion.

(d) Sampling representative of recent or long-term exposure

Samples should be collected towards the end of the workweek.

(e) Populations of special concern

No populations of special concern have been identified.

(f) Ethnic differences (enzyme deficiency, environment, diet)

No ethnic differences have been identified.

3.2.9 Assessing exposure to fenvalerate

This method involves analysis of urine for intact fenvalerate. It can also be used to detect intact deltamethrin.

3.2.9.1 Toxicokinetics

Although fenvalerate and deltamethrin are metabolized and excreted rapidly, these pesticides have been detected in urine within 24 h of exposure from subjects engaged in packaging and spraying operations (8, 13).

3.2.9.2 Biological sampling

(a) Sampling time and specimen

Urine samples should be collected at the end of the workshift at the end of the workweek.

(b) Contamination possibilities

Special precautions are necessary to avoid contamination of the sample especially when voiding urine. Contamination is likely from work clothes or contaminated skin.

(c) Sampling device and container

Urine samples should be collected in clean plastic containers.

(d) Preservative, shipment and stability

Fenvalerate and deltamethrin are unstable in water and their concentrations in water decrease with time. Urine samples should be acidified with hydrochloric acid (1 mL acid to 100mL urine) and should be stored at a low temperature (1–4°C) or deep frozen until required for analysis.

3.2.9.3 Recommended analytical methods

This method can be used for the analysis of both fenvalerate and deltamethrin at the same time. The most widely used technique for the determination of fenvalerate in water, plants, soil and biological fluids is gas chromatography using packed columns (50, 55–58, 59) or capillary columns (60) with electron capture detection (GC-ECD). Very few methods have been developed for the determination of fenvalerate in biological fluids. A method using GC-ECD is the only one which can meet the required limit of detection.

(a) Principles of the method

Urine is extracted with hexane followed by clean-up through a Florisil column. The eluting solvent is concentrated and analysed by GC-ECD using a packed glass column.

(b) Reagents required

Analytical grade hexane and benzene (purified by distillation) and anhydrous sodium sulfate. Micro-columns (150 x 6 mm) packed with a cotton plug and 8 cm Florisil (100–200 mesh) and a supply of nitrogen are also required.

(c) Equipment required

A gas chromatograph equipped with an electron capture detector with nitrogen as carrier gas. Separatory funnels, centrifuge and water bath are required for sample preparation. Solvent extraction and elution procedures should be performed in a fume cupboard.

(d) Procedures

i) Calibration

A calibration curve should be prepared in urine from an individual with no exposure to pyrethroids. The calibration range used for either fenvalerate or deltamethrin was 5–100 µg/L.

ii) Extraction and clean-up

Urine (10 mL) was shaken vigorously with hexane (5 mL) in a separatory funnel. The hexane layer was collected and this procedure repeated twice more with the same volume of hexane. The combined hexane extracts are centrifuged for 20 min at 2500 rev/min. After centrifugation the organic phase were transferred to a clean tube and concentrated to approximately 1 mL under a flow of nitrogen in a water bath at 50°C.

A micro-column was washed with hexane (2 mL) just prior to use and a known volume (1 mL) of the extract placed on the column and eluted with benzene (12 mL). An aliquot (12 mL) of the eluting solvent was collected in a test tube and concentrated to 1 mL under a stream of nitrogen in a water bath at 50°C. The residue was mixed with anhydrous sodium sulfate and 1 µL volume of the organic phase injected onto the GC column.

iii) Chromatographic analysis

Analyses were performed using a gas chromatograph with a ^{63}Ni electron capture detector. A glass column (1 m x 2 mm i.d.) packed with 3% OV-101 on 80–100 mesh Chromosorb W AW DMCS was used.

Operating temperatures for injector, column and detector were 250°C, 240°C and 310°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min. Under the above conditions fenvalerate (which consists of four isomers) and deltamethrin eluted separately as single peaks at 4.6 and 5.9 min, respectively. No compounds that interfered with the pyrethroid peaks were found.

(e) Criteria of analytical reliability

i) Trueness

Trueness was determined by recoveries of spiked samples and average recovery was 92% and 95% for fenvalerate and deltamethrin, respectively.

ii) Precision

A within-day precision of 7% relative standard deviation can be expected for urine samples containing 10 µg/L fenvalerate.

iii) Detectability

The detection limit is estimated at 0.2 µg/l for a 10 mL urine sample based on three times the signal to noise ratio. Detection limit may be improved further by taking more urine or by reducing the final extract volume prior to sample injection.

(f) Quality assurance

i) Special precautions

No external proficiency testing programmes are available. Internal quality control must be conducted by using spiked urine samples carried through the analytical procedure. Blanks are also necessary to ensure the quality of the results obtained.

ii) Interferences

Interferences have not been seen using this method.

(g) Sources of possible error

i) Pre-analytical

Urine samples must be collected at the end of the workshift since the initial elimination half-time of deltamethrin is rapid. Meticulous attention must be paid to avoid contamination during sample collection.

ii) Analytical

Urine samples should be analysed as soon as possible after collection.

(h) *The reference to the most comprehensive description of the method*

Wu YQ, et al. Determination of deltamethrin and fenvalerate in human urine by gas chromatography. J Inst Health 1988;15:39–42 (in Chinese).

Wu YQ, et al. Determination of deltamethrin and fenvalerate in human urine by gas chromatography. Biomed Environ Sci 1994;7:216–21.

(i) *Evaluation of the method*

The above method has been used to detect deltamethrin in urine of sprayers (13) and workers engaged in packaging pyrethroids (8) and in patients who had ingested deltamethrin accidentally. Results have shown the presence of these pyrethroids in urine confirming absorption had occurred. A summary of some of the data is given in table 3.2.6 with atmospheric pyrethroid levels and, where appropriate, estimates of dermal absorption rate.

3.2.9.4 Other analytical methods

Urine clean-up by solid phase extraction (48) can be used. An analytical method for the fenvalerate metabolite CPBA has been reported (53) but does not appear to have been widely used.

3.2.9.5 Guide to interpretation

(a) *Measured values in groups without occupational exposure*

Deltamethrin and fenvalerate are not products of endogenous metabolism. With current use patterns and under normal conditions of use, environmental exposure is expected to be very low. Exposure of the general population to deltamethrin or fenvalerate should be rare.

(b) *Published biological action levels*

No reference values are available. However, data on urinary levels of intact fenvalerate and deltamethrin are given below in table 3.2.6a and 3.2.6b.

Table 3.2.6. Data from workers exposed to deltamethrin and fenvalerate during (a) packaging of pyrethroids and (b) spraying

(a) *Workers engaged in packaging pyrethroids*

Air concentration (mg/m ³)	Urine pyrethroid concentration (µg/L)	
	Deltamethrin	Fenvalerate
0.003±0.002	0.2–0.4 (n=5)	
0.012±0.001	0.3–0.8 (n=4)	
0.013±0.004		0.2–0.7 (n=10)
0.055±0.013		1.02–18.6 (n=5)

(b) Sprayers

Respiratory exposure*	Dermal uptake	Urine pyrethroid elimination (μg)**	
$\mu\text{g/h}$	(mg/h)	Deltamethrin	Fenvalerate
0.07 ± 0.06	0.59 ± 0.30	0.15 ± 0.07 (n=5)	
0.65 ± 0.32	3.97 ± 1.88		1.16 ± 1.07 (n=5)

* air concentration ($\mu\text{g}/\text{m}^3$) x ventilation volume ($0.01\text{m}^3/\text{min}$) x 60min = $\mu\text{g/h}$

** total collection between 12–24 h after start of spraying

(c) Non-analytical interference

- i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

No other chemicals are known to give rise to excretion of deltamethrin or fenvalerate in urine. Several pyrethroids can be detected using the same extraction and chromatographic conditions but elute at different retention times. There are no compounds in the urinary extract that appear to interfere with the deltamethrin or fenvalerate peaks. No data are available concerning the effects of other pesticides, chemicals, medication or alcohol on deltamethrin or fenvalerate excretion.

- ii) Diet and environment

No information is available regarding effects of diet and environment on deltamethrin or fenvalerate excretion.

(d) Sampling representative of recent or long-term exposure

Measurement of deltamethrin or fenvalerate in urine samples taken at the end of the shift is suitable for monitoring same day exposure. The amount of pyrethroid detected in urine of sprayers working for three days was greater than that found in sprayers working for one day. This indicates that there may be some accumulation of these pesticides during the workweek. Measurement of deltamethrin or fenvalerate in urine samples obtained towards the end of the week may reflect exposure over more than one day.

(e) Population of special concern

No populations of special concern were identified.

(f) Ethnic differences (enzyme deficiency, environment, diet)

No ethnic differences in excretion of deltamethrin or fenvalerate have been observed.

3.2.10 Research needs

Further work is required to investigate the viability of measuring urinary CPBA as an indicator of exposure to fenvalerate. Volunteer studies with oral and dermal exposure to pyrethroids under controlled conditions are required to aid the interpretation of biologi-

cal monitoring data of pyrethroids collected in the field. The effects of different formulations on the rate of dermal penetration of pyrethroids and the relative importance of the roles of exposure via dermal absorption, inhalation and ingestion in man are poorly understood. Such information is required before suitable biological exposure indices for pyrethroids can be set.

Analytical methodologies for detection of pyrethroid metabolites in urine at the low $\mu\text{g/L}$ level are often complex. Simpler, reliable methods are required so that monitoring for exposure to these pesticides can be performed more easily.

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Chapter 4. Biological monitoring and other selected compounds

4.1 Aromatic amines

Methylenebis(2-chloroaniline) (MbOCA) and methylenedianiline (MDA)

4.1.1 Introduction

Methylenebis(2-chloroaniline) (MbOCA) and methylenedianiline (MDA) are aromatic diamines used in a wide range of industries. MbOCA is used in the manufacture of some polyurethanes and elastomers with a range of applications from wear resistant linings for pulley wheels and metal containers to seals and gaskets. MDA also has a wide range of applications from potting and encapsulation of electrical components to high performance composite materials used in the aerospace industry. About 95% of MDA is used in an enclosed process to make methylene diphenyl di-isocyanate (MDI), but it is the use of the remaining 5% as a curing agent in a range of epoxy resins and some polyurethane systems which offers the greatest potential for occupational exposure. Both MbOCA and MDA in their pure form are solids at room temperature with low vapour pressures and unless used in a form which generates dusts (e.g. flaked MDA) the major route of absorption is via the skin rather than inhalation (1–3). There is concern about occupational exposure to these chemicals since both are genotoxic *in vitro* and carcinogenic in at least two animal species. There are reviews of the toxicity of these chemicals (1, 2, 4–9).

From the early use of MbOCA in 1971 (10) to the present day use of MDA (11) biological monitoring has proved a consistently useful tool for monitoring occupational exposure to these aromatic amines and there are numerous reviews and examples of its application (3, 12–18). Although biological monitoring has been used for many years, there are insufficient data to set health-based occupational limits. However, there are sufficient data to provide action levels or “benchmark” values based on occupational practice and thus guide improvements in occupational hygiene to reduce exposure to potential carcinogens.

4.1.2 Physical-chemical properties

Table 4.1.1. Physical-chemical properties of MbOCA and MDA

Property	4,4'methylenebis -(2-chloroaniline) (MbOCA)	4,4' methylenedianiline (MDA)
Synonyms	Dichloro-diamino-diphenylmethane, MOCA	Diamino-diphenylmethane DADPM, DDM MDA
CAS No	101-14-4	101-77-9
RTECS No.	CY 1050000	S42,500
Empirical formula	$C_{13}H_{12}N_2Cl_2$	$C_{13}H_{14}N_2$
Molecular weight	267.17	198.30
Physical form	pale cream crystalline solid	pale cream crystalline solid
Melting point	110°C	89-93°C
Specific gravity	1.44	1.10
Solubility	Almost insoluble in water soluble in alcohol & ether	Almost insoluble in water, sol- uble in alcohol, acetone & ether
Vapour pressure	1.3×10^{-3} Pa at 25°C 4.8×10^{-3} Pa at 100°C	1.3×10^{-4} Pa at 20°C 0.4 Pa at 100°C
Conversion factors	1 µg/L = 3.745 nmol/L 1 µmol/L = 267 µg/L	1 µg/L = 5.043 nmol/L 1 µmol/L = 198.3 µg/L

4.1.3 Possible occupational and non-occupational exposures

Occupational exposure to MbOCA and MDA can occur during their production, formulation and use. The wide range of products using these amines as curing agents or hardeners, either as pure compounds or components of mixtures, means that many thousands of workers may potentially be exposed to them (2, 13).

MbOCA and MDA are not naturally occurring chemicals and non-occupational exposure is unlikely, other than from contaminated clothing or food. There are reports of MDA being released from some polyurethane medical devices after sterilization by irradiation (19) and conflicting reports of its release from polyurethane medical plastics by heat (20, 21) but the significance of this is not clear.

4.1.4 Summary of toxicokinetics

4.1.4.1 Absorption

(a) Inhalation

No quantitative data are available on the toxicokinetics of MbOCA or MDA following inhalation. The low vapour pressure of both MbOCA and MDA make it unlikely that inhalation is a significant route of occupational exposure except where workers handle

solid material and there is the potential for inhalation of particulates. A recent occupational hygiene survey of workers manufacturing and formulating flaked MDA reported that urinary MDA levels could rise rapidly from none detected in pre-shift samples to over 1400 nmol/mmol creatinine in post-shift samples (2).

(b) *Dermal*

There are several studies which show that MbOCA and MDA are readily absorbed by the dermal route in animals (22–24). There are also *in vitro* studies with excised human skin which show significant absorption of MbOCA and MDA (25, 26). The most recent of these showed that MDA was better absorbed dermally than MbOCA with $13 \pm 4.3\%$ of a dose of MDA found in the receptor fluid compared to $2.4 \pm 1.4\%$ (mean + SD) of a dermal dose of ^{14}C MbOCA detected in the receptor fluid after 72 hours. The absorption of MDA increased to $32.9 \pm 9\%$ if the skin was occluded. A considerable amount of material remained within the skin with 23–58% for MDA and 31–66% for MbOCA being measured at the end of the experiment (26). A recent dissertation described a human volunteer experiment where 5 people were dermally exposed to MDA for 1 hour to 0.75–2.25 μmol MDA dissolved in isopropanol, by the use of a patch technique. Determination of MDA remaining in the patch units after exposure showed that a median of 28% (range 25–29%) were absorbed (27).

A consistent theme of occupational exposure assessments is that dermal absorption is the main route of entry into the body, rather than inhalation or oral routes, for both MbOCA and MDA (3, 7, 10, 14, 15, 17, 18).

(c) *Gastrointestinal*

No quantitative information is available on the gastrointestinal absorption of MbOCA or MDA in humans. Studies with ^{14}C MbOCA in rats show that it is well absorbed orally with 16–24% of the dose being excreted in the urine (23, 28). Qualitative data exist on the acute oral toxicity of MDA from the 'Epping Jaundice Incident' of 1965 when 84 people ate bread made with flour contaminated with MDA and developed hepatitis. The dose in each case was unknown but was in the order of 3 mg/kg (29).

4.1.4.2 Metabolic pathways and biochemical interactions

There are few data on the metabolism of MbOCA or MDA in humans. In animals, MbOCA is extensively metabolized, with many of the metabolites arising from C-hydroxylation and subsequent conjugation with sulphate or glucuronic acid (28, 30–32) (figure 4.1.1). Oxidation of the aromatic ring of MbOCA yields 5-hydroxy MbOCA and as the sulphate conjugate this is the major metabolite of MbOCA found in canine urine (30, 31). Oxidation of the methylene bridge yields 4,4-diamino-3,3'-dichlorobenzhydrol, 4,4-diamino 3,3'-dichlorobenzophenone and ultimately bridge cleavage to give an amino chlorophenol and a derivative of 2-chloro-4-methyl-aniline (32). But it is the N-hydroxylation of MbOCA which gives rise to concern, since this is the mechanism of activation that produces electrophilic metabolites which may damage DNA. An *in vitro* study with two human liver specimens showed a marked preponderance for N-hydroxy-

lation over the detoxifying C-hydroxylation process (32). Other workers have shown that cytochrome P450 enzymes were responsible, in particular P450 3A4 and to a lesser extent P450 2A6 (33, 34). It has been shown that N-hydroxy MbOCA can bind to DNA *in vitro* to form an adduct N-(deoxyadenosin-8yl)-4-amino 3-chlorobenzyl alcohol (35, 36) and this adduct has been found *in vivo* in exfoliated urothelial cells from a worker acutely exposed to MbOCA (37). MbOCA has also been shown to bind to cellular macromolecules, such as haemoglobin and these have been proposed as possible biological markers (38, 39).

In addition to the oxidation pathways discussed above, MbOCA may be conjugated, either by the polymorphic N-acetyl transferase (40) or by glucuronyl transferases to give an N-glucuronide both of which have been found in workers' urine (41, 42). However, in terms of their potential for biological monitoring, few of the metabolites found in animals have been found in human urine. The 5-hydroxy metabolite and some of the methylene bridge cleavage metabolites have been looked for in workers' urine but not found (28, 30). The only human urinary metabolites of MbOCA found so far, are MbOCA itself, the N-acetyl and N,N'-diacetyl and N-glucuronide conjugates (41, 43). The acetyl metabolites of MbOCA in humans are minor metabolites usually present in urine in smaller amounts than MbOCA itself, whereas the N-glucuronide is present in greater amounts than 'free' MbOCA, typically 2–3 times the amount (41, 42, 44).

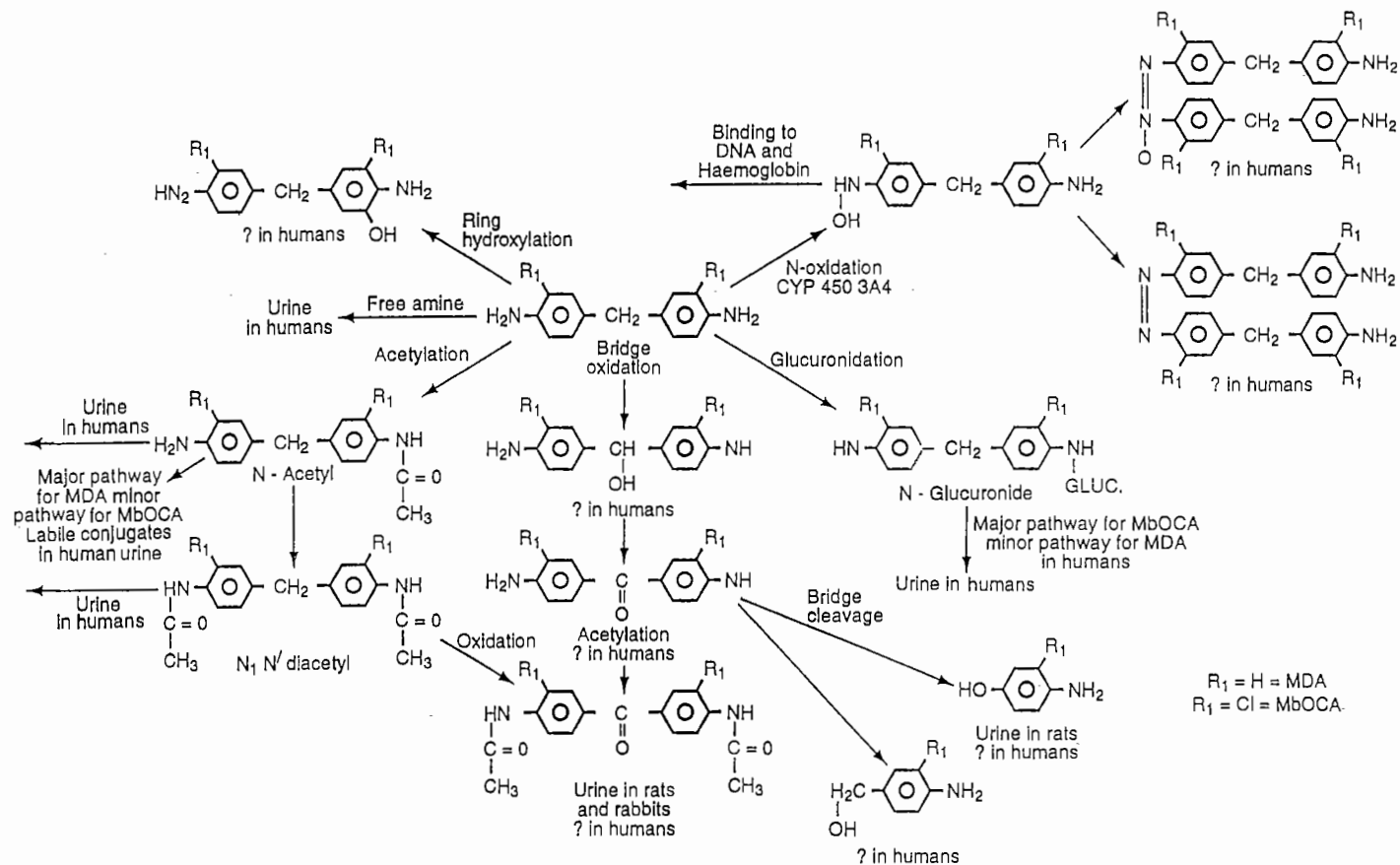
The metabolism of MDA has been less well studied but shows qualitative similarities to MbOCA. In rats dosed i.p. with MDA at least 17 metabolites were produced although the analysis was incomplete (45). Metabolism of MDA was principally via N-acetylation and oxidation of the methylene bridge. The most prominent metabolite was N,N'-diacetyl 4,4'-amino-benzhydrol. There was also some evidence of ring hydroxylation, N-hydroxylation and N-glucuronidation (45). A recent study using liquid chromatographic mass spectrometric techniques has identified two novel metabolites azo-MDA and azoxy-MDA and it was suggested that these may be formed by condensation from N-hydroxy MDA (46).

Like MbOCA, few of the metabolites of MDA reported in animals have been identified in humans. The only ones so far reported are the N-acetyl and N,N'-diacetyl metabolites and their labile conjugates (34, 41). Also like MbOCA, MDA has been shown to bind to haemoglobin in both animals and humans (39, 47, 48). Unlike MbOCA the acetyl metabolites of MDA (and their labile conjugates) are major metabolites and are excreted in greater amounts than the parent amine.

4.1.4.3 Distribution

There are no data on the distribution of MbOCA or MDA in humans. Studies in rats given ^{14}C MbOCA i.v, i.p, or p.o. show extensive distribution with the highest concentrations in liver, small intestine, adipose tissue, lung, kidney and skin with no deposition in these tissues (28, 49). However, with dermal dosing of MDA in rats, up to 26% of the dose was retained in the skin and was not removed by washing but was recoverable by 'extraction and solubilization' (24).

Figure 4.1.1. Metabolism of MbOCA or MDA in humans



4.1.4.4 Elimination

The major route of elimination of MbOCA and MDA depends on the species. In rats, where one of the major biliary metabolites is the N-glucuronide of MbOCA (32), the major route is via the faeces with 59–73% of a dose of MbOCA and 56% of a dose of MDA being eliminated this way (28, 49, 50). However, in rabbits and monkeys, only 10–20% of the dose of MDA is eliminated via the faeces, with up to 80% eliminated in the urine (45, 50). It is thought that this may be due to the higher molecular weight threshold for biliary excretion in rabbits and monkeys. If this explanation is correct, then it is likely that in humans, with a similar higher molecular weight threshold, the major route for elimination of MbOCA and MDA will be via the urine.

There are few data on the rates of elimination of MbOCA or MDA. However, two studies of accidental exposures to high concentrations of MbOCA report rapid absorption of MbOCA and elimination half-time of MbOCA in urine of 23–24 hours (51, 52). In the case of MDA, a dermal absorption study in 5 volunteers showed half-times for urinary MDA (total) of 4.6–11 h (median 7h) and a half-time for plasma MDA of 9.2–19 h (median 13h) (27).

4.1.5 Summary of toxic effects

There are few data on the acute toxicity of MbOCA or MDA in humans. One report of a worker sprayed in the face with molten MbOCA describes conjunctivitis, and an 'upset stomach' (51). The 'Epping Jaundice Incident' showed that people who ingested MDA could develop an intrahepatic cholestasis with jaundice (29). Occupational exposure to MbOCA or MDA only rarely leads to acute poisonings, rather, it is the potential for long-term exposure that causes concern. There are numerous studies to show that both MbOCA and MDA are genotoxic *in vitro* and carcinogenic in at least two animal species *in vivo* but the epidemiological proof of human carcinogenicity is lacking (1, 2, 4–6, 9). However, there are data to show that human tissues can metabolize MbOCA and MDA to mutagenic products, N-hydroxy metabolites and produce haemoglobin and DNA adducts (32, 37, 39, 47, 53, 54). Most regulatory bodies treat MbOCA and MDA as potential human carcinogens.

4.1.6 Biological monitoring indices

Table 4.1.2 lists the biological monitoring indices that have been suggested for monitoring occupational exposure to MbOCA or MDA.

Table 4.1.2. *Biological monitoring indices for MbOCA and MDA*

Biological monitoring indices	Suitability	Ref.
MbOCA in urine (after hydrolysis of labile conjugates)	Widely used, with proven utility for monitoring occupational exposure - the recommended method for MbOCA	10, 12, 13, 43, 55-60
MbOCA-DNA adducts in exfoliated urothelial cells	Limited application and data	37
MbOCA in blood: Haemoglobin adducts	Limited application and data	61
MDA in urine after hydrolysis of labile & acetyl conjugates)	Widely used, with proven utility for monitoring occupational exposure - the recommended method for MDA	16, 62-66
MDA in blood:	a) Has not been used for occupational exposure assessment	a) 19, 67,
a) free		
b) haemoglobin adducts	b) limited application and data	b) 39, 48

The analysis of MbOCA and MDA after hydrolysis of their labile and acetyl conjugates in urine are the most widely used biological indicators of exposure to these chemicals. Urine sampling is non-invasive, the analysis is comparatively simple and there are guidance or target values to help in the interpretation of results. In addition, there have been numerous studies over the last 20 years which have shown that urinary monitoring for MbOCA or MDA can be used to encourage improvements in occupational hygiene and demonstrate the effectiveness of control measures.

The analysis of adducts of MbOCA and MDA to haemoglobin and of MbOCA to DNA, have been recently applied to a small number of samples from exposed workers (37, 39, 48, 61). These methods have demonstrated that MbOCA and MDA can bind to cellular macromolecules and this increases the concern about their occupational exposure. So far, however, the methods which are based on sophisticated analytical techniques, have not been adopted widely by others. Therefore, only the procedures for analysis of MbOCA, MDA and their conjugates in urine are discussed further below.

4.1.7 MbOCA in urine

4.1.7.1 Toxicokinetics

Little has been published on the human pharmacokinetics of MbOCA excretion in humans, but two studies of accidental exposures to high concentrations of MbOCA report rapid absorption of MbOCA and elimination half-time of MbOCA in urine of 23-24 hours (51, 52).

4.1.7.2 Biological sampling

(a) Sampling time and specimen

The majority of biological monitoring studies for MbOCA involve collecting urine samples at the end of shift. In view of the long half-time of MbOCA in urine, if exposure is consistent throughout a workweek, the highest urinary concentrations would be expected in end-of-shift end-of-week samples.

(b) Contamination possibilities

Sample contamination is possible but in practice uncommon. Suspected contamination can be investigated by analysing fresh urine samples before and after hydrolysis of glucuronide metabolites. If no increase in MbOCA concentration is seen after hydrolysis contamination may be suspected.

(c) Sampling device and container

Urine may be collected in any clean container but storing samples at -20°C in polystyrene bottles is recommended.

(d) Preservative, shipment and stability

Early studies used citric acid as preservative (10, 43), but later studies have not used a preservative. The concentration of "free MbOCA" in urine increases if samples are left at room temperature for up to four days due to the spontaneous hydrolysis of conjugates (56). No special conditions are required if samples can be delivered to the laboratory within 48 hours. If analysis is likely to be delayed the samples should be frozen at -20°C, under which conditions they are stable for at least three months (56, 60).

4.1.7.3 Recommended analytical methods

There are numerous methods for the analysis of MbOCA in urine, based on either gas chromatography or high-performance liquid chromatography (HPLC) and in skilled hands all are suitable for biological monitoring. However, those based on HPLC require less sample preparation and are easier to use. The method described below uses HPLC with either solvent extraction or solid phase extraction of MbOCA from urine.

(a) Principles of the method

The analysis of MbOCA in urine is based on the hydrolysis of labile conjugates of MbOCA by heat, followed by extraction from the urine and HPLC analysis with electrochemical detection. Calibration standards are prepared in urine and taken through the procedure.

(b) Reagents required

MbOCA, 3,3'-dichlorobenzidine (internal standard), sodium dihydrogen phosphate, disodium hydrogen phosphate, HPLC grade methanol, water, acetonitrile and for the solvent extraction method sodium hydroxide and peroxide free diethyl ether are re-

quired. (Note: MbOCA and 3,3'-dichlorobenzidine are potential carcinogens and care should be taken to avoid exposure, particularly when weighing out solid material).

(c) Equipment required

A waterbath or dry-block capable of heating to 100°C is needed for sample hydrolysis. If extraction of MbOCA is to be carried out using a solvent extraction method, the equipment required comprises a rotary tumbler/mixer, a centrifuge and solvent evaporation equipment. Where solid-phase extraction is used, disposable extraction columns packed with 100 mg C₁₈ stationary phase, and an extraction manifold are required. Separation and detection of MbOCA uses an HPLC system with electrochemical detection; a 100mm x 4.6mm HPLC column packed with 3µm Spherisorb ODS2 (octadecylsilane 12% w/w carbon loading) is suggested.

(d) Procedures

i) Calibration

There are no certified reference materials for MbOCA so the purity of the standard material used must be verified. Standard solutions of MbOCA (267mg/L, 1 mmol/L) and internal standard dichlorobenzidine (326mg/L, 1 mmol/L) are prepared in methanol, stored in the dark at 4°C and discarded after 1 month. Daily working solutions of MbOCA (1.07 mg/L, 4µmol/L), and DCB (1.63mg/L, 5 µmol/L) are prepared by dilution in water. The MbOCA working solution is used to spike aliquots of urine (2mL) from an unexposed individual to give a calibration curve from 0 to 500 nmol/L MbOCA; standards are taken through the procedure below.

ii) Procedure

Aliquots of urine (2mL in duplicate) are transferred to screw capped glass tubes, spiked with the internal standard DCB (100 µ/L of 1.63 mg/L), capped, mixed and heated at 100°C for 30 minutes. After the samples have cooled to room temperature the MbOCA can be extracted, either by solvent extraction or by solid phase extraction.

For solvent extraction, sodium hydroxide (1mL, 1M) and diethyl ether (8 mL) are added to each tube and the contents mixed for 30 minutes. The tubes are then centrifuged at 900 g for 10 minutes to separate the aqueous and organic phases. The diethyl ether layer is transferred to a clean tube and the solvent evaporated to dryness at 40°C under a gentle stream of oxygen free nitrogen. Once dry the residues are reconstituted using HPLC mobile phase (1mL) and subjected to HPLC analysis.

For solid phase extraction, the disposable extraction columns (DEC) packed with 100mg of C₁₈ are each conditioned with 2mL of methanol followed by 2mL of water. Aliquots of hydrolysed samples (0.8mL) are transferred to the DEC and washed sequentially with water (800µL) and 45% acetonitrile in water (800µL). The MbOCA is then eluted into collection tubes with 90% acetonitrile in water (0.3mL) and diluted with phosphate buffer (400µL 50 mmol/L pH7) before injection (200µL) into the HPLC system.

HPLC analysis for extracts prepared either by solvent extraction or by solid phase extraction uses a single pump with an isocratic mobile phase (phosphate buffer (50 mmol/L, pH7): acetonitrile:methanol (12:10:1)) at a flow rate of 1 mL/min into an 100 mm x 4 mm 3µm ODS column connected to an electrochemical detector with a polarising voltage of +0.8V. Peak area (or height) ratios are calculated for the MbOCA and DCB peaks which elute after about 7.5 and 6 minutes respectively under the conditions described above. The concentration of MbOCA in the samples is calculated by comparison of the peak area ratio to the calibration curve determined by linear regression analysis of the calibrants prepared and analysed at the same time as the unknown urine samples.

(e) Criteria of analytical reliability

i) Trueness

The extraction recovery for MbOCA if solvent extraction is used is 94% (56) and >95% if solid phase extraction is used (59). However, no correction is necessary as samples with unknown amounts of MbOCA are compared to calibration curves derived from urine spiked with known amounts of MbOCA. Comparison of the solvent extraction-HPLC procedure with a GCMS method showed a correlation coefficient of 0.976 and a slope of 1.075 showing good agreement between the two methods (52) in the range 8–3000 µg/L. Comparison of the solvent extraction method with the solid phase extraction method showed a coefficient of determination of 0.9925 and a slope of 1.021 again showing good agreement between the methods (59).

ii) Precision

The precision, expressed as relative standard deviation at 250 nmol/L (67 µg/L) is typically 3% and 7% for within-run and between-run analyses respectively for the solvent extraction procedure. The within-run and day-to-day relative standard deviations for the solid phase procedure are typically 1% and 4%, respectively (59).

iii) Detectability

The limit of quantitation using this method, based on peaks >3 times background noise and half the lowest point on the calibration curve has been found to be 25nmol/L (6.7 µg/L) (59). Although this figure could be improved if necessary, simply by taking more urine, it is adequate for detecting occupational exposure below the proposed action limits.

(f) Quality assurance

i) Special precautions

Since 1996 there is an external proficiency testing programme run by the Deutsche Gesellschaft für Arbeits-, Sozial- und Umweltmedizin e.V. Institut & Poliklinik für Arbeits- und Umweltmedizin, Schillerstrasse 25, Erlangen, Germany. Each laboratory performing MbOCA analysis should also prepare and use internal quality control (QC) samples either by pooling urine samples from exposed individuals or by spiking filtered urine from an unexposed individual. Aliquots of QC material should either be stored at

-20°C or freeze dried. It is recommended that QC samples should be run before and after each batch of 5 samples.

ii) Interferences

Interferences have not been reported for the HPLC determination of MbOCA.

(g) Sources of possible error

i) Pre-analytical

The most likely source of pre-analytical error is sample contamination during collection, but as discussed in 4.1.7.2 above this can be checked if suspected and in practice is not a common problem.

ii) Analytical

There are no specifically reported problems or especially critical stages in the method. However, it is recommended that in addition to care with weighings and dispensings, attention is given to ensuring an adequate hydrolysis stage, and if ether is used it should be peroxide-free.

iii) Post-analytical

The debate about whether to correct results for urine dilution by either creatinine or specific gravity is not confined to MbOCA in urine and will not be discussed here (see WHO Guidelines on biological monitoring of occupational exposure at the workplace: Vol I). There is little data on which to judge the merits of creatinine correction for MbOCA. A study measuring the elimination of MbOCA in multiple timed urine samples after an acute exposure found a slightly better fit to an exponential curve if no correction was made than if the results were corrected for creatinine (R^2 0.94 cf R^2 0.86). The literature of reported biological monitoring surveys for MbOCA is equally divided into those that correct for creatinine (12, 14, 58, 68) and those that do not (10, 15, 43, 57).

(h) The reference to the most comprehensive description of the method

The method outlined above is taken from Nutley BP, Connelly C. Manual and automated methods for the routine analysis of 4,4'-methylenebis (2-chloroaniline) (MbOCA) in urine samples. Internal Report of the Health & Safety Laboratory, Health & Safety Executive, Broad Lane Sheffield, UK. S3 7HQ. Available from the author.

(i) Evaluation of the method

There are no published reports on the evaluation of the HPLC analytical method described above, although the method and its many modifications are in widespread use. Looking wider at the application of biological monitoring for occupational exposure to MbOCA by analysis of MbOCA in urine there are numerous reports of its utility (12, 13, 15, 58, 68).

4.1.7.4 Other analytical methods

Other HPLC methods for MbOCA in urine have been described, but these lack some of the elements of the method above. There are HPLC methods involving solvent extraction or solid phase extraction UV detection but these have less sensitivity and specificity (55, 57). There are also HPLC methods using solid phase extraction and electrochemical detection or photoconductivity detection but which omit the essential hydrolysis stage (43, 69).

MbOCA has also been determined in urine by gas chromatography, usually as a per-fluoroacyl derivative using electron capture detection and MDA as internal standard (56) or negative ion chemical ionisation mass spectrometry using deuterated benzidine as internal standard (60) or without derivatization, but using a nitrogen/phosphorous detector (58).

4.1.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

MbOCA has not been detected in the urine of workers without occupational exposure to MbOCA (55, 58).

(b) Published biological action levels

Table 4.1.3 shows the published biological action levels for MbOCA in end-of-shift urine samples. The action levels are not health based, rather, the UK and Australian levels are based on what is achievable by the majority of the industry. The basis for the Californian OSHA limit is less clear.

Table 4.1.3. Biological action levels for MbOCA

Country & organisation	Biological action level	Ref
UK Health & Safety Commision	15 nmol/mmol after hydrolysis (approx. 50 µg/L)	70
USA California Occupational Safety & Health Administration	100 µg/L "free MbOCA" corrected to a specific gravity of 1.024 (approx. 30 nmol/mmol)	71
Australia DOSHWA	45 µg/L "free MbOCA" (approx. 15 nmol/l)	68

The CAL/OSHA and DOSHWA limits are for "free MbOCA", i.e., MbOCA extracted from unhydrolysed urine samples. Extensive work with over 800 urine samples analysed before and after hydrolysis showed that on average the hydrolysed values were approximately 2.5 times greater than the unhydrolysed values (56). More recent work with only ten urine samples showed an average 4.8 fold increase after hydrolysis (60). Thus, the three action levels are broadly similar. However, in view of the spontaneous hydrolysis of conjugates the use of "free MbOCA" should be confined to samples analysed immediately after collection. In most cases where samples are transported to the

laboratory, or if analysis may be delayed, the samples should be hydrolysed before analysis.

(c) Non-analytical interference

- i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

The effects of ethanol, and medication on MbOCA in urine have not been reported in humans.

- ii) Diet and environment

MbOCA is not a normal constituent of either the diet or environment.

(d) Sampling representative of recent or long-term exposure

The measurement of MbOCA in end of shift urine samples is indicative of the same day exposure. The reported half-time of 23 hours (52) would indicate that if exposure was continuous throughout the workweek there may be some accumulation. Samples taken at the end of the week may therefore reflect exposure not just on the day of sampling but also may reflect some of the exposure during the week.

(e) Population of special concern

No specific information has been located on populations of special concern due to exposure to MbOCA (or MDA). However, an individual's susceptibility to MbOCA or (MDA) will depend on the balance of activities in activation and detoxification metabolic pathways, many of which have polymorphic enzymes. In the case of MbOCA and (MDA) it would appear that poor acetylators may be at greater risk than fast acetylators, since acetylation appears to be a detoxification mechanism with the acetyl and diacetyl metabolites being less mutagenic (53, 72). The role of other enzymes and metabolites in the toxicity of MbOCA and (MDA) is less clear. A review discusses the possibilities for aromatic amines in general (73), but in summary it is still difficult at present to identify particular populations at special risk at this stage.

(f) Ethnic differences (enzyme deficiency, environment, diet)

No information was found on ethnic differences in MbOCA metabolism or excretion.

4.1.8 MDA in urine

4.1.8.1 Toxicokinetics

There are few data on the pharmacokinetics of MDA in humans. Some information comes from occupational hygiene studies where a few workers have provided multiple urine samples but there are insufficient data to calculate half-times of elimination. However, qualitatively the elimination of MDA appears to be similar to MbOCA. The results of occupational hygiene studies have shown that, where exposure to MDA was most likely via inhalation of particulate MDA during handling of flaked material, absorption

and elimination of MDA was rapid with the levels of MDA and its alkaline hydrolysable metabolites ('total MDA') rising rapidly from < 1 nmol/mmol creatinine (approximately 0.02 µg/L) to over 1432 nmol/mmol creatinine (approximately 28 µg/L) by the end of the shift and fell to 290 nmol/mmol creatinine (approximately 6 µg/L) by pre-shift the next day (2). However, when absorption was most likely via the skin, the urinary levels of "total MDA" again rose rapidly, but the peak urinary concentrations of "total MDA" were delayed at least 5 hours after the end of the shift and elimination was incomplete by the start of the next shift. This suggests that elimination of MDA is being prolonged by continued absorption. More recently, a human volunteer study looked at dermal exposure of MDA in 5 people exposed to 0.75–2.25 µmol MDA for 1 h in isopropanol, by use of a patch test technique. Half-times of elimination from urine were 4.1–11 h (median 7h) and from plasma were 9.2–19 h (median 13h) (27). Studies with ¹⁴C MDA in animals also report that MDA is readily absorbed via the skin, that the elimination of a dermal dose is delayed compared to an intravenous dose (24, 50) and that the skin may act as a reservoir for prolonged absorption of MDA (26).

4.1.8.2 Biological sampling

(a) Sampling time and specimen

A biological monitoring survey of 411 workers exposed to MDA in 45 factories attempted to collect urine samples post-shift and pre-shift the next day (11). In those cases where both samples were collected and the most likely route of exposure was via the skin (n=117), the highest urinary MDA concentrations were found in the pre-shift (next-day) samples. Where exposure was to MDA dust and the most likely route was inhalation, the highest urinary 'total MDA' concentrations were found in post-shift samples (2, 11).

(b) Contamination possibilities

Sample contamination is possible, particularly where MDA is present as a dust but in practice it is uncommon. An occupational hygiene survey checked for contamination (male workers only) by using two open sample containers taped together. After-urine was collected into one container the other was half filled with water, capped, shaken and an aliquot taken for analysis. No MDA was detected in any of the water samples (3). If contamination is suspected it can be checked by analysing the sample before and after alkaline hydrolysis and looking for a significant increase due to the hydrolysis of the acetyl metabolites. If there is no increase contamination should be suspected.

(c) Sampling device and container

Urine may be collected into any clean container but for storing samples at -20°C polystyrene bottles are recommended.

(d) Preservative, shipment and stability

Studies of the stability of MDA in urine from exposed workers showed that at 2–4°C there was no loss of 'total MDA' over 16 days (63). Urine samples spiked with MDA

(which may be more reactive than acetyl metabolites) and stored at room temperature for 41 hours showed recoveries of 90% without preservative, but the recovery decreased to 75% after 65 hours. Addition of acid to take the urine to pH3 maintained the 90% recovery at room temperature for 65 hours (74). Therefore, it is unlikely that there will be significant loss if samples are transported to the laboratory at room temperature within 40 hours. If analysis is likely to be delayed the samples should be frozen at -20°C, under which conditions they are stable for at least six months (63).

4.1.8.3 Recommended analytical methods

There are several methods for the analysis of MDA and its hydrolysable metabolites in urine, based on either gas chromatography or high performance liquid chromatography (HPLC), which in skilled hands are suitable for biological monitoring. However, those based on HPLC require less sample preparation and are easier to use. The method described below uses HPLC with electrochemical detection after either solvent extraction or solid phase extraction of MDA from alkaline hydrolysed urine.

(a) Principles of the method

The analysis of "total MDA" in urine is based on the alkaline hydrolysis of labile and acetyl conjugates of MDA, followed by extraction and HPLC analysis with electrochemical detection. Calibration standards are prepared in urine and taken through the procedure.

(b) Reagents required

MDA, 4,4'-ethylenedianiline (EDA) (internal standard), sodium dihydrogen phosphate, disodium hydrogen phosphate, HPLC grade methanol, water, acetonitrile and either diethyl ether (for solvent extraction) or benzene (for solid phase extraction) are required. NB: care should be taken when using MDA, EDA and benzene if the solid phase extraction method is used.

(c) Equipment required

A waterbath or dry-block capable of heating to at least 80°C is needed for sample hydrolysis. If extraction of MDA is to be carried out using a solvent extraction method, the equipment required comprises a rotary tumbler/mixer, a centrifuge and solvent evaporation equipment. Where solid-phase extraction is used, disposable extraction columns packed with 500 mg of 40 µm-preparative-grade Bondesil Octadecyl (C₁₈) bonded silica stationary phase, and an extraction manifold are required. Separation and detection of MDA uses an HPLC system with electrochemical detection; a 100 mm x 4.6 mm HPLC column packed with 3µm Spherisorb ODS2 (octadecylsilane 12% w/w carbon loading) or 250mm x 6.4 mm 5µm APEX ODS (or equivalent) is suggested.

(d) Procedures

i) Calibration

There are no certified reference materials for MDA, so the purity of the standard material must be verified. Standard solutions of MDA (198 mg/L, 1 mmol/L) and internal standard EDA (212 mg/L 1 mmol/L) are prepared in HPLC grade methanol, stored in the dark below 4°C and discarded monthly. Daily working solutions of MDA (0.792 mg/L, 4 µmol/L) and EDA (1.06 mg/L, 5 µmol/L) are prepared by dilution in water and used to spike aliquots (2 mL) of urine from an unexposed individual to give a multipoint (>5) calibration curve from 0 to 500 nmol/L (99 µg/L) MDA, standards are taken through the procedure below.

ii) Aliquots of urine

Aliquots of urine (2mL in duplicate) are transferred to screw capped glass tubes, spiked with internal standard (EDA 200 µl of 1 µmol/L, 1.06 mg/L), made alkaline with sodium hydroxide (2mL, 10 mol/L), capped, mixed and heated to at least 80°C for at least 90 mins. After the samples have cooled to room temperature the MDA can be extracted, either by solvent extraction or by solid phase extraction.

For solvent extraction, diethyl ether (8 mL) is added to each tube and the contents mixed for 20 mins, then centrifuged at 900 g for 5 minutes to separate the aqueous and organic phases. The diethyl ether layer is transferred to clean tubes and the solvent evaporated under a gentle stream of oxygen-free nitrogen at 40°C. Once dry, the residues are reconstituted using 20% methanol in water (200 µL) and mixed thoroughly before transfer to the HPLC.

For solid phase extraction, HPLC grade water (6 mL) is added to each tube before the tubes are centrifuged at 450 g for 5 min. Extraction columns are conditioned with 3 mL of methanol followed by 5 mL of HPLC grade water. Each sample is transferred to an extraction column and allowed to pass through the column at a flow rate of 1–2 mL/min before being rinsed with sodium hydroxide (3 mL, 1 mmol/L) and allowed to dry for 2–3 min. The remaining water is removed by centrifugation at 450 g for 5 mins before the MDA is eluted with benzene (2x3 mL) at a flow rate of 1–2 mL/min. The benzene is removed under a stream of nitrogen at 35°C and the residues reconstituted in methanol (200 µL) before transfer to the HPLC.

HPLC analysis for extracts prepared either by HPLC or solvent extraction uses a single pump with an isocratic mobile phase (phosphate buffer (50 mmol/L, pH7): acetonitrile: methanol (50:25:20)) (or alternatively, sodium acetate (10 mmol/L): acetonitrile:methanol (62:27:11)) at a flow rate of 1 mL/min at ambient temperature into a reverse phase ODS HPLC column connected to an electrochemical detector with a glassy carbon electrode operating at +0.7 to +0.8 volts.

Peak area (or height) ratios are calculated for MDA and EDA peaks which elute in that order. The concentration of MDA in unknown samples is calculated by comparison of the peak area ratio to the calibration curve determined by linear regression analysis of

the calibrants which are prepared and taken through the procedure at the same time as the unknowns.

(e) Criteria of analytical reliability

i) Trueness

The extraction recovery of MDA from urine if solvent extraction is used is >80% (56, 66) and 68% for MDA and 53% for EDA if solid phase extraction is used (63). However, no correction is necessary as samples with unknown amounts of MDA are compared to a calibration curve derived from urine spiked with known amounts of MDA and taken through the extraction procedure. Both solvent extraction and solid phase extraction variants of the HPLC method have been investigated by comparing results obtained with these procedures to those obtained by a GCMS method and in both cases correlation coefficients of >0.95 were obtained (63, 75). The solid phase variant was used to analyse over 300 urine samples from exposed workers and found only 5 false positives (negative by GCMS) and no false negatives (63).

ii) Precision

The precision, expressed as relative standard deviation is typically 3.5% at 250 nmol/L (50 µg/L) for within batch analysis and 9.8% at 50 nmol/L (10 µg/L) and 8% at 500 nmol/L (100 µg/L) for batch to batch analysis for the solid phase extraction method (63). The corresponding figures for the solvent extraction method were 4% at 350 nmol/L (69 µg/L) for within batch analysis and 10% at 350 nmol/L (69 µg/L) for batch to batch analysis for the solvent extraction method (76).

iii) Detectability

The limit of detection defined as >3 times background noise is the same for both extraction methods and is quoted as 10 nmol/L and 2.5 µg/L. This figure could be improved if necessary by taking more urine, it is comparable with GCMS and GC-ECD assays (65–67) and is sufficient to detect occupational exposures.

(f) Quality assurance

i) Special precautions

Since 1996 there is an external proficiency testing programme run by the Deutsche Gesellschaft für Arbeits-, Sozial- und Umweltmedizin e.V. Institut & Poliklinik für Arbeits- und Umweltmedizin, Schillerstrasse 25, Erlangen, Germany. Each laboratory performing MDA analysis should also prepare internal quality control samples, either by pooling urine samples from exposed individuals or by spiking filtered urine from an unexposed individual. Aliquots of QC material should be stored at -20°C. It is recommended that QC samples should be run before and after each batch of 5 samples.

ii) Interferences

The HPLC MDA assay has been checked for interference by other common aromatic amines; benzidine, 3,3'-dichlorobenzidine, MbOCA, m-phenylenediamine, o-tolidine, o-toluidine, 3,3'-dimethoxybenzidine and 4-methoxy-1,3-phenylenediamine, and no inter-

ference was found. However, 4-phenyl-1,4-phenylenediamine could potentially co-elute with the internal standard and reduce the calculated concentration of MDA (63).

(g) Sources of possible error

i) Pre-analytical

The most likely source of pre-analytical error is sample contamination but, as discussed in 4.1.8.2 above, this can be checked if suspected, but in practice it is not a common problem.

ii) Analytical

There are no specifically reported problems or especially critical stages in the method. However, it is recommended that in addition to care with weighings and dispensings, attention is given to ensuring an adequate hydrolysis stage, and if ether is used it should be peroxide-free.

(h) The reference to the most comprehensive description of the method

Peterson JC, Estiva EC, Lyttle DS, Harris RM. High-performance liquid chromatographic determination of 4,4'-methylenedianiline. *J Chromatogr* 1991;564:205–12.
Waine EM. Development of an HPLC method for the measurement of methylenedianiline in urine. An Internal report from the Health & Safety Laboratory of the Health & Safety Executive, Broad Lane Sheffield UK S3 7HQ. Available from the author.

(i) Evaluation of the method

No other published reports of comprehensive method evaluation were found.

4.1.8.4 Other analytical methods

There are a number of other HPLC methods for MDA. A very similar method to the solid phase extraction method described above has been reported, but without the analysis of urine samples from exposed workers (74). The use of UV as a detector for HPLC has been tried but other compounds in urine interfere (63) unless MDA is derivatized with pentafluoropropionic anhydride (62, 64).

There are a number of methods based on capillary gas chromatography of the perfluoroacyl derivatives of MDA with selected ion mass spectrometry detection, either in the positive ion mode (65, 66, 77) or negative ion mode (78). However, this latter paper does not use any hydrolysis stage or report the analysis of any samples from workers exposed to MDA.

The separate measurement of MDA and N-acetyl MDA, rather than 'total MDA' in urine has also been reported (41) and uses GCMS with selected ion monitoring of the pentafluoropropyl derivatives.

4.1.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

MDA has not been detected in the urine of 70 workers without occupational exposure to MDA (41, 66).

(b) Published biological action levels

The only guide to the interpretation of results is the UK Health & Safety Executive's recommended "benchmark" value of 50 nmol/mmol creatinine (approximately 100 µg/L for "total MDA" in urine (MDA and alkaline hydrolysable conjugates). This is the value below which 90% of the urinary MDA values fell in an occupational survey of 411 workers exposed to MDA in 45 factories in the UK (2).

(c) Non-analytical interference

- i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication

The effects of ethanol and medication on MDA in urine have not been reported.

- ii) Diet and environment

MDA is not a normal constituent of either the diet or the environment.

(d) Sampling representative of recent or long-term exposure

The measurement of MDA in the end-of-shift urine samples is indicative of exposure to MDA the same day and to a lesser extent the previous day. The measurement of MDA in pre-shift samples reflects exposure the previous day. The delay in the elimination of MDA if absorption is via the skin means that if exposure is continuous throughout the week there may be some accumulation and samples taken at the end of the week will therefore reflect exposure, not just on the day of sampling but also during the past week. In addition, elimination might be incomplete after a two-day break in exposure.

(e) Populations of special concern

There are no reports of populations of special concern due to exposure to MDA but see comments under this heading for MbOCA.

(f) Ethnic differences (enzyme deficiency, environment, diet)

No information was found on ethnic differences in MDA metabolism or excretion.

4.1.9 Research needs for MbOCA and MDA

There is a clear need for more information on the pharmacokinetics of MbOCA and MDA in humans to improve existing biological monitoring methods. Ethical considerations may limit the use of volunteer studies, but additional data could be obtained from more detailed occupational hygiene and biological monitoring studies. If possible, these

should also include the evaluation of the potential of haemoglobin adducts for biological monitoring. There is a need for more data to link the observed levels of exposure with any quantitative risk.

In terms of new biological monitoring methods, there is a need for further research into the development of analytical methods for DNA adducts which would be suitable for large population studies. In terms of identifying populations at risk, there is a need for further research into non-invasive methods for phenotyping workers for acetylator and cytochrome P450 3A4 status may be helpful.

4.1.10 References

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4.2 Polycyclic aromatic hydrocarbons: 1-Hydroxypyrene in urine

4.2.1 Introduction

Polynuclear aromatic compounds (PAC) are chemical compounds with 2 or more condensed aromatic rings which originate from incomplete combustion processes or pyrolysis of organic material. PACs include heterocyclic compounds containing nitrogen and oxygen and alkylated or nitro polynuclear compounds.

PACs are present in the environment as trace contaminants. Polycyclic aromatic hydrocarbons (PAH) are unsubstituted polynuclear aromatic hydrocarbons from the elements carbon and hydrogen and are a sub-family of the PACs.

This chapter deals with biological exposure monitoring of PAHs. Benzo(a)pyrene and pyrene have been used as single indicators of PAHs. Benzo(a)pyrene is an example of a carcinogenic PAH and pyrene is an example of a dominant PAH, which is always present in PAH mixtures. The composition of the PAH-mixture in different work environments will vary. When a single compound is used as an indicator or marker, one has to realize that the relative proportion of an individual PAH in the PAH mixture has to be established. When exposure data from different worksites are compared using a single indicator, it is necessary to establish the proportion of the marker in the total PAH content of the different worksites.

4.2.2 Physical-chemical properties

PAHs are mostly non-volatile compounds. Airborne PAHs with less than 3 benzene-rings (molecular weight = 128–178) are present as gaseous compounds in the work environment. PAHs with 4 rings (molecular weight = 202) are present both as gaseous compounds and as particulate matter. PAHs with larger molecular weights (> 228) are bound to airborne particulates. The solubility of PAH in water is low, but PAH are highly soluble in fat. The octanol/water partition coefficient ($\text{Log } P_{ow}$) varies from 3.5–6.6.

4.2.3 Occupational and non-occupational exposures

4.2.3.1 Occupational exposure

The most important sources of occupational exposure to PAH are coal tars and derived products. Crude coal tar is a by-product of coke (and in former times gas) works. Crude coal tar is usually distilled and blends of distillation fractions are used for various purposes: wood conservation, paints, roadtars, roofing materials, etc. PAH concentrations in coal tar products may range up to approximately 10 wt%.

A second source of PAHs are petroleum-distillates. However, heavy petroleum-distillates contain much lower concentrations of PAH, namely in the ppm-range.

A third source is burning or pyrolysis of organic material at the workplace. Pyrolysis of organic material may result in PAH-emission. Examples of this type of source are: fire fumes, diesel exhaust gas, rubber fumes, waste incinerator fumes, etc.

Traditionally, the exposure of workers is assessed by air quality measurements. In most studies coal tar pitch volatiles (CTPV) in air is used as the indicator of airborne PAH. Another approach is the measurement of 16 EPA-PAH (gaseous and particulates), or a single marker namely benzo(a)pyrene (b(a)p) in workroom air. A ranking of PAH exposure levels in the occupational environment was presented by Lindstedt & Sollenberg (1) (table 4.2.1).

Table 4.2.1. Benzo(a)pyrene exposure levels for different workplaces

1.	Very high b(a)p exposure ($> 10 \mu\text{g}/\text{m}^3$)
-	Gas and coke work (topside work)
-	Aluminium works (some jobs, e.g. pin setters, potmen, cranemen, etc)
-	Manufacturing of carbon electrodes (pitch bin workers, etc)
-	Handling of molten tar or pitch (roofing, paving, insulation coating, etc)
-	Chimney sweeping (from top)
-	Asphalting if asphalt is mixed with tar (some jobs)
2.	Fairly high b(a)p exposure ($1-10 \mu\text{g}/\text{m}^3$)
-	Gas and coke works in general (non-topside work)
-	Blast furnaces
-	Steel works (some jobs)
-	Manufacturing of carbon electrodes (in general)
-	Aluminium works (in general)
-	Asphalting if asphalt is mixed with tar (in general)
3.	Moderate b(a)p exposure ($0.1-1 \mu\text{g}/\text{m}^3$)
-	Steel works (in general)
-	Foundries (some jobs)
-	Welding of rails on track
-	Manufacturing of Soderberg electrode paste
4.	Low b(a)p exposure ($0.01-0.1 \mu\text{g}/\text{m}^3$)
-	Automobile repair shops
-	Asphalt manufacturing (from petroleum)
-	Foundries (in general)
-	Construction of tunnels and rock chambers
-	Aluminium electrolysis halls with prebaked electrodes
5.	Very low b(a)p exposure ($< 0.01 \mu\text{g}/\text{m}^3$)
-	Iron mines
-	Garages

Adapted from Lindstedt & Sollenberg (1)

4.2.3.2 Non-occupational sources

Inhabitants of communities are exposed to PAH from the environment. Pathways are inhalation (air, smoking), ingestion (diet, drinking water) and dermal absorption (medicinal drug for skin, children on contaminated soil). It is estimated that smoking and certain food constituents are the most important sources of the regular daily intake of PAH (2, 3). Inhalation of urban air may be an additional source. Dermal treatment with products derived from tar or creosotes may be a significant source of dermally absorbed PAHs (4).

4.2.4 Summary of toxicokinetics

4.2.4.1 Uptake

It has been demonstrated that PAH can be absorbed in the respiratory tract, in the gastrointestinal tract and can penetrate through the skin. The dermal exposure may be very significant in some industries. The dermal absorption of pyrene among cokeoven workers and creosote impregnating workers is reported to be 50–90% of the total pyrene uptake (5, 6).

4.2.4.2 Metabolism

Extensive knowledge is available about the metabolism of benzo(a)pyrene (b(a)p), one of the carcinogenic PAHs. In many studies on chemical carcinogenesis, b(a)p has been used as a model substrate. The intermediary epoxide-b(a)p and dihydrodiol-epoxide-b(a)p may covalently bind to DNA to form DNA-adducts. These metabolites are thought to be carcinogenic intermediate metabolites of b(a)p and, in general, of the PAH. The metabolism of pyrene is less complicated. It is mainly metabolized to the intermediary 1-hydroxypyrene to form 1-hydroxypyrene-glucuronide which is excreted (7). 1,2-dihydroxy-1,2-dihydropyrene has been reported to be present in human urine as a minor metabolite (8).

4.2.4.3 Excretion

The excretion of PAH metabolites has been studied in experimental animals. In rats most of the excreted metabolites (85–95%) were found in the faeces after inhalation of b(a)p. A small proportion was excreted in urine. After dermal application of [^{14}C] pyrene in rats, 20–23% of the dose was found in urine and 24–33% was found in the faeces over 6 days, suggesting equal excretion in urine and faeces (9). In man, 1-hydroxypyrene is excreted in urine as a conjugated metabolite following exposure to pyrene (7).

4.2.5 Summary of toxic effects

4.2.5.1 Cancer in workers exposed to PAH

Workers from industrial settings with high airborne PAH levels like gas works, coke works, primary aluminium industry show excess rates of cancers. Aluminium produc-

tion, coke production, coal gasification and coal tar pitches/coal tar fumes are carcinogenic to humans according to IARC classification (10). No reports were available in which workers were exposed to isolated PAH.

4.2.5.2 Experimental animals

The International Agency for Research on Cancer (11) reported that there is sufficient evidence that 11 PAHs are carcinogenic to experimental animals (table 4.2.2).

Table 4.2.2. PAH of which sufficient evidence is available that these compounds are carcinogenic to experimental animals

Chemical name	C.A.S. Reg number
benz[a]anthracene	56-55-3
benzo[b]fluoranthene	205-99-2
benzo[j]fluoranthene	205-82-3
benzo[k]fluoranthene	207-08-9
benzo[a]pyrene	50-32-8
dibenz[a,h]anthracene	53-70-3
dibenzo[a,e]pyrene	192-65-4
dibenzo[a,h]pyrene	189-64-0
dibenzo[a,i]pyrene	189-55-9
dibenzo[a,l]pyrene	191-30-0
indeno[1,2,3-cd]pyrene	193-39-5

4.2.5.3 Other health effects in workers exposed to PAH

Most of the toxic effects, other than cancer, of PAH-containing coal tars and creosote oils on humans concern the skin and eyes as targets; adverse effects to the skin include dermatitis, chronic tar dermatosis, tar or pitch warts, cutaneous phototoxicity and chronic melanosis, folliculitis and pitch acne. A review is given by the IARC (12).

4.2.6 Biological monitoring indices

Uptake of PAHs may be monitored by several biomarkers, e.g. metabolites in urine, urinary thioethers, urinary mutagenicity, protein-adducts and DNA-adducts. Both urinary mutagenicity and urinary thioethers are non-specific indicators of exposure to mutagenic agents. The latter two methods lack sensitivity in the case of occupational exposure to PAH and smoking is a very strong interfering confounder (4, 13, 14). The methods are not suitable for routine application.

Hemoglobin-adducts of benzo(a)pyrene have been reported as possible biomarkers of exposure. There is still little experience and the first results show limited usefulness (15). A large effort has been made to use the extent of binding of PAH to DNA as a biomarker of exposure. White blood cells are used as surrogate target DNA. Enzyme

immunoassays (16) and 32P-postlabeling assays (17) have been suggested. Both methods may be subject to appreciable variation, as was demonstrated in a recent trial (18). A clear relationship of PAH exposure and PAH-DNA-adducts has not yet been established. Moreover, at the moment, the present methods are too laborious for routine application.

A specific metabolite of pyrene, 1-hydroxypyrene, in urine was suggested as a biomarker of human exposure of PAH (19). Pyrene is a dominant PAH in PAH-mixtures. Many reports from different sources confirmed the potential of the methodology (20–32). At present, urinary 1-hydroxypyrene is a widely used biological indicator of exposure to PAH. The conclusion of the first international workshop on 1-hydroxypyrene was that the analytical method is robust, that the baseline excretion varies from country to country and that urinary 1-hydroxypyrene is a solid biological exposure indicator (33).

The measurement of various hydroxylated phenanthrenes has also been reported as a biomarker of exposure (8, 34). However, the experience is still limited. It appears that urinary 1-hydroxypyrene is a sound biomarker and that the analytical method is robust and non-laborious. This makes this biomarker suitable for routine application. Table 4.2.3 summarizes the characteristics of methodologies.

Table 4.2.3. Biological monitoring methods of PAH

Biological indicator	Suitability
Hydroxyphenanthrenes in urine	Limited experience
1-Hydroxypyrene in urine	Cost effective indicator of exposure, high sensitivity, sound method
Urinary thioethers	Low sensitivity, strong confounding by smoking
Mutagenicity in urine	Low sensitivity, strong confounding by smoking
Hemoglobin-benzo(a)pyrene	Limited experience
PAH-DNA adducts in blood cells	Indicator of exposure, laborious method with unexplained variability

4.2.7 1-Hydroxypyrene in urine

4.2.7.1 Toxicokinetics

The half-time for the urinary excretion of 1-hydroxypyrene in occupationally exposed workers is 18 h (mean of 15 workers) (26) or 6–35 h (range of 18 workers) (22).

Boogaard et al. (32) reported a half-time of 13 (4–27) hr in 16 workers. Therefore, urine sampling should preferably be done at the end-of-shift at the end of the week.

4.2.7.2 Biological sampling

(a) Sampling time

Urine should be sampled at the end-of-shift at the end of a routine workweek.

(b) Contamination

Sample contamination is not likely since 1-hydroxypyrene is a metabolite formed in the body.

(c) Sampling device

Urine can be collected in any clean container. Approximately 25 ml of urine will be sufficient for a duplicate analysis.

(d) Preservative, storage, stability

Urine is collected in a container without preservative. When the samples are stored in the dark at -18°C, the samples can be kept for at least a year without losses of 1-hydroxypyrene. The long-term stability of 1-hydroxypyrene in urine is good.

4.2.7.3 Recommended analytical method

(a) Principles of the method

The total of free and conjugated 1-hydroxypyrene is determined with high pressure liquid chromatography (HPLC). After enzymatic hydrolysis to release the conjugated part of 1-hydroxypyrene the analyte is separated from the matrix and enriched by reversed phase column extraction. The components of the eluate are separated by HPLC and 1-hydroxypyrene is determined with a fluorescence detector. The results are corrected for urinary creatinine content.

(b) Reagents

1-Hydroxypyrene (purity > 98%) is presently available at several sources (e.g. from the US NCI, Carcinogen Repository; Janssen Chimica, Belgium). β -glucuronidase/aryl sulphatase solution (100.000 Fishman U/ml and 800.000 Roy U/ml e.g. from Boeringer, Mannheim, Germany); Glacial acetic acid, p.A.; NaOH p.A.; HCl p.A.; HPLC-grade methanol; HPLC-grade water (conductivity > 18 MOhm.cm⁻¹); 0.1 M Acetate buffer (pH=5); A stock solution of 1-hydroxypyrene in methanol (0.2 mmol/L) is prepared every 2 months and stored in a refrigerator at -18°C. The standard addition solution (2 μ mol/L) is prepared from the stock solution and prepared twice a month. This solution is also stored at -18°C.

(c) Equipment

A high pressure liquid chromatograph (HPLC) with a column thermostat, capable of forming a binary gradient; fluorescence spectrophotometer; plotter or printer/plotter; an electronically controlled rotary shaking bath; research pH meter; centrifuge (600 x g = 2000 rpm); thermostatic waterbath (max. 50°C); cartridges packed with C-18 reversed phase material (e.g. Sep-pak, Waters, USA).

(d) Procedures

After thawning the urine sample is manually mixed by gently rotating. An aliquot of 10 mL urine is transferred to a conical flask, 20 mL 0.1 M acetate buffer (pH 5.0) are added to reach a total volume of 30 mL. The pH of diluted urine sample is adjusted to 5.0 with 4.0 N HCl. This mixture is incubated overnight (16 h) in a conical glass flask with 12.5 μ L solution of β -glucuronidase/aryl sulphatase in the flask sealed with parafilm in a shaker water bath (210 r.p.m.) at 37°C.

Sample enrichment and purification is carried out using a cartridge packed with C-18 reversed phase material to extract the metabolite. After priming the cartridge with 5 mL methanol, followed by 10 mL distilled water, the hydrolysed sample passed the cartridge at a rate of approximately 10 mL/min by means of a slight vacuum. Subsequently the cartridge is washed with 10 mL distilled water. Retained solutes are eluted with 9 mL methanol. The solvent is evaporated at 40°C in a gentle flow of nitrogen and the residue is redissolved (ultrasonic bath, 4 min) in 2.0 mL methanol. After centrifugation (600xg, 5 min) an aliquot of 1.5 mL is pipetted in a vial. The vial is sealed and an aliquot is analysed by HPLC.

Note:

- (i) Do not use plastic flasks, tubes or containers for the urine samples after hydrolysis, due to possible adsorption effects.
- (ii) An alternative procedure is washing the cartridge with a fixed volume (4 mL) of 20% methanol instead of water after loading of the urine sample. This is done in order to improve the cleaning of the sample.
- (iii) The evaporation step is unnecessary if the remaining volume of methanol after elution of the cartridge is constant.

Calibration samples are prepared in blank urine. Samples of enzymic hydrolysed urine of non-exposed persons (blank urines) are spiked with the given analyte. These calibration samples are processed and analysed as assay samples. The calibration curves for 1-hydroxypyrene are set up in hydrolysed urine across the working range (approximately 0, 10, 20, 40, 100 and 250 nmol/L). Calibration curves are calculated with the least squares method. The standard curves are linear with a correlation coefficient of at least 0.99. As the calibration samples are prepared in blank urine, the value measured for the blank urine is taken into account; the 1-hydroxypyrene concentration of the urine samples under investigation are directly read from the calibration curve with correction of background hydroxypyrene in the blank urine.

Instrumental parameters for HPLC

For the injection a 20 μL sample loop is used. Separation is performed on a 150 x 4.6 mm ID reversed phase (Lichrosorb RP 18–5 μm) column. The column temperature is maintained at 40°C. The solvent gradient is as follows: 5 min 46/54 % methanol/water, a linear gradient to 94/6 % methanol/water in 35 min. This is held for 10 min. The solvent flow is 0.8 mL/min. Retention time of 1-hydroxypyrene is 30 min. The excitation and emission slits of the fluorescence spectrophotometer are both set on 10 nm. Emission wavelength is 388 nm, excitation wavelength is 242 nm (7).

This method was developed for co-determination of several hydroxylated PAH-metabolites. When only 1-hydroxypyrene has to be determined a gradient with a shorter retention time (16 min) can be applied (35) or even an isocratic elution (acetonitrile–water 70:30) with a short retention time of 3.5 min (36). Since the three-dimensional fluorescence plot of 1-hydroxypyrene has several peaks, different instrumental settings of excitation and emission wavelengths can be used. The optimum is strongly dependent on the detector.

(e) Analytical reliability

(i) Trueness

The recovery of the analyte was determined in spiked urine samples at three concentrations (40, 200 and 400 nmol/L). The mean recovery (SD) from four determinations was 88% (9.0), 83% (4.4), and 84% (2.9), respectively. Fluorescence excitation and emission scanning of the HPLC-eluate with the flow stopped at the retention time of 1-hydroxypyrene allow the identification of this analyte.

(ii) Precision

Urine samples of workers ($n=10$, range 40–150 nmol/L) were analysed in duplo to test the within-day variation. The corresponding relative standard deviation was 3.7%. In the course of 12 months, 24 aliquots of the quality control samples (180 nmol/L) were assayed. The relative standard deviation was 12.6%. The latter may be used as an estimation of the between-day variation.

(iii) Detection limit

The detection limit of urinary 1-hydroxypyrene is 1.0 nmol/L (signal/noise > 3), when a sample volume of 20 μL is injected with the autosampling and auto-injection system using 2 mL vials. When the instrumental parameters are further optimized, more concentrated samples can be analysed and the detection limit may be lowered by a factor 5.

(f) Quality control

(i) Procedures

Internal quality control must be performed. The following procedure is recommended:

A bulk urine sample from several highly exposed individuals should be prepared for internal quality control (IQC). The urine is divided in aliquots of 20 mL and stored at -18°C (IQC-samples). At every series of urine samples, two IQC samples are added to the sample series. When the IQC samples are beyond the range of mean $\pm 1.96 \cdot \text{SD}$, the variation is too high and an alert is given. Since 1993 urinary 1-hydroxypyrene is included in the German external quality control scheme (Dr. Schaller, personal communication).

(ii) Interferences

1-Hydroxypyrene is baseline separated in urine samples with the given HPLC/fluorescence method. Fluorescence excitation and emission scans made at both sides of the 1-hydroxypyrene peak are similar and equal to the reference analyte. Sample blanks (ultrapure water) are included in sample series to monitor for interferences.

(g) Possible errors

- (1) Highly diluted or concentrated urine samples may lead to erroneous results.
- (2) Deconjugated 1-hydroxypyrene may absorb on plastic ware. No plastic material should be used after the hydrolysis of 1-hydroxypyrene.

(h) Reference to the most comprehensive description of the method

Jongeneelen FJ, et al. (7). Recent adapted method descriptions are available: Boos KS, et al. (35) and Hansen AM, et al. (36).

(i) Method evaluation

Angerer J, Schaller KH. (37). In this report the method was examined by four independent laboratories and the quality of the method was confirmed.

4.2.7.4 Other analytical methods

A gas chromatographic mass spectrometric (GC-MS) method for hydroxylated PAH-metabolites in urine, including 1-hydroxypyrene, was applied (8, 34). However, the sample clean-up procedure for the gas chromatographic analysis is very laborious.

4.2.7.5 Guide to interpretation

(a) Normal value among non-exposed referents

A trace amount of 1-hydroxypyrene in urine is found in referents' urine. Non-occupational uptake of PAH is reflected in urinary metabolite excretion: among community residents a background excretion of 1-hydroxypyrene in urine is found. Some reports are available on the assessment of PAH-intake in urban areas using the urinary 1-hydroxypyrene method (23, 38, 39). Smoking habits affect the concentration of 1-hydroxypyrene in urine. A overview of the background concentration of smokers and non-smokers is shown in table 4.2.4. It seems that background values vary from country to country, probably due to variations in the environmental PAH background and/or dietary intake of PAH. The upper limit of normal value in residents from the Netherlands,

defined as the 95-percentile in controls, is reported to be 0.66 in non-smokers and 1.31 $\mu\text{mol/mol}$ creatinine in smokers (21).

Table 4.2.4. Urinary 1-hydroxypyrene concentrations of smoking and non-smoking non-occupational exposed controls

1-Hydroxypyrene in urine ($\mu\text{mol/mol creat}$) – median (number of persons)					
Group	Country	Non-smoking	Smoking	P	Ref.
University controls	Netherlands	0.26 (52)	0.28 (38)	0.07	21
Industrial controls	Netherlands	0.17 (14)	0.51 (28)	0.003	22
University controls	Netherlands	0.12 (39)	0.25 (37)	0.0001	47
University controls	Turkey	0.24* (15)	0.33* (14)	0.01**	27
Industrial controls	Denmark	0.16* (20)	0.26* (26)	0.01**	29
Industrial controls	Belgium	0.08* (9)	0.17* (9)	---	26
Urban controls	China	0.68* (74)	0.76* (84)	0.33	24
Controls	Germany	0.05 (10)	0.22 (10)	---	31

* Arithmetic mean

** p-value of ANOVA of smoking among controls and exposed workers

Conversion factor

1 $\mu\text{mol/L}$ = 218 $\mu\text{g/L}$

1 $\mu\text{mol/mol creatinine}$ = 1.9 $\mu\text{g/g creatinine}$

(b) Biological action level

No authorized biological exposure limit (USA ACGIH: BEI or Germany DFG: BAT) has yet been proposed. Individual authors have reported on the relation of airborne PAH and 1-hydroxypyrene in urine. Jongeneelen (40) proposed a biological exposure limit of 2.3 $\mu\text{mol/mol}$ for cokeoven workers. Tjoe Ny et al. (41) proposed 4.3 $\mu\text{mol/mol}$ for Söderberg potroom workers. Note that these tentative limits are based on the TLV of airborne PAH-concentration and the relationship between airborne PAH-concentrations and urinary 1-hydroxypyrene concentrations.

The proposed tentative biological exposure limit is not valid for all work environments because the relative proportion of pyrene in the PAH mixture of different work environments may vary. Moreover, dermal uptake appears to be a significant pathway of PAH and the extent of dermal PAH exposure in different occupational settings may vary.

(c) Non-analytical interferences

Cytochrome P-450 1A is responsible for the metabolism of pyrene. The capacity of cytochrome P-450 1A may vary between individuals and inter-individual differences may

exist. Intake of the drug cimetidine (Tagamet) may affect the PAH-oxidating metabolism and may enlarge the inter-individual differences.

(d) Representatives of 1-hydroxypyrene as indicator of exposure

In vitro experiments with human liver fractions suggested that the formation of 1-hydroxypyrene is a good indicator for the activation of pre-mutagens from crude coal tar (42). In urine of coal tar treated psoriatic patients a good correlation of 1-hydroxypyrene and mutagenicity was found (4). Keimig et al. (43) reported that initial treatment of urine with heat and strong acid to hydrolyse 1-hydroxypyrene conjugates did not increase the yield of free analyte. Enzymatic hydrolysis with a mixture of glucuronidase and sulphatase appeared to be very effective and necessary for measuring total urinary 1-hydroxypyrene. The most intense hydroxylated-PAH peak in urine of coke workers corresponds to 1-hydroxypyrene as established with GC-MS (34). 1-Hydroxypyrene is a metabolite of pyrene and pyrene is always present in PAH mixtures. However, the relative proportion of pyrene in the PAH-source is not constant. Comparative measurements of PAH of airborne particulate matter in a certain worksite have shown that the relative contribution of each PAH (PAH-profile) is relatively constant, but the relative proportion of pyrene in the PAH-profiles of different worksites may differ significantly (44, 45). The variation of relative pyrene levels at various workplaces makes obligatory the additional analysis of air at each site where workers are being monitored for 1-hydroxypyrene. The value of 1-hydroxypyrene as biomarker of PAH exposure lies primarily in comparing exposure in longitudinal studies, rather than between different worksites.

4.2.8 Research needs

- (i) Prospective epidemiological studies concerning the dose-response of PAH, with urinary 1-hydroxypyrene as a parameter of dose and cancer mortality rates as a parameter of effects are needed to set a sound biological exposure limit. The relation of end-workweek urinary 1-hydroxypyrene (as year average) and long-term effects might be established in long-term prospective epidemiological studies of highly exposed workers, such as cokeoven workers or carbon anode production workers.
- (ii) Initial studies show that the dermal uptake of PAH among workers might be very significant (6, 32, 46). It is estimated that more than 50% of the uptake of pyrene among cokeoven workers is absorbed through the skin (5). However, knowledge of the extent of the dermal route in workers is still very limited. More occupational hygiene studies on the extent of dermal exposure of PAH are needed.
- (iii) It would be preferable to use several metabolites of different PAH in urine as biomarker instead of one metabolite of one parent PAH. Additional metabolites of carcinogenic PAH in urine should be traced and identified.

4.2.9 References

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