Commission of the European Communities

Industrial health and safety

Human biological monitoring of industrial chemicals series

Benzene
R. Lauwerys

Cadmium
L. Alessio, P. Odone, G. Bertelli, V. Foà

Chlorinated Hydrocarbon Solvents
A.C. Monster. R.L. Zielhuis

Lead
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Editorial board

Preface

The evaluation of the exposure of workers to dangerous agents is one of the measures insuring a better health protection. This evaluation is called monitoring.

Two approaches are available for the monitoring :
- ***ambient monitoring*** already in use for many years and
- ***biological monitoring*** of more recent development.

The need for clear definitions and for establishing the respective roles of these two types of monitoring has become necessary recently. In 1980 in Luxembourg at an international seminar organized jointly by the CEC and the United States authorities (Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health) on the Assessment of Toxic Agents at the Workplace, the following definitions were agreed :

- ***ambient monitoring*** is the measurement and assessment of agents at the workplace and evaluates ambient exposure and health risk compared to an appropriate reference;
- ***biological monitoring*** is the measurement and assessment of workplace agents or their metabolites either in tissues, secreta, excreta, expired air or any combination of these to evaluate exposure and health risk compared to an appropriate reference.

In addition, the term "Health Surveillance" was also defined as the periodic medico-physiological examinations of exposed workers with the objective of protecting health and preventing occupational related disease. The detection of established disease is outside the scope of this definition.

The definitions of biological monitoring and health surveillance separate components of a continuum which can range from the measurements of agents in the body through measurements of metabolites, to signs of early disease. A problem left unresolved concerns the precise place within these definitions of certain biochemical tests such as zinc protoporphyrin (ZPP), delta aminolaevulinic acid dehydrase (ALA-D), delta aminolaevulinic acid (ALA) in the blood and urine, etc., which are, in fact, indicators of metabolic effects which have occurred as a consequence of exposure.

Ambient monitoring is carried out for different reasons, for example :

a. determining ambient concentrations in relation to an established legal standard or consensus guideline;
b. determining the relationship, if any, between the concentrations of agents at the workplace and the health of the workers;
c. ensuring the effectiveness of control measures;
d. evaluating the need for controls in the vicinity of specific emission sources;
e. indicating trends in relation to an improvement or determination at the workplace;
f. providing an historical record.

Biological monitoring measures or evaluates exposure from all routes. It sometimes allows a better evaluation of health risk than ambient monitoring especially in cases where exposure through different routes has to be considered.
Biological monitoring takes into account individual variability, the impact of factors such as personal activity, biological characteristics and life styles of the individual.

The two types of monitoring are complementary in increasing the protection of workers' health. If both are carried out simultaneously, information should be produced on the relationships existing between external exposure and concentration of the substance in biological samples, and between this concentration and early effects.

Detailed knowledge of the metabolism of the toxic agent in the human organism and of the alterations that occur in the critical organ is essential in selecting the parameter to be used as indicator.

Unfortunately, however, such knowledge is usually insufficient and thus limitations exist in most biological monitoring programmes.

The conditions necessary for successful biological monitoring are :

- existence of indicators,
- existence of analytical methods that will guarantee technical reliability in the use of these indicators,
- possibility of measuring the indicators on readily accessible biological specimens,
- existence and knowledge of dose-effect and dose-response relationships.

In carrying out a biological monitoring programme, it is indispensable to know exactly what the characteristics and behaviour of the indicators under study are in relationship to length of exposure, time elapsed since beginning and end of exposure, and all physiological and pathological factors other than exposure that could give a false interpretation of the results obtained.

Conditions for biological monitoring application include adoption of analytical methods yielding values comparable throughout the different laboratories.

This long time adopted approach has already permitted the CEC to standardize in 1972 a method for erythrocyte ALAD determination and develop programmes for inter-laboratory comparisons for lead and cadmium determination in biological media.

The Council of Ministers of the European Communities in adopting in 1978 the First Action Programme on Safety and Health at Work proposed by the Commission stressed the need to increase protection against dangerous substances; it emphasized the need to promote new monitoring and measuring methods for the assessment of individual exposure, in particular through the application of sensitive biological indicators.

In August 1982 the Council adopted a directive on the protection of workers exposed to lead. The monitoring of blood lead levels as well as the determination of ALAU, ALAD and ZPP are among the tools to be used for monitoring worker exposure to lead. A comparison of the results with action levels and limit values allows appropriate action to be taken.

Considerable data concerning the biological monitoring of a number of industrial chemicals has been published in the international literature.

Nevertheless, the difference in approaches used in the research, the variety of analytical methods and the frequent discordances in the results, usually make it difficult to formulate a conclusive synthesis permitting the transfer of literature data into practice.

The aim of this series dedicated to human biological monitoring of industrial chemicals in occupational health is based on the considerable experience acquired by the authors in the specific topics.

For the draft of the monographs, the following outline, suggested by R.L. Zielhuis and R. Lauwerys, has been used :

- a review of metabolism and/or mechanism of action;
- potentially useful biological parameters for evaluation of exposure and/or body burden and/or early reversible effects;
- a critical evaluation of each parameter :
  - predictive validity in regard to exposure;
  - quantitative relationship between levels of external exposure and internal exposure, and between exposure and effects;
  - limitations of the test;
- a proposal for one or several tests for biological monitoring.

Because of the considerable gaps in scientific knowledge it has not been possible to

follow this outline strictly in every single one of the monographies. It is hoped that future research will fill these gaps.

It must be recognized that the biological monitoring approach for other toxic agents must still be developed and that considerable research is still necessary.

The Council in the above mentioned action programme and in the directives recently adopted in this field stressed the need to provide adequate information at all levels. It is considered that these monographs will be of benefit to the occupational health physicians, the industrial hygienists, the employers and the trade-union representatives, by giving the scientific rationale on which a number of biological monitoring programmes are based.

Human biological monitoring of industrial chemicals series

Benzene

R. Lauwerys

Summary

This document reviews the liquid benzene as related to occupational exposure and the possibilities of the biological monitoring of exposure.
Absorption of benze occurs mainly through inhalation of vapours and secondarily through skin contact with the liquid form. Benzene can cause aplastic anaemia, leukemia and erythroleukemia.

Benzene gives rise to phenol in vivo and therefore the measurement of urinary phenol excretion has some practical application for evaluating current exposure to benzene. A phenol concentration exceeding 20 mg/l at the end of a work shift suggests that workers have been exposed to benzene if the analytical method used was sufficiently sensitive and if other circumstances which might cause increased phenol excretion have been excluded.

Determination of benzene in exhaled air is a valuable method of confirming exposure to benzene. It is highly sensitive and more specific than phenol determination. Not enough data exists, however, to correlate benzene concentration in exhaled air with integrated exposure. A benzene concentration in breath of 0.12 ppm, measured 16 hours after the end of exposure, is proposed as a tentative biological threshold resulting from an 8 hour exposure to 10 ppm benzene.

Further investigations are required to define the usefulness of the various biological parameters in the routine control of workers exposed to benzene.

Benzene

Chemical and Physical Properties

Benzene is a colourless liquid at normal temperatures. Several of its chemical and physical properties are given in Table 1.

Table I - Chemical and physical properties of benzene

Boiling point (760 mm Hg)	80.1 °C
Vapour pressure (20 °C)	74.66 mm Hg
Vapour density	2.77
Molecular formula	C_6H_6
Structural formula	
1 ppm	3.247 mg/m^3
1 mq/m^3	0.308 ppm

Biological Changes Related to Chronic Benzene Toxicity

Benzene can cause aplastic anaemia, leukemia and erythroleukemia.

In its initial stages, benzene toxicity can manifest itself as a paradoxial alteration of the blood picture. Polycythemia and aneamia, leucocytosis and leucopenia, thrombocytosis and thrombocytopenia have all been reported in exposed workers. With continued exposure, however, the trend is toward decreased levels of circulating erythrocytes, leucocytes and thrombocytes. As the disease intensifies, circulating blood cell levels decrease further and pancytopenia develops (Kocsis and Snyder, 1975).

Aksoy et al. (1972) found that workers exposed to benzene and suffering from pancytopenia displayed increased levels of HbF. Depression of leucocytes is usually apparent prior ro the onset of anaemia (Hunter, 1939; Mitnik and Genkin, 1931).

However, Kocsis and Snyder (1975) have rightly stressed that despite the general agreement that benzene toxicity results in leukopenia, effective screening for benzene toxicity should not be restricted to the determination of leucocyte levels alone.

It has also been suggested that thrombocytopenia may be among the earliest signs of benzolism (Goldwater, 1941; Nikulina and Titowa, 1934) and that the ability of platelets to aggregrate is also depressed by benzene (Saita and Sbertoli, 1954). Lee at al. (1973, 1974) have demonstrated, based on cell counting techniques in animals, that a reduction in the incorporation of ^{59}Fe into haemoglobin of maturing red cells can occur even through no inhibition of white cell production is apparent.

Thus, the effects of benzene on erythrocyte production occur quite soon after exposure. Detection of the earliest stages of reduced haemopoiesis is not possible with classical cell counting techniques, however, due to the long life span of the red cells.

Earlier studies performed by Truhaut et al. (1959) on rabbits suggested that the response of the erythropoietic system was quite variable, depending on the routes and the intensity of exposure.

Hypofunction of one system, however, may be associated with hyperfunction of another system, and therefore no valid judgement can be made as to whether or not hypofunction is invariably seen in advance of hyperfunction (Deutsche Forschungsgemeinschaft, 1974). Since these changes in peripheral blood are not necessarily reversible, they cannot be regarded as sufficiently sensitive for the biological monitoring of workers exposed to benzene. Furthermore, their lack of specificity is well known and does not require further comment.

Cytogenetic studies have revealed that chromosomal aberrations can be found in bone marrow and peripheral leucocytes of humans exposed to benzene (Berlin et al., 1975; Forni and Moreo, 1969; Forni et al., 1971a,b; Hartwich and Schwanitz, 1972; Prost et al., 1976; Tough and Court Brown, 1965; Tough et al., 1970; Vigliani e Forni, 1969).

No dose-effect relationship has so far been demonstrated for benzene-induced chromosome aberration and the implication of this finding for the occurrence of benzene leukemia is still not clear (Truhaut and Murray, 1978).

Metabolism

Metabolic Pathways

Absorption of benzene occurs mainly through inhalation of vapours and secondarily through skin contact with the liquid form.

According to Hanke et al. (1961) the rate of human skin absorption of liquid benzene applied under a closed cup is 0.4 mg/cm²/h.

Srbova et al. (1965) exposed human vlunteers to 47-110 ppm benzene vapours for 2 hours and found that about 50% of the inhaled benzene was absorbed.

Hunter (1966) found that at a benzene concentration of 35 ppm, the amount of benzene reached a relatively steady state in approximately 5-7 minutes and amounted to about 47% of the benzene in the inhaled air.

Docter and Zielhuis (1967) have pointed out that it is not the proportional retention as such which is the relevant parameter but the amount of benzene absorbed, which includes not only the proportional retention but also the ventilation parameters. The amount of benzene absorbed has been estimated at about 0.4 mg/min when the subject is exposed to 10 ppm with a ventilation of 25 l/min (Docter and Zielhuis, 1967).

Since benzene is highly lipophilic, highest levels are found in fat and bone marrow (Truhaut and Murray, 1978).

A fraction of the absorbed benzene is excreted unchanged in the exhaled air. Several authors found that in man, the fraction eliminated in the exhaled air varies between 10 and 50%, depending on the metabolic activities and the quantity of the fat (Srbova et al., 1956; Teisinger et al., 1952). The remaining fraction is metabolised (Hasegawa et al., 1967; Parkes and Williams, 1953a, b; Porteous and Williams, 1964a, b).

The first reaction catalysed by the microsomal mixed function oxidase system of various tissues is the transformation of benzene into benzene epoxide (Harper et al., 1973). This compound is a very reactive intermediate that either binds directly to cellular constituents (e.g. DNA, proteins), or is further transformed into other benzene derivatives. Benzene epoxide is suspected of being responsible for the myelotoxic action of benzene (Daly et al., 1972; Jerina et al., 1968).

Benzene epoxide may be transformed non-enzymatically into phenol, which is then conjugated with glucuronic acid or sulphate. The glucurono and sulphoconjugates of phenol are then excreted in the urine (Porteous and Williams, 1949a, b; Snyder, 1974). Phenol (free or conjugated) constitutes the main urinary metabolite of benzene.

The epoxide may also react with glutathion and the product formed will be S(1,2-dihydro-2-hydroxyphenyl) glutathione. The subsequent action of a glutathionase in the presence of a glutamine acceptor, a peptidase and acetyl CoA acetyltransferase, results in the formation of premercapturic acid, i.e. S(1,2-dihydro-2-hydroxyphenyl)

acetyl-L-cysteine premercapturic acid, which is excreted as such in the urine. When urine is treated with mineral acids, the premercupturic acid is transformed into mercapturic acid.

Trans-1,2-dihydroxybenzene is formed under the action of the enzyme epoxide hydrase, and is quickly transformed into catechol (Jerina et al., 1968). An enzyme (cis-benzene glycol dehydrogenase), which catalyses the conversion of cis-benzene glycol into catechol in the presence of NAD^+, has been isolated from bacteria (Axcell and Geary, 1973). Very slight amounts of hydroquinol and 1,2,4-trihydroxybenzene have also been identified in urine.

After a ring scission, catechol is transformed into trans-trans muconic acid, which is excreted in urine, and carbon dioxide, which is excreted with the exhaled air (see Fig. 1).

This metabolic pathway was first unravelled in animals but has also been confirmed in man. Teisinger et al. (1952) repeatedly exposed 15 volunteers to about 100 ppm benzene for 5 hours a day. They observed that 33 to 65% of inhaled benzene was absorbed. Of the total quantity absorbed, an average of 12.1% (3.8 to 27.8 %) was eliminated by the pulmonary route. In urine, phenol (averaging 28.8%), catechol (2.9%) and hydroquinol (1.1%) were found, as well as a very small proportion of unchanged benzene (0.1 to 0.2% of the quantity absorbed). On the other hand, Hunter and Blair (1972) exposed 5 volunteers to 22 ppm for 6 hours, and found that 74 to 87% of the benzene absorbed is excreted as phenol and about 12% is eliminated unchanged in the exhaled air. Sherwood (1976) has also estimated that about 80% of absorbed benzene is biotransformed into phenol. All the investigations agree, however, that phenylmercapturic acid, catechol, hydroquinol and muconic acid are minor metabolites of benzene.

It has been demonstrated that benzene metabolism occurs not only in the liver, but also in other tissues like the bone marrow (Snyder et al., 1977). This observation may have some bearing on benzene toxicity. It should be recalled that as for the majority of voltatile organic substances, the elimination of benzene and its metabolites is rapid. Excretion of the metabolites is usually completed within 24-48 hours after a single exposure which represents a biological half life of less than 12 hours (Sherwood and Carter, 1970). However, tissues with high fat content may retain a slight quantity of benzene for several days after the end of exposure.

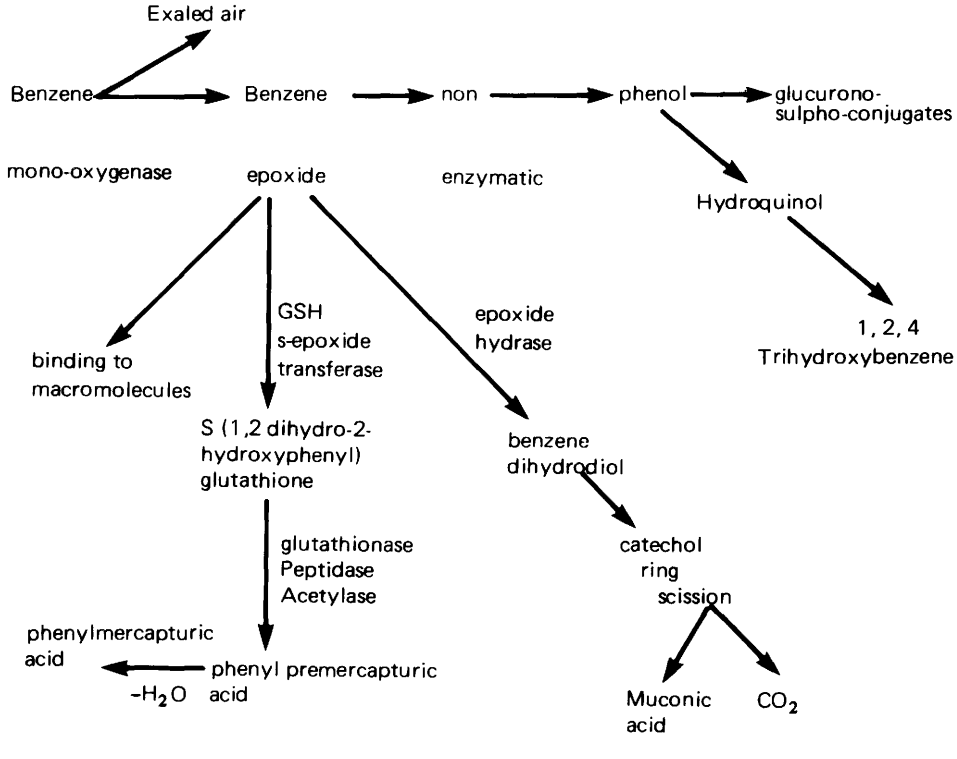

Figure 1 - Metabolic biotransformation of benzene in vivo

Factors Influencing Benzene Metabolism

Animal experiments suggest that benzene metabolism can be stimulated by microsomal enzyme inducers such as phenobarbital (Ikeda and Ohtsuji, 1971; Snyder et al., 1967) and that benzene can stimulate its own metabolism. On the other hand, Sato et al. (1967) have observed that in vitro some metabolites of benzene (phenol, catechol and hydroquinol) can inhibit benzene hydroxylation.

Ikeda et al. (1972) have also found that in rats, conversion of benzene to phenol is suppressed by the simultaneous administration of toluene. Phenobarbital pre-treatment, which stimulates benzene metabolism, reduces the leukopenic action of benzene in rats (Ikeda and Ohtsuji, 1971).

Biological Indicators

Background Information

Information about the fate of benzene in vivo has given rise to several biological exposure tests :

- the determination of the ratio between inorganic and organic sulphates in urine;
- the measurement of total (free and conjugated) phenol in urine; and
- the measurement of benzene in blood and exhaled air (Lauwerys, 1975).

Pheripheral blood examination and cytogenetic techniques are also suggested for detecting early biological manifestations of benzene.

It should be stressed that since the biological half-life of benzene metabolites is usually short (less than 12 hours), the time of sampling of biological material in relation to exposure is very important. When biological monitoring involves sampling and analysing urine, the collection methods and the means of expressing the results should be standardised.

Several methods of urine collection can be considered :

1 Collection of urine for 24 hours and expression of the results in amount excreted per 24 hours. This procedure is the most accurate, but it is also impractical for the routine monitoring of workers.

2 Collection of urine during a well-defined period of time (e.g. for two to four hours during the second part of the work shift) and expression of the results in quantity/unit time. This method is more accurate than spot sample analysis, but often too elaborate for routine control of workers.

3 Spot specimen collection at a well-defined time after the beginning or end of exposure (at the end of work shift or 16 hours after the end of exposure, i.e. before starting a new work period). This seems to be the most widely used method. It is advisable to correct the results for the dilution of the urine. Two methods of correction have been used : a) expression of the results per gram of creatine; b) adjustment to a constant specific gravity.

   Although creatine adjustment is not superior to specific gravity adjustment, the former is better for very concentrated and very dilute samples (Elkins et al., 1974). Furthermore, in the case of glucosuria and probably proteinuria, the specific gravity adjustment may give inaccurate results. Whatever the method of collection, analyses performed on very dilute urine specimens (specific gravity less than 1.010) are not reliable. The results of urine analyses can only be interpreted if renal excretion is not impaired (no evidence of kidney disfunction).

4 When a large interindividual variability and/or a high "background" level of the biological parameter selected makes the interpretation of a single measurement difficult, it is sometimes useful to analyse biological material collection before and after the exposure period.

   If there is no important circadian rythm in the urinary excretion of the substance, the change in the biological parameter due especially to exposure can then be better assessed.

When comparing results on benzene metabolite excretion as reported in the literature, it must be kept in mind that since different methods of urine collection may have been used, results may not be comparable. Furthermore, the different degree of specificity and the sensitivity of the analytical method selected for urine analysis must also be taken into consideration.

When one attempts to correlate the changes of a biological parameter with the intensity of benzene exposure, one must also realise that in several investigations the determination of the exposure was necessarily inaccurate due to semi-quantitative determination with detector tubes, a limited number of spot samples and the failure to take skin contamination into consideration. Exposure has rarely been well-characterized in the terms proposed by Sherwood (1971).

Ratio between Inorganic and Organic Sulphates in Urine

The ratio between inorganic and total sulphates in urine is normally more than 85%. In 59 non-exposed individuals, Teisinger and Bergerova-Fiserova (1955) have found a mean ratio of 92.5% with a standard deviation of 4.6%. Exposure to benzene produces a decrease in this ratio, since some metabolites of benzene are eliminated as sulphoconjugates (Yant et al., 1936a, b).

A tabulation of rather "old" data from the literature on the relationship between benzene exposure and inorganic-total sulphate ratio has been prepared by the German Working Group for the Establishment of MAK-values, of the Senate Commission for the Examination of Hazardous Industrial Materials (Deutsche Forschungsgemeinschaft, 1974). It is partially reproduced in Table 2.

Table II - Urinary inorganic sulphate after exposure to benzene (8 hours daily)

Number of subjects exposed	Concentration of benzene (ppm)	Average inorganic sulphate (% of total sulphate)	Reference
14	13 - 23	85	Yant et al., 1936b
8	19 - 50	70	
22	123 - 132	72	
13	158 - 372	55	
	40 - 45	71 (67 - 79)	Hardy and Elkins, 1948
	55 - 70	54	
	50 - 70	68	
	80	67	
8	0	86	Bowditch and Elkins, 1939
6	40	81	
11	<40 - 75	61	
11	75 - 100	42	
9	100 - 125	34	
4	10.5 - 31.5	>80	Teisinger and Bergerova-Fiserova, 1955

It is clearly evident that on a group basis, a significant decrease in the ratio, i.e. below 70%, is only observed when benzene exposure exceeds 40 ppm.

The sensitivity of this test is thus too low as an exposure on the order of 10 ppm, the time-weighted average exposure recommended in 1974 by the National Institute for Occupational Safety and Health (NIOSH, U.S.A.), and in 1976 by an international group of experts (Truhaut and Murray, 1978), would not significantly influence this ratio (Teisinger and Bergerova-Fiserova, 1955; NIOSH, 1974). The specificity of this test is also limited since numerous hydroxylated organic chemicals are also excreted in urine as sulphoconjugates. This test can no longer be recommended for evaluating benzene exposure.

Concentrations of Total Phenol in Urine

Contrary to substituted benzene derivatives, e.g. toluene and xylene, which are mainly metabolised on the side chain, benzene gives rise to phenol in vivo and therefore the measurement of urinary phenol excretion has been proposed as an exposure index. The half-life of phenol lies in the range of 4-8 hours (Sherwood and Carter, 1970).

Assuming that 30% of the retained benzene is oxidized into phenol (Teisinger et al., 1952), about 60 mg phenol should be produced in workers exposed to 10 ppm benzene for 8 hours with a ventilation of 25 l/min. Individual variability in pulmonary ventilation

and in the proportion of benzene biotransformed into phenol (80% according to Sherwood, 1976) makes the preceding estimation valid only on a group basis.

Several methods have been developed for the analysis of free and conjugated phenol in biological material. As indicated above, interpretation of the reported results must take the specificity of these methods into consideration. Briefly, in order of increasing specificity, these include : the colorimetric method of Theis-Benedict (diazotised p-nitroaniline as reagent) and the colorimetric method, using 4-aminoantipyrine as a reagent, the colorimetric method of Gibbs (2,6-dichloroquinone chlorimide as reagent) and the various gas liquid chromatographic methods.

The 4-aminoantipyrine method determines many phenol derivatives (Gottlieb and Marsh, 1946).

The method of Theis-Benedict, which measures not only the phenol but also the ortho-, meta- and paracresol and other hydroxy compounds (Müting at al., 1970), gives values for phenol cntent in urine about 30% greater than those obtained with the method of

Table III - Phenol concentration in urine of persons non-occupationally exposed to benzene

Number of subjects	Phenol concentration	Method for phenol determination	Reference
	$\bar{X}$ = 25 mg/24 H (range: 11 - 42 mg/24 h	1 +	Deichmann and Schafer, 1942
	$\bar{X}$ = 30 mg/l (S.G. 1024) (range: 15 - 50 mg/l)	1	Walkley et al., 1961; Pagnotto et al., 1961
	$\bar{X}$ = 17.8 mg/l (range: 3.2 - 41.3 mg/l)	1	Teisinger and Bergerova-Fiserova, 1952
	$\bar{X}$ = 7 mg/l	2	Bardodej et al., 1962
12	$\bar{X}$ = 17 mg/l (S.G. 1016) (range. 9.3 - 34.4)	3	Buchwald, 1966
	(range· 5 - 10 mg/l)	4	Porteous and Williams, 1949b
328	$\bar{X}$ = 8.2 mg/24 h (S.D. 5.9) 97.5th percentile = 20 mg/l	4	Teisinger and Bergerova-Fiserova, 1952
54	$\bar{X}$ = 7.8 mg/l (S.G. 1024) (S.D. 3.7) 97.5th percentile = = 16 mg/l	4	Docter and Zielhuis, 1967
20	$\bar{X}$ = 7.5 mg/l (S.G. 1024) (range. 2 - 18 mg/l)	5	Van Haaften and Sie, 1965
10 (Male)	$\bar{X}$ = 10.4 mg/24 h 95th percentile = 12.4 unit	5	Lebbe et al., 1966
10 (Female)	$\bar{X}$ = 11.3 mg/24 h 95th percentile = 14.8 unit		Lebbe et al., 1966
20	$\bar{X}$ = 4 7 mg/G creatinine 9.4 mg/l (uncorrected) 97.5th percentile = 14.7 mg/G creatinine	5	Buchet et al., 1972
9	$\bar{X}$ = 1.8 mg/l (all $<$ 3.5 mg/l)	5	Sherwood, 1972b
13	$\bar{X}$ = 7.6 mg/l (S.G. 106) (range: 3.2 - 14.7 mg/l)	5	Dirmikis and Darbre, 1974

+ = See methods

Methods

1. Colorimetric method of Theis-Benedict
2. Amino-antipyrine method
3. Modified Theis-Benedict method
4. Colorimetric method of Gibbs
5 Gas chromatographic method

Gibbs (Rainsford and Davies, 1965). With the latter method, ortho- and metacresol can also interfere (Docter and Zielhuis, 1967; Sherwood and Carter, 1970), but since, of the three cresol isomers, only p-cresol is normally present in significant quantity in urine (Sherwood and Carter, 1970), the interference of the other two isomers is usually minimal. Buchwald (1966) has proposed a modification of the method of Theis-Benedict using a stabilized diazonium salt of p-nitroaniline. The author claims that although ortho- and metacresol interfere with this technique, paracresol does so only slightly.

Gas chromatographic methods (Bakke and Sheline, 1969; Buchet et al., 1972; Dirmikis and Darbre, 1974; Lebbe et al., 1966; Sherwood and Carter, 1970; Van Haaften and Sie, 1965) are certainly superior in specificity to colorimetric methods, though not that superior to Gibbs' method (Van Haaften and Sie, 1965). Gas chromatographic methods are not only very specific, but also very precise when used with an internal standard (Buchet et al., 1972). Whatever the method used, it is necessary to control that the hydrolysis of conjugated phenol is complete before extraction. Enzymatic hydrolysis seems to be the method of choice.

A summary of control values reported in the literature for phenol concentration in urine by persons who have not been occupationally exposed to benzene is presented in Table 3.

It is obvious that the Theis-Benedict method gives results which are too high due to p-cresol interference. It is known that p-cresol, which is not a metabolite of benzene, can amount to up to 58% of "total phenols" in urine (Van Haaften and Sie, 1965). One must conclude that the colorimetric method of Theis and Benedict cannot be relied upon for detecting slight exposure to benzene. Since phenolic compounds other than p-cresol are only found in minute quantities in normal urine, the results obtained with Gibbs' method are rather close to those obtained by gas chromatography.

Considering only the results obtained with the last two techniques, it can be concluded that in persons non-occupationally exposed to benzene, phenol concentration in urine does not exceed 20 mg/l.

The results of studies which have attempted to correlate benzene exposure with urinary phenol excretion are presented in graph form in Fig. 2, where the regression

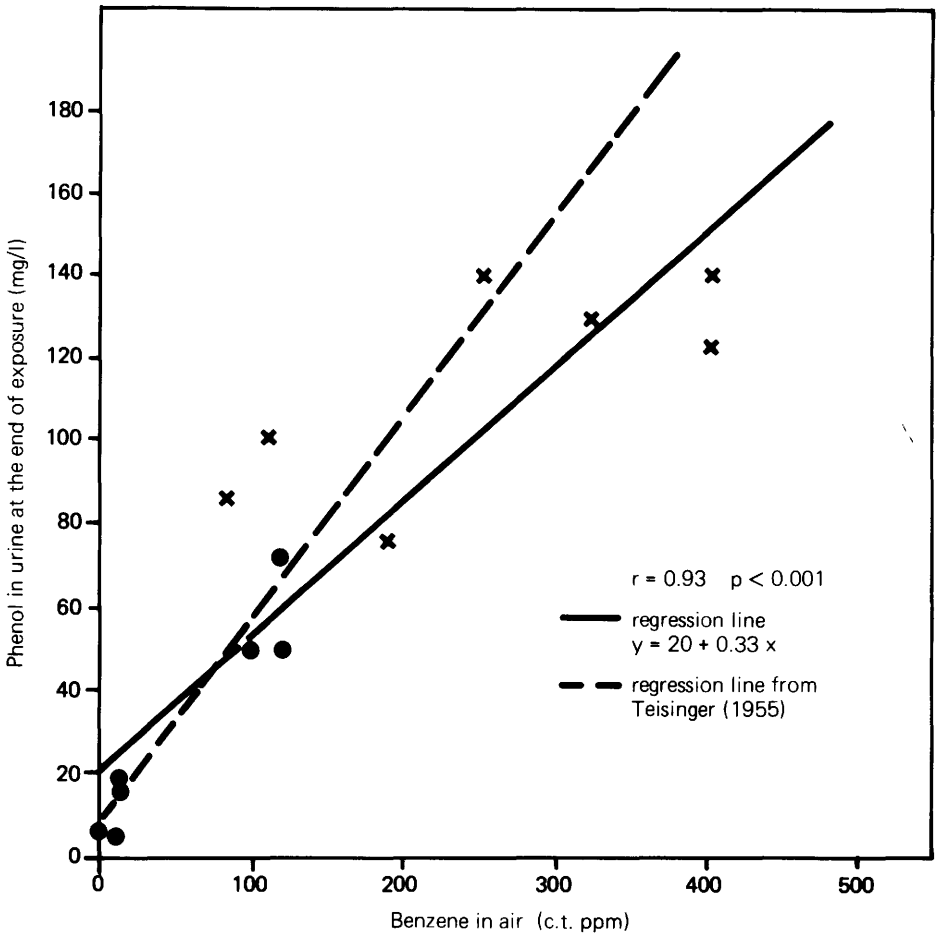

Figure 2 - Relationship between the phenol concentration in post shift urine samples and benzene exposure. Each point represents the mean value found in a group of workers. See Table 4. Phenol was determined by the method of Gibbs (X) or by gas chromatography (*)

Table IV - Relationship between benzene exposure and phenol in urine (Colorimetric method of Gibbs and gas chromatography technique)

Number of subjects	Benzene conc. (ppm)	Duration expos. (hrs.)	Total expos. (C.t.)	Concentration phenol-urine before exposure (mg/l) (A)	Concentration phenol-urine after exposure (mg/l) (B)
43	<10	8	–	28 (12 - 44)	33 (19 - 74)
10	7 - 15	8	≈88	52 (24 - 144)	87 (14 - 176)
8	12 - 15	8	≈108	55 (37 - 98)	100 (60 - 195)
8	7.5 - 50 (24)	8	≈192	37 (25 - 46)	74 (52 - 124)
7	40 - 60	8	≈400	60 (41 - 91)	126 (61 - 310)
6	10 - 70	8	≈320	37 (21 - 56)	129 (50 - 254)
5	10 - 70	6	≈240	79 (59 - 177)	140 (107 - 210)
5	20 - 80	8	≈400	68 (52 - 111)	140 (87 - 224)
14	25 - 150	5 - 6	≈480	39 (19 - 69)	177 (113 - 278)
3	>500	1	>500	–	132 (82 - 188)
10			0.55	5.1 (1.5 - 14.4)	5.1 (2.1 - 14.7)
10			1.31	4.9 (0.3 - 12.5)	6.5 (1.6 - 23.4)
10			10.20	8.5 (2.2 - 32)	15.4 (5.9 - 39.2)
1	25	4 1/2	115	±6	50
5	0.52	5.3	2.8	2.8 (1 - 5)	9.8 (5 - 18)
2	2.15	3	6.45	1.5 (1 - 2)	5.5 (5 - 6)
2	2.0		9.4	±5	12.0 (9 - 15)
1	20.0		114.0	±8	71.0
1			100		50
					20

Table IV (cont.d) - Relationship between benzene exposure and phenol in urine (Colorimetric) method of Gibbs and gas chromatography technique)

Time of urine sampling	(B) - (A) (mg/l)	Methods for phenol determination	Remarks	Reference
Immediately after exposure	5	Gibbs	Atmoshperic benzene concentration determined by spot samples during the shift	Rainsford and Davies, 1965
	35	Gibbs		
	45	Gibbs		
	37	Gibbs		
	66	Gibbs		
	92	Gibbs		
	61	Gibbs		
	72	Gibbs		
	138	Gibbs		
	—	Gibbs		
	0	Chromatog.	Personal sampler	Berlin et al., 1975
	1.6	Chromatog.	Personal sampler	
	6.9	Chromatog.	Personal sampler	
End of exposure	±44	Chromatog.	Sedentary subject (lab exposure)	Sherwood and Carter, 1970
		Chromatog.		
End of exposure	7 (4 - 15)	Chromatog.	Personal sampler	Parkinson, 1975
	4	Chromatog.	Personal sampler	
End of exposure	7	Chromatog.	Personal sampler	Sherwood, 1972b
	63	Chromatog.	Personal sampler	
End expos. 16h after exposure		Chromatog.	Personal sampler	Sherwood, 1976

line proposed by Teisinger and Bergerova-Fiserova (1955) has been reproduced. These results are also summarized in Table 4. Only the results obtained with the colorimetric technique of Gibbs or by gas chromatography have been presented in this table, since, as we have indicated above, they are comparable. The results have also been presented in graph form, i.e. Fig. 2 and Fig. 3. Fig. 3 shows the relationship between the change in urine phenol concentration during exposure to benzene and the concentration of benzene in air. Although the data are derived from different investigations, they clearly indicate that on a group basis there is a highly significant correlation ($r = 0.93$; p less than 0.001) between exposure to benzene (C.t.) and the concentration of phenol found in a urine sample collected at the end of exposure.

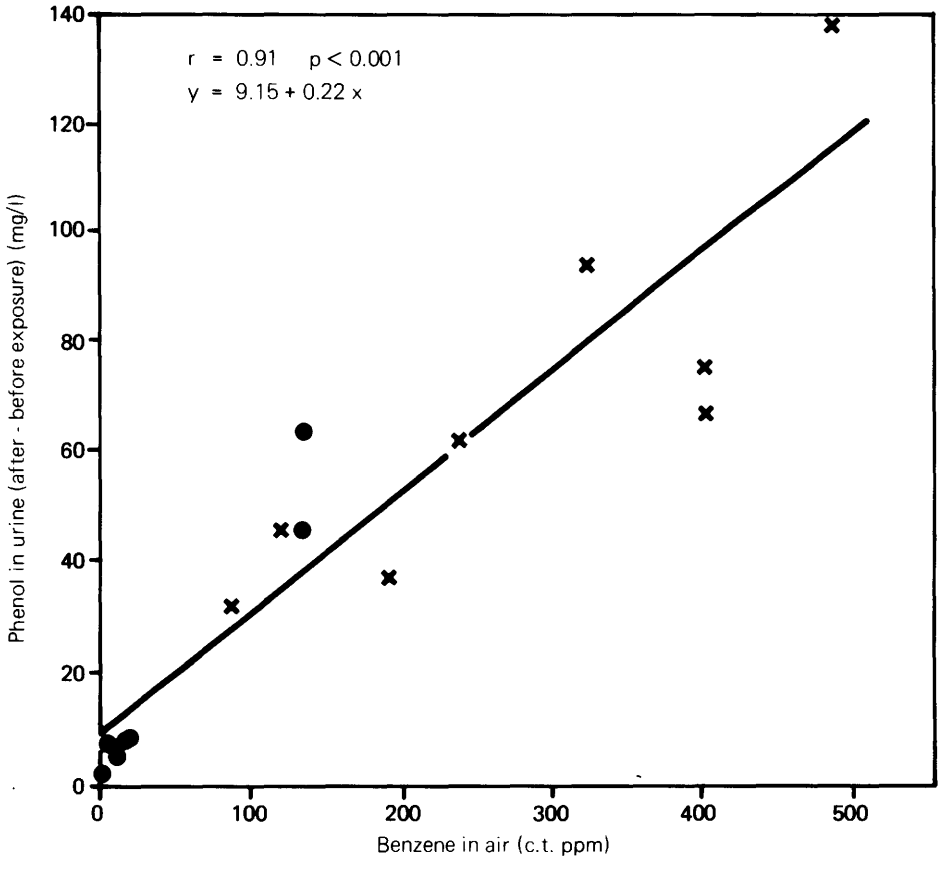

Figure 3 - Relationship between change in urine phenol concentration during exposure to benzene and concentration of benzene in air. Each point represents the mean value found in a group of workers. See Table 3. Phenol was determined by the method of Gibbs (X) or by gas chromatography (*)

These results suggest for a 6 hour exposure to a benzene level of approximately the current ACGIH (American Conference of Governmental Industrial Hygienists) TLV (10 ppm), the average phenol concentration in the postshift urine samples would be around 40 mg/l (corrected for a specific gravity of 1.016), provided a specific method of analysis were used (Gibbs, chromatography). When the average phenol concentration exceeds 20 mg/l, exposure to benzene has occurred.

However, in using 20 mg/l as the threshold, very slight exposure to benzene (e.g. exposure to 1 ppm benzene which is the time-weighted average concentration recently proposed by the American Occupational Safety and Health Administration) may be overlooked, since the pre-exposure level may be much lower. In that case, comparison of phenol concentrations between pre- and post-shift samples may be useful, since it seems that in control individuals there is no significant difference in phenol concentration between morning and afternoon urine samples (Van Haaften and Sie, 1965).

Data published by Bethlehem Steel Corporation (NIOSH, 1974), Pagnotto (NIOSH, 1974), Pagnotto et al. (1961) and Walkley et al. (1961) also indicate that urinary excretion of phenol has a linear relationship to the atmospheric concentration of benzene to which workers are exposed. However, these results were obtained with the method of Theis and Benedict and the interference of cresol makes interpretation of the data for a moderate benzene exposure (less than 10 ppm) difficult. The data of

Pagnotto and Bethlehem Steel Corporation are published in NIOSH (1974) and are summarized in Table 5. The data published by Walkley et al. (1961) are reproduced in Fig. 4. It is evident that below an exposure level of 10 ppm (C.t. less than 60 ppm x h) no clear change in urinary metabolite excretion can be demonstrated when using the Theis-Benedict technique, probably because of p-cresol interference.

Table V - Summary of data published by Pagnotto and Bethlehem Steel Corporation

Sampling (hr)		4 - δ	
ppm		100	200
Uncorrected (mg/ml)	Mean	3 - 09	8 - 19
	SD	0 - 70	2 - 62
Corrected (mg/ml)	Mean	2 - 81	5 - 85
	SD	0 - 66	1 - 24
Rate (mg/min)	Mean	3 - 10	4 - 61
	SD	0 - 84	0 - 80

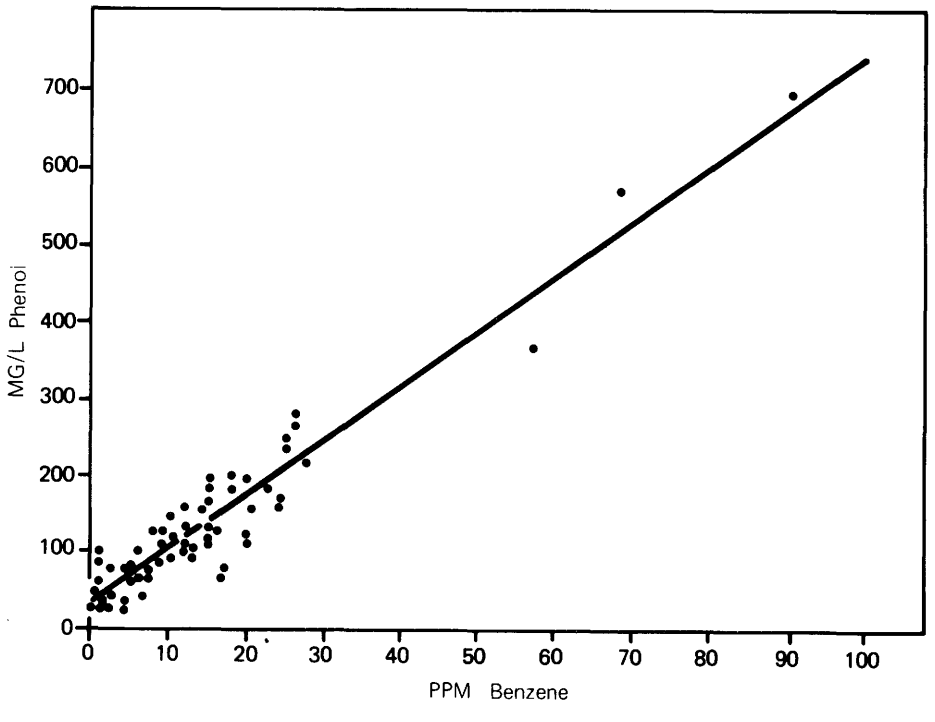

Figure 4 - Relationship between phenol concentration in post-shift urine sample and benzene exposure. Phenol was determined by the method of Theis-Benedict. Data are from Walkley et al., (1961)

The ingestion of phenacetin, caffein, saccharin, aspirin and salicylic acid does not affect the excretion of phenol (Docter and Zielhuis, 1967; Walkley et al., 1961).

It has been stated that in non-exposed subjects there is no difference in excretion between the sexes, not between smokers and non-smokers; the concentrations in the morning and afternoon do not differ significantly (Van Haaften and Sie, 1965).

There are, however, several factors which influence phenol excretion.

Dermal application of phenol-containing preparations, exposure to phenol itself, some gastrointestinal disorders favouring the bacterial degradation of tyrosine and phenylalanine (Duran et al., 1973) and ingestion of phenylsalicylate containing drugs (Fishbeck et al., 1975); Kociba et al., 1976) increase urinary phenol concentration (Lauwerys, 1975).

The proportion of benzene eliminated as phenol increases after ethanol consumption (Sherwood, 1976).

In summary, on a group basis the following relationship between benzene exposure and phenol excretion can be proposed if a specific method is used for phenol determination in urine.

A phenol concentration exceeding 20 mg/l at the end of the working period suggests that workers have been exposed to benzene, at least if Gibbs' method or a gas chromatographic method is used for phenol determination and if the other circumstances causing increased phenol excretion (see above) have been excluded.

Table VI - Relationship between benzene exposure and phenol excretion

Benzene exposure (C.t.) (ppm x h)	Phenol concentration in urine collected at the end of the working day (mg/l) +
0	< 20
40 ++	30 - 35
80	45 - 50
100	50 - 55
200	85 - 90

+ Corrected for a S.G.: 1016

++ 10 ppm for 4 hours

Concentrations of Benzene in Exhaled Air

The usefulness of this biological method was primarily investigated by Sherwood. Elimination curves for benzene in exhaled air, initially obtained by Sherwood and Carter (1970) and Sherwood (1972a), demonstrated a two or more phase elimination process comprising a rapid phase with a half-time in the vicinity of 1 hour and a low phase with a half-time of 1 day or more. However, results recently obtained by Sherwood (1976) showed three distinct phases of elimination : a very rapidly falling rate during the first one or two hours after exposure, a less rapid fall over the next few hours and then a steady decline to natural background levels over a period of as much as 70 hours.

During the first period, the concentration/time relationship is not logarithmically linear, and according to Sherwood (1976), it is likely that several compartments of the body contribute during this period. The second period, which may define the release of benzene from lean tissues, is generally marked by a half-life of 3 to 4 hours. The third period, which may indicate loss of benzene from fatty tissues, has a characteristic half-life of between 20 and 30 hours.

As in the case of many volatile solvents, it is to be expected that the content of benzene in exhaled air on the morning following exposure best reflects the integrated exposure, i.e. exposure-dose (ppm x h), during the preceding day. The last parameter may therefore be a good index of the risk of chronic disease (Sherwood, 1972a). Two methods have been developed for sampling exhaled air : breath sampling tubes, and breath sampling respirator (Sherwood and Carter, 1970). Sherwood (1972a) has briefly listed the advantages and disadvantages of both methods.

«The former (sampling tube) has the advantage of instantaneous collection, complete absence of chemical pre-treatment before gas chromatography and

ready collection of duplicate samples. Although the latter requires a 10 minute sampling period for each operator, it provides a more consistent measure of elimination, as all exhaled breath over the period is sampled and it reduces the risk of interference from ambient benzene vapour.»

The results obtained by several authors who measured benzene in exhaled air have been tabulated (see Table 7). Results found 16 hours after the end of exposure (pre-shift sample) are plotted in Fig. 5. Although the results do not allow any firm conclusion to be drawn (sometimes only 1 subject per experiment) they suggest that an 8 hour exposure to 10 ppm benzene produces a benzene concentration in breath of around 0.12 ppm 16 hours after the end of exposure.

Table VII - Benzene in exhaled air

Benzene in exhaled air (ppm)								
Number of subjects	Benzene conc. (ppm)	Duration of expos. (h)	Total expos. (ppm x h)	During exposure	After exposure		Remarks	References
				Conc.	Time	Conc.		
8	—	—	Control	0 013	—	0 013	Field study on workers (physical activity)	Berlin et al , 1975
10	—	—	1 31	—	Variable	0 04		
					16 h	0 03		
10	—	—	10.20	—	End expos	0 43		
					16 h	0.11		
10	—	—	0 5	—	End expos	0.08		
					16 h	0.04		
1	25	4 5	115	—	End expos.	2.0	Sedentary subject	Sherwood and Carter, 1970
					16 h	0 2		
—	—	—	200	—	16 h	0 3	—	Barretta +
—	—	—	182	—	16 h	0 13	—	Blair +
10	1 44	0 7	1	—	End of expos	0 41	Field study	Parkinson, 1975
2	2 0	—	9 4	—	End of expos.	0.16	Field study	Sherwood, 1972b
1	20 0	—	114	—	End expos	0 84	Field study	Sherwood, 1972b
					16 h	0 19		
3	26 5-28 5	6	159-171	—	17 h	0 06-0 34	Volunteer study	Hunter and Blair, 1972
1	24 6	3-4	73 8-98 4	—	End of expos	12 3-13 5	Volunteer study	Hunter and Blair, 1972
1	—	—	100	—	16 h	0 15	Sedentary subject	Sherwood, 1976

+ = Cited by Sherwood and Carter, 1970

For routine monitoring, Sherwood (1971) has proposed taking samples at the end of the work shift and analysing them promptly. Any follow-up samples needed can then be taken before the next shift commences, which will allow a better estimation of the integrated exposure during the preceding day.

Sherwood and Carter (1970) have also indicated that if the longer half-life of one day (slow phase of elimination) is confirmed, some accumulation of benzene may occur over a working week when exposure is repeated. According to their data, after 5 days sedentary exposure to 115 ppm x h/day, concentration in breath on the morning of the sixth day would be twice that of the second morning, that is, about 0.4-0.3 ppm.

Sherwood (1976) has, also reported that ethyl alcohol could accelerate benzene elimination in exhaled air and as phenol in urine. The same author has also found a good correlation (0.75 to 0.94) between phenol in urine and benzene in breath for the period of 20 hours following the end of the exposure.

In summary, determination of benzene in exhaled air is certainly a valuable method of confirming exposure to benzene. It is highly sensitive and more specific than phenol determination. It may, however, be relevant to stress that benzene is present in very high concentrations in cigarette smoke (47 to 64 ppm) (Egle and Gochberg, 1976; Newsone et al., 1965). Therefore, although determination of benzene in exhaled air is specific, its detection does not necessarily imply exposure to chemicals containing benzene. This may explain the results of Berlin et al. (1975), who found benzene in exhaled air of control workers (although the authors suggest that it mainly derives from motor fuel).

More experimental work is required to validate the correlation between integrated exposure to benzene (ppm x h) and its concentration in exhaled air on the morning following exposure.

Concentration of Benzene in Blood

The measurement of benzene in blood has rarely been studied as a method of evaluating exposure (Truhaut, 1968). It is likely, however, that benzene in blood follows the same pattern of change as that in breath, with which it is in equilibrium.

Sato et al. (1975) exposed three volunteers to 25 ppm benzene for two hours. At the end of the exposure period benzene in blood was approximately 200 µg/l and decreased up to about 10 µg/l 5 hours after the end of exposure. Gas chromatography techniques have been reported for detecting benzene concentration in blood (Angerer et al., 1973; Sato et al., 1975; Snyder et al., 1975; Szadkowski et al., 1971; Withey and Martin, 1974).

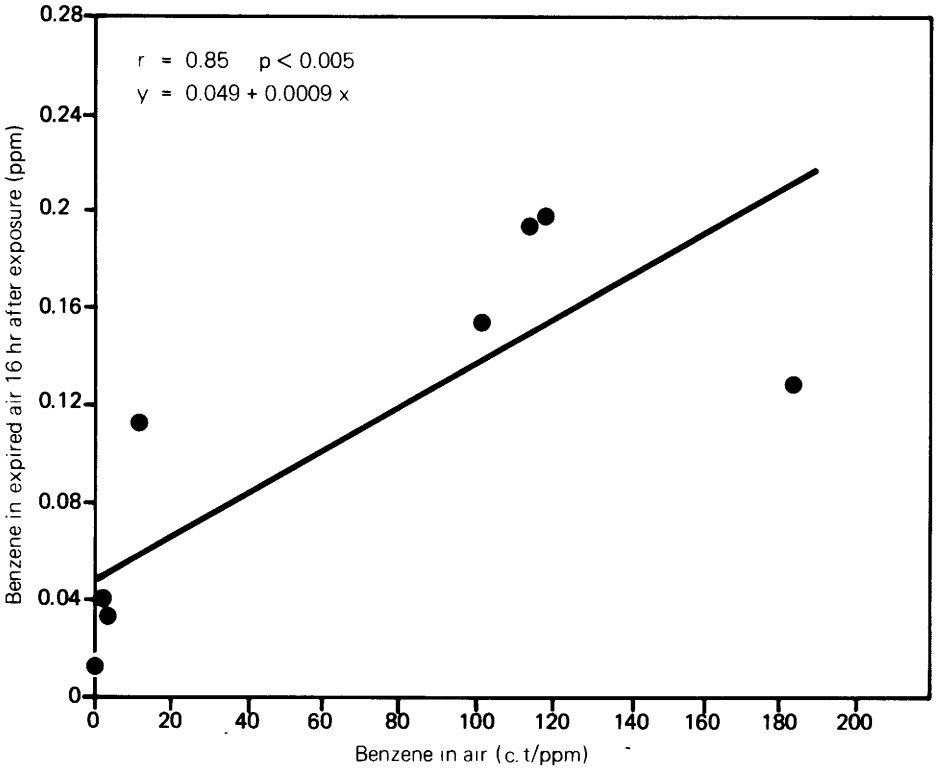

Figure 5 - Relationship between benzene exposure and benzene concentration in exaled air 16 hr after the end of exposure

Cytogenetic Studies

Several cytogenetic studies have confirmed the mutagenicity of benzene. Berlin et al. (1975) have recently investigated the frequency of chromosome aberrations in peripheral lymphocytes of workers currently exposed to benzene. Their results are summarized in Table 8.

The road tanker drivers (current exposure 1.31 ppm) show a greater proportion of chromosome damage than do individuals from the other two occupational groups. Ship tanker crews who sustain the highest current exposure do not, however, have such high levels of chromosome damages. The authors stress the point that these workers are young and the labour turnover in these coastal tankers is high. According to the authors, the highest long term integrated exposure is to be found among road tanker drivers. The authors recognize that they cannot conclude with certainty that the chromosome damage was caused by benzene exposure, since petrol contains many other components that could conceivably give rise to such damage. Previous cytogenetic studies have demonstrated that benzene can cause chromosomal aberrations in man, but the integrated exposure of the workers could not be estimated in any of them. The results of these cytogenetic studies are summarized in Table 9.

At present, cytogenetic studies are possibly useful in confirming previous exposure to benzene if the influence of other physical and chemical agents on chromosomes is also taken into account. It would be interesting to know whether chromosomal aberrations are precursor signs of haematological changes and whether individual

susceptibility plays a role in the development of these chromosomal changes. In the latter case, such tests would have a place in the routine screening of workers to benzene.

Table VIII - Frequency of chromosome aberrations in peripheral lymphocytes

Group	No.	Mean age	Estimation of current exposure to benzene (C.t.) (ppm)	% of cells with chromosome and chromatid-type aberrations	% of cells with chromosome type aberrations
Road tanker drivers	11	45.1	1.31	11.5 +	2.2 +
Ship tanker crews	9	30.7	10.20	5.3	1.0
Petrol station staff	9	42.5	0.5	6.1	0.4

+ = significantly different from the two other groups

From Berlin et al , 1975

Table IX - Cytogenetic studies

Group	No.	Age range (yr)	% of cells with chromosome aberrations	% of cells with chromosome and chromatid aberrations	References
Workers examined 2 yr after exposure to benzene for 1 - 20 yr	20	25 - 64	2 5		Though et al , 1970
On site controls	5		1.0		
Other controls	38		1 4		
Workers examined 14 - 15 yr after exposure to benzene for 1 - 22 yr	10	36 - 54	2.28		Forni et al , 1971a
Controls	34		0.7		
Workers studied 1 - 18 yr after recovery from benzene poisoning	25	21 - 61	3 11		Forni et al., 1971b
Controls	25		0 53		
Refinery workers exposed to benzene for 3 to 7 yr	9			10 4	Hartwich and Schwanitz, 1972
Controls (blood donors)				5 1	
Road tanker drivers	11	27 - 64	2.2	11 5	Berlin et al., 1975
Ship tanker crews	9	17 - 62	0.2	5 3	
Petrol station staff	9	26 - 65	0.2	6 1	

Peripheral Blood Examination and Other Biological Tests

It is known that periodic haematologic testing may fail to reveal chronic benzene poisoning at a stage when the damage done to the blood producing organs is still reversible (Van Haaften and Sie, 1965). Furthermore, there is no quantitative relationship between intensity of exposure and response. Thus these tests can only be used to detect a lesion in exposed workers, but cannot be considered as sensitive enough for the early detection of excessive exposure.

Girard et al. (1970) have reported that benzene and toluene exposure reduces leukocyte alkaline phosphatase activity. A reduction of the plasma levels of the immunoglobulins IgG and IgA and an increase in the level of IgM have also been found in workers exposed to benzene (Lange et al., 1973). The specificity and the sensitivity of these tests are, however, unknown. Presently they cannot be proposed for screening workers potentially exposed to benzene.

Conclusions

Among the tests which have been proposed for evaluating current exposure to benzene, two appear to have some practical application: determination of the total phenol concentration in urine and measurement of the benzene concentration in exhaled air.

The limitations of these tests must, however, be kept in mind. When phenol is measured with a specific method, any value exceeding 20 mg/l, for example in urine collected at the end of the work shift, indicates exposure to benzene. That is, if other causes of increased phenol excretion have been excluded.

In workers with normally low urinary phenol excretion, slight exposure to benzene (C.t. less than 10 ppm x h) may not be detected by measuring phenol in a postshift urine sample alone. Increased sensitivity can be obtained by comparing the concentration of phenol in pre- and postshift urine samples.

When benzene exposure is very low (C.t. less than 1 ppm, i.e. 0.15 ppm for 8 hours), this technique is not sensitive enough for confirming exposure. For integrated exposure exceeding 10 ppm x h (C.t.), the correlation between phenol excretion and benzene exposure is only valid on a group basis. It has been estimated that in a group of workers exposed to 10 ppm benzene for 8 hours, the mean phenol concentration in urine samples collected at the end of the exposure period would amount to 45-50 mg/l (S.G. 1.016).

The determination of benzene in exhaled breath is a very specific and sensitive method for confirming exposure to benzene. However, not enough data exists to correlate benzene concentration in exhaled air with integrated exposure. It must also be kept in mind that since benzene is present in cigarette smoke, its detection in exhaled breath does not necessarily imply occupational exposure to benzene.

Nevertheless, a benzene concentration in breath around 0.12 ppm, detected 16 hours after the end of exposure, is proposed as a tentative biological threshold resulting from an 8 hour exposure to 10 ppm benzene.

Peripheral blood analysis of workers exposed to benzene is not useful as a test of exposure, but can be used to detect those who might be particularly susceptible to the myelotoxic action of benzene.

Further investigations are required to define the usefulness of cytogenetic investigations in the routine control of workers exposed to benzene.

Research Needs

The following are recommendations for further research :

- Further investigation of the possibility of determining benzene concentration in exhaled air for evaluating the intensity of exposure;
- Epidemiological studies to determine the level of long-term exposure to benzene not associated with chromosomal aberration and evaluation of these changes as possible precursor signs of haematological disorders;
- Study of individual variability in susceptibility to benzene to help identify subjects potentially more at risk when exposed;
- Investigation of the potential action of benzene on the immunological defences of the organism with a view to the development of biological tests for the early detection of the toxic action of benzene;
- Basic investigation of the interaction of benzene metabolites with critical cellular sites as relevant for the detection of excessive exposure.

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Human biological monitoring of industrial chemicals series

Cadmium

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Summary

Cadmium is a metal widely used in industry. In human exposure the two main target organs are the kidney and the lung. Still uncertain is the carcinogenic effect.

The main route of absorption in occupational exposure is the respiratory tract. Cadmium is retained in the body, having a calculated half-life of 10-20 years. It is principally excreted with urine.

For the biological monitoring of cadmium-exposed subjects both indicators of internal dose and indicators of effect are available.

Cadmium concentration in urine mainly reflects body burden at low exposure conditions and in absence of renal damage. Cadmium concentration in blood mainly reflects recent exposure. The most sensitive indicators of effect are urinary $beta_2$-microglobulin and retinol-binding protein, which enable detection of early tubular proteinuria. Electrophoresis of urinary proteins is also useful, because it permits distinguishing between proteinuria of tubular and of glomerular type, the latter has been also described in cadmium-exposed subjects. Therefore also the quantitative determination of high molecular weight proteins, such as albumin and transferrin, may be useful. It is uncertain if the above mentioned indicators of effect are sufficient to detect a renal damage, which is still reversible.

As an "operative limit value" for CdU 10 μg/g creat. has been suggested in order to prevent reaching critical concentration in kidney cortex.
No sufficient data are at present available for setting limit values for CdB and for the various indicators of effect.

Abbreviations

CdU	cadmium concentration in urine
CdB	cadmium concentration in blood
CdA	cadmium concentration in air
TCA	trichloroacetic acid
SSA	sulphosalicilic acid
$beta_2$-MG	$beta_2$ microglobulin
RBP	retinol-binding protein
MT	metallothionein

Cadmium

Chemical and physical properties

Cadmium is a silver-white, soft metal, symbol Cd, atomic number 48, atomic weight 112.4, specific gravity 8.6, melting point 320.9°C, boiling point 765°C.
Starting from temperatures of 400-500°C there are considerable vapour losses with the consequent formation of cadmium oxide. Cadmium has a valence +2. Of the many inorganic cadmium compounds, several are quite soluble in water, e.g. fluoride, chloride, acetate and sulphate, whereas oxide, sulphide and carbonate are insoluble. Cadmium also forms organic compounds, but these are rather unstable in environmental conditions.
Cadmium is found in nature mainly together with zinc in the form of sulphide deposits (greenockite); cadmium-zinc ratio in these minerals ranges from 1:100 to 1:1000.
Cadmium production is constantly growing; in Europe it has risen from 216,900 tons in 1966 to 585,000 tons in 1977; and consumption also, though considering trade fluctuations, has an increasing trend (Stubbs, 1979).

The framework Directive of EEC (November 1980) considers cadmium as a toxic agent from the workplace view point, and the Commission will prepare an individual directive.

Chronic effects in humans

The lungs and kidneys are the two target organs after long-term exposure to cadmium (Friberg, 1950).
However, data concerning occupationally exposed workers, as well as the general population of cadmium polluted areas suggest that the kidney is the "critical organ" (Nordberg, 1976).
Kidney damage is classically characterized by a tubular proteinuria (Friberg, 1948). There is an increased urinary excretion of low molecular weight proteins, coming from the plasma, due to an impaired reabsorption (Piscator, 1961). More recent investigations indicate that the cadmium proteinuria may consist not only in the tubular proteinuria, but also in a proteinuria of glomerular type (Bernard et al., 1976).

The literature mainly reports cadmium toxicity as follows: cadmium bound with metallothionein is filtered through the glomeruli. Protein is normally reabsorbed in the proximal tubules and metabolized, and the absorbed metal is bound in the tubular cells with other metallothionein. When more cadmium accumulates in the kidney than can be bound by metallothionein, cadmium then exchanges with zinc in enzymes involved in the reabsorption and catabolism of proteins; this may cause tubular proteinuria and tubular damage (Nordberg, 1972; Friberg et al., 1974).

In intense and prolonged exposure conditions, lung disturbances may occur. Early investigations reported emphysema (Baader, 1951; Kazantzis et al., 1963); in more recent studies only a mild obstructive syndrome has been found (Materne et al., 1975; Stanescu et al., 1977).

Cadmium can provoke bone lesions which are similar to osteomalacia; it is yet to be demonstrated whether they are directly caused by the action of the metal on the bone tissue, or secondary to the renal damage possibly associated with a disturbance of vitamin-D metabolism (Lauwerys, 1978).

The renal disturbances may also affect the handling of calcium and phosphorus with possible formations of stones (Way-Yee Chan and Rennert, 1981).
In workers excessively exposed to cadmium, slight hypochromic anemia (Tsushiya, 1967), modest functional liver disturbances (Friberg, 1950), and anosmia (Adams and Crabtree, 1966) have been described. Actually the hypertensive action of cadmium in humans is still controversial, and further studies are required (Friberg, 1979).

A carcinogenic effect of cadmium was first suspected in 1965 after the report of 5 cancer cases in 74 cadmium exposed workers; 3 were prostate cancers (Potts, 1965).
On the basis of the few available data, IARC (1978) concluded that "occupational exposure to cadmium in some form (possibly the oxide) increases the risk of prostate cancer in man. In addition, one of these studies suggests an increase of respiratory tract cancer".
According to Piscator (1981a) the role of cadmium in the development of cancer of the prostate is still controversial, also with regard to the complex ethiology of this neoplasm, and further investigations are necessary, however.

Metabolism

Absorption

The respiratory and gastrointestinal tracts are the two main routes of absorption of cadmium in man. Percutaneous absorption is practically negligible.
For the respiratory route in test animals, an absorption ranging from 10% to 40% of the inhaled cadmium dose has been observed. The variation depends upon the particle size and the chemical form of cadmium. For example, cadmium oxide and cadmium chloride are more readily absorbed than cadmium sulphide (Poots et al., 1950; Princi and Geever, 1950).
There are no human data for the respiratory absorption of cadmium in working conditions. Considering the cadmium concentration in cigarettes and autopsy data of smokers, an absorption rate of 25% - 50% has been calculated (Lewis et al., 1972; Friberg et al., 1974; Elinder et al., 1976).
Gastrointestinal absorption in test animals is lower than 10% of the oral dose, and is about 2% in many cases. The absorption is considerably increased when there is calcium, protein, iron or zinc deficiency (Suzuki et al., 1969; Hamilton and Valberg, 1974; Spivey Fox et al., 1979).
The absorption is higher in young rats and mice than in adults (Nordberg, 1975; Kostial et al., 1979).
In humans the gastrointestinal absorption rate varies from 1.5% to 29% (Nomiyama, 1980), and is also influenced by the amount of the iron depots (Flanagan et al., 1978).

Distribution

Blood

After absorption cadmium is transported through the organism by the blood circulation.
In rats, after a single injection, cadmium is found mainly in the plasma and it undergoes a rapid clearance (Shaikh and Lucis, 1972). After repeated injections in rabbits the level of cadmium in blood increases progressively until a ceiling is reached (Friberg, 1955); in this situation cadmium is contained prevalently in the erythrocytes (Truhaut and Boudene, 1954).
In man blood cadmium is found mainly in the erythrocytes (Szadkowsky, 1972; Wilden, 1973). However, the ratio between cadmium in plasma and cadmium in red blood cells decreases with the rising of the concentration in whole blood; in cadmium exposed workers Bernard et al. (1977) have found 13% of the circulating cadmium in plasma against 31% in the control group.
Most of the erythrocytic cadmium is probably bound to metallothionein (Nordberg, 1972).
More details on the significance of cadmium in blood in man will be given in the paragraph "Indicators of Internal Dose".

Tissues

Cadmium accumulates in the body, and the body burden in non-occupationally exposed subjects in Europe and North America has been estimated as 9.5 mg - 40 mg (Lauwerys, 1978).
Cadmium deposit increases with age and is greater in smokers (Lauwerys, 1979).
The newborn tissues are almost free of cadmium, due to the barrier role of the placenta (Roels et al., 1978). However, in blood and urine the metal is always present (Baglan et al., 1974; Odone et al., 1982).

a) Liver and kidneys

In chronic exposure experiments in animals about 75% of cadmium injected or absorbed by the intestine is found in liver and kidney (Friberg, 1979).

In humans, after long-term low-level exposure, about 40% - 80% of the retained cadmium is found in the liver and kidneys, and about one third in the kidney alone (Friberg, 1974). In these organs, cadmium is mainly bound to metallothionein.

Metallothionein is a low molecular weight protein (PM 10,000 - 12,000) with high cysteine residues content and deficient in aromatic amino acids (Kagi and Vallee, 1961; Margoshes and Vallee, 1957).
In man, metallothionein has been found not only in liver, kidneys and erythrocytes, but also in heart, brain, testis and skin epithelial cells (Lucis et al., 1970). In animals, the protein has been detected also in placenta, spleen, and in intestinal mucosa (Wolkowsky, 1974, Amacher and Ewing, 1975). Not only cadmium but other metals, in particular Zn, Cu, Hg, Ag, Sn can bind metallothionein in vivo (Sabbioni and Marafante, 1975a). Cadmium can induce synthesis of metallothionein in kidneys, liver, and intestine (Piotrowsky et al., 1974; Sabbioni and Marafante, 1975b; Sugawara, 1975; Bryan et al., 1979). In lungs and kidneys of rats metallothionein synthesis is stimulated also by zinc (Oberdoerster and Kördel, 1981). Metallothionein is filtered through the glomeruli and then reabsorbed in the proximal tubules.
After a single injection of $CdCl_2$ in mice, the greatest concentration is found in the liver and a small amount in the kidney. On the contrary, after administration of cadmium-thionein a higher concentration is measured in the kidney than in the liver (Nordberg et al., 1975)

Cadmium level in the kidney and in particular in the renal cortex increases progressively with age (Friberg et al., 1974). This depends not only from the amount of the metal chronically absorbed, but also from the probable transfer of cadmium from other tissues to the kidney (Friberg, 1979).
It has been observed that in non occupationally exposed subjects when they reach 50-60 years, the level of cadmium in the kidneys tends to diminish (Schroeder et al., 1967; Piscator and Lind, 1972; Miller et al., 1976; Kowal et al., 1979). Many factors may have contributed in determining this situation, in particular, the occurrence of age-dependent renal changes (Travis and Maddock, 1980) and the increased exposure of the population to cadmium over the last 50 years (Friberg et al., 1974).
Smoking significantly increases the level of cadmium in kidney tissue (Hammer et al., 1973; Elinder et al., 1976; Ellis et al., 1979).
Once renal damage has occurred cadmium excretion rises markedly and the level in the kidney diminishes (Friberg et al., 1971).

b) Lungs

In non-occupationally exposed subjects, the lungs contain about 2% of the cadmium body burden (Schroeder et al., 1967; Sumino et al., 1975).
The concentration of cadmium in this organ appears to increase with age (Tipton and Shafer, 1964; Friberg et al., 1971) and is also affected by smoking (Lewis et al., 1972).

c) Other organs

Cadmium has been found in various other human organs: muscle, testicles, pancreas, etc. (Sumino et al., 1975). In exposed persons the cadmium pancreatic concentration is also increased.
In bone, cadmium concentrations are usually very low. Animal and human observations suggest that cadmium does not easily penetrate into the brain (Sumino et al., 1975).

Excretion

Urinary

The urinary system is the main route of excretion for cadmium. In non-occupationally exposed subjects, daily excretion corresponds to approximately 0.004% - 0.015% of the total body burden (Friberg et al., 1974).
In animals, after a single dose of cadmium, only a small part of the metal is found in the urine (Miller et al., 1969; Lucis et al., 1969). Long-term experiments in rats have demonstrated a good correlation between urinary excretion and body burden of cadmium during the accumulation phase. This phase is followed by a sharp increase in cadmium urinary excretion which corresponds to the onset of proteinuria, indicating tubular damage (Nordberg, 1972).
The significance of urinary cadmium excretion in humans will also be discussed in the paragraph "Indicators of Internal Dose".

Fecal

In animals the gastrointestinal tract is an important route of elimination of absorbed cadmium. In rabbits and rats before proteinuria occurs, fecal excretion is usually higher than urinary excretion (Axelsson and Piscator, 1966).
No correlation exists between body burden of cadmium and fecal cadmium (Nordberg, 1972).
In humans, fecal excretion of cadmium is suggested as being less than 0.1% (Rahola et al., 1972); it mainly indicates the amount of ingested cadmium.

Other routes

Other routes of excretion are less important than urinary and fecal (CEC, 1977). Cadmium in hair is hardly useful because of the difficulty of distinguishing endogenous cadmium from that deposited on the surface (Nishiyama and Nordberg, 1972).

A recent study reports markedly higher concentrations of cadmium in the saliva of exposed workers as against that of unexposed subjects (Gervais et al., 1981).

Biological half-life

Mathematical models have been developed for evaluating the metabolism of cadmium in man. According to a one-compartment model, which considers only the renal cortex, a half-life of 20 years has been calculated (Friberg, 1979). A more elaborate eight-compartment model gives shorter half-times (8-14 years) for each compartment (Kjellstroem and Nordberg, 1978).

Critical Organ Concentration

The kidney is the critical organ for cadmium, i.e. the organ which first reaches the critical concentration.
Many efforts have been undertaken to determine the critical concentration of cadmium in renal cortex.
Friberg et al. (1974), when relating cadmium concentrations measured in kidney tissue obtained from ***autopsies and biopsies*** of cadmium exposed subjects with clinical findings of tubular disturbances, indicate 200 μg/g wet weight as the critical concentration in kidney.
Experiments in rats and rabbits have shown functional and morphological kidney changes occurring at kidney cortex concentrations of 200-400 μg/g (Bonnell et al., 1960; Axelsson et al., 1968; Stowe et al., 1972). In larger animals, i.e. monkeys, a critical concentration of 380-470 μg/g has been found (Nomiyama, 1980).
Considering the available data, a WHO group (1977) concluded that in man the critical level may be between 100 and 300 μg/g, with 200 μg/g as the most likely estimate.

Neutron activation analysis permits ***in vivo*** measurement of cadmium in liver and kidney (McLellan et al., 1975). The application of this technique has been recently

enabled the construction of a transportable equipment (Vartsky et al., 1977). Until now, only a few studies on cadmium workers have been carried out.
Roels et al. (1979) have reported their conclusion of a critical concentration lying between 200 and 250 μg/g. In this study no difference was observed in the cumulative frequency distribution of kidney cadmium between normal subjects and subjects with kidney lesions. This fact may be explained with the fall in kidney cadmium concentration after the onset of proteinuria. Moreover in workers with renal damage cadmium concentrations in the liver are markedly higher as against subjects without renal damage. These results have been substantially confirmed by the same authors in a more recent study (Roels et al., 1981).
Much the same observations have been reached by Ellis et al. (1981), but on the basis of their measurements they have indicated a critical level of 300-400 μg/g.

Biological Indicators

Indicators of internal dose

Concentration of cadmium in urine

In non-occupationally exposed subjects, the mean urinary excretion - though with some fluctuations - generally does not exceed 1 μg/g creat. when the analysis is made by atomic absorption spectroscopy (Table 1). In the past, when the analyses were conducted with less accurate methods, higher values were reported (Smith and Kench, 1957; Imbus et al., 1963).

Table I - Some examples of urinary cadmium concentration, determined by AAS, in not occupationally exposed subjects

Country	No.	Mean	Range	Unit	Reference
BDR	10	0.98	0.34 - 1.57	μg/24 h	Lehnert et al., 1968
S	10	0.39	0.05 - 0.77	μg/24 h	Linman and Lind [°]
U.S.A. (Chicago)	189	0.64	0.02 - 2.06	μg/l	Kowal et al., 1979
U.S.A. (Dallas)	86	0.59	0.11 - 2.14	μg/l	Kowal et al., 1979
J (Nagasaki)	30	4.8 ± 2.9		μg/l	Tsushiya et al., 1979
J (Akita)	30	6.1 ± 4.2		μg/l	Tsushiya et al., 1979
B	88	0.88	0.77 - 1.93	μg/g creat	Buchet et al., 1980
GB	542	0.52 ± 0.46		μg/l (24 h)	Strehlow and Baltrop, 1981
NL	34	0.48 [°°]	0.21 - 1.06 [°°°]	μg/g creat	Wibowo et al., 1982
I	268	0.75 [°°] 0.56 [°°]	0.10 - 4.50 0.08 - 4.54	μg/l μg/g creat	Alessio et al., 1982

[°] Quoted by Friberg et al., 1971

[°°] Geometric mean

[°°°] 67% range

Values referred for the general population of Japan are higher, probably due to a more intense environmental exposure (Tsushiya, 1978). The urinary excretion of cadmium is affected by tobacco smoke. Higher CdU values are reported in smokers than in non-smokers (Elinder et al., 1978; Kowal et al., 1979; Cohn et al. 1979).

Age also influences the urinary excretion of cadmium. It has been demonstrated that, on a group basis, CdU increases with age (Lauwerys et. al., 1976; Tsushiya, 1978; Elinder et al., 1978; Kowal et al., 1979; Alessio et al., 1982).

Starting from the sixties CdU seems to decrease (Tati et al., 1976). This fact is in accordance with the diminution of cadmium kidney tissue concentration mentioned before (see "Distribution - Tissues").

No statistically significant differences by sex have been reported when urinary cadmium concentration is expressed in μg/l. Adjusting CdU per gram creatinine females present higher values; this is probably due to a difference in urinary creatinine excretion in the two sexes (Kowal et al., 1979; Alessio et al., 1982).

In occupationally exposed subjects, cadmium excretion varies widely in relation to different exposure conditions.
Smith et al. (1980) analysing 27 workers chronically exposed to cadmium have found a significant correlation between the time-weighted cumulative exposure and CdU (Fig. 1).

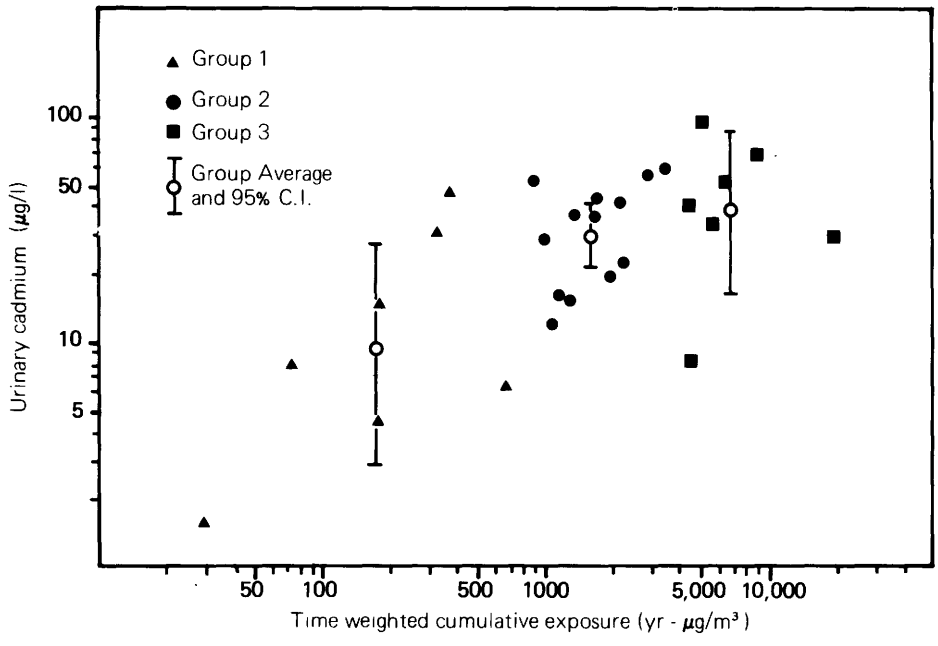

Figure 1 - Association between CdU and Time-Weighted Cumulative Exposure in 3 groups of subjects exposed to increasing cadmium concentrations (from Smith et al., 1980)

Harada (1976) reports that in male and female workers of a battery producing plant, average CdU values tend to increase with the airborne concentration of cadmium. Increase in CdU is more marked among workers with more than 6 months exposure, although increases in CdU are not infrequent even during the first 6 months of exposure (Fig. 2). These discrepancies may be explained by different exposure levels.
Lauwerys et al. (1979a) have followed the behaviour of CdU in 11 cadmium salts factory workers for about a year after the start of an excessive exposure (CdA values 110-2125 μg/m³). They observed three phases in cadmium excretion, i.e. first phase (0-15 days) with a rapid increase of up to about 15 μg/g creat., a second phase (15-120 days) of slower increase followed by another phase of rapid increase. It should be noted that of the examined workers only 4 were newly exposed to cadmium.
The same research group has made a study on about 200 workers, subdivided as subjects with "low exposure" and those with "high exposure". On the whole, the airborne cadmium concentration was usually below 90 μ/m³. In the first group CdU and duration of employment were significantly correlated, and no correlation was found between CdU and CdB. In the second group, CdU and duration of employment were not correlated, while a significant correlation was present between CdU and CdB. The workers of both groups all had a normal electrophoretic pattern of urinary proteins (Lauwerys et al., 1976).

They also report a slower decrease of CdU than CdB in relation to the decrease of exposure.
In retired workers the persistence of rather high CdU levels has been observed and a correlation between CdU levels and length of past cadmium exposure has been found (Odone et al., 1982b)).

Neutron activation has permitted to couple the in vivo measurement of cadmium in kidney with CdU. In 221 workers without signs of kidney disfunction and with cadmium kidney cortex concentrations below the indicated critical level of 200-250 μg/g, a good correlation was found between CdU and cadmium in kidney cortex (Fig. 3). The critical

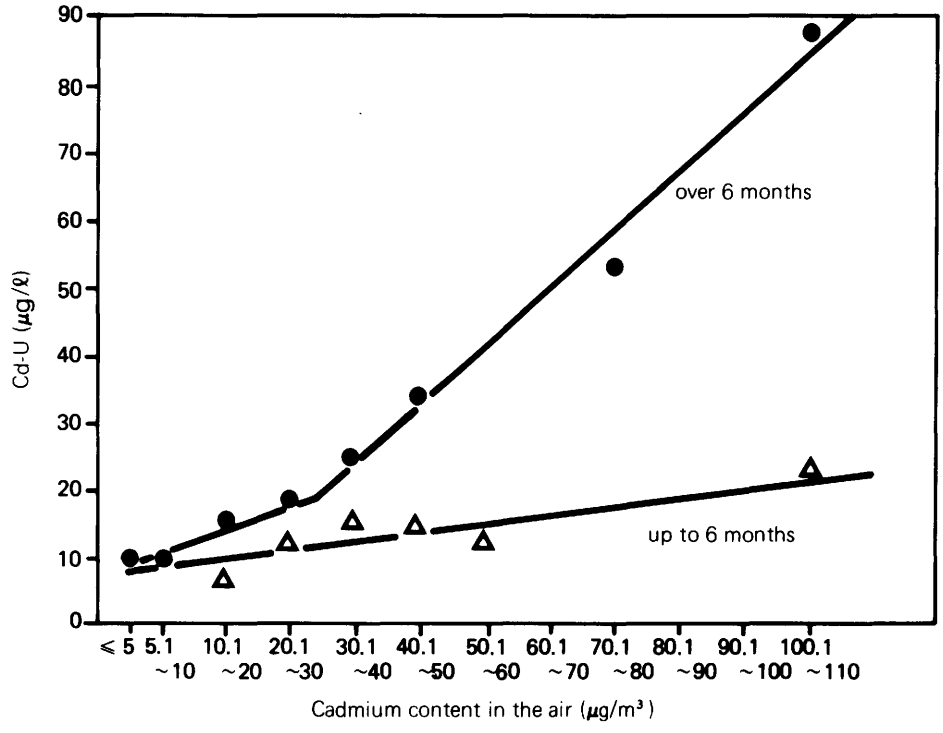

Figure 2 - Relationship between cadmium concentration in air and CdU in workers with up to 6 months and more than 6 months of exposure (from Harada, 1976)

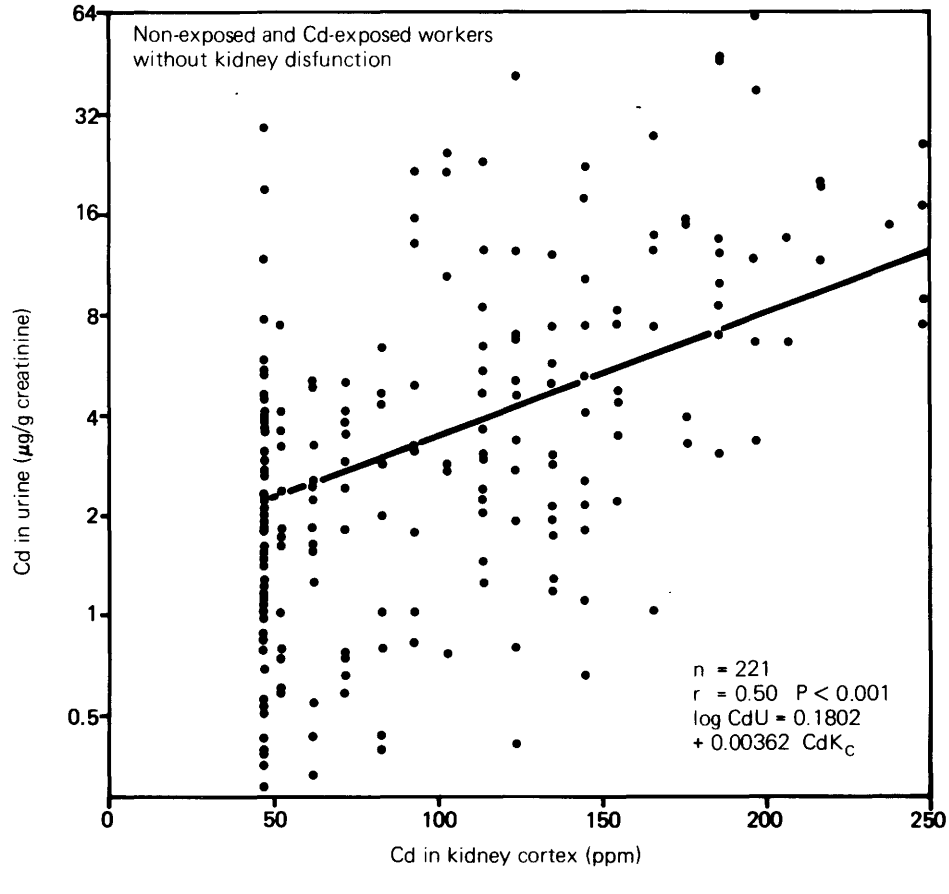

Figure 3 - Relationship between CdU and Cadmium in kidney cortex in subjects without kidney disfunction (from Lauwerys et al., 1979 b)

level of 200-250 μg/g in kidney cortex corresponds to a CdU level of 10-15 μg/g creat. (Roels et al., 1979).

Considering the data reported above, it can be concluded that, in low exposure conditions, with kidney cadmium concentrations below the critical level, and in general in absence of signs of kidney impairment, CdU mainly reflects cadmium body burden. On the other hand, in high exposure conditions CdU is prevalently influenced by current exposure.

Concentration of cadmium in blood

In non occupationally exposed subjects mean cadmium blood concentrations usually do not exceed 0.5 μg/100 ml (Table II). The wide range among the reported values is probably mainly determined by the presence of smokers in the considered groups. It has been widely demonstrated that smokers have significantly higher CdB values than non smokers (Ulander and Axelsson, 1974; Beevers et al., 1976; Kowal et al., 1979; Gervais et al., 1981; Morgan et al., 1981). CdB values increase with the number of cigarettes smoked (Zielhuis et al., 1977). Smoking less than 10 cigarettes is enough for a significant rise in CdB concentration (Alessio et al., 1982). Kowal et al. (1979) have also observed that CdB values are significantly higher in present smokers than in former smokers.

Table II - Some examples of blood cadmium concentration, determined by AAS, in not occupationally exposed subjects

Country	No.	Mean μg/100 ml	Range	References
U.S.A.	243	0.5	0.5 - 14.2	Kubota et al., 1968
BDR	18	0.35		Lehnert [o]
S	150	0.2		Piscator [o]
BDR	>500	0.15	0.03 - 0.8	Stoeppler and Brandt, 1978
U.S.A. (Colorado)	105	0.12	0.04 - 0.69	Wysowski et al., 1978
U.S.A. (Chicago)	168	0.11	0.02 - 0.33	Kowal et al., 1979
B	88	0.23	0.02 - 1.16	Buchet et al., 1980
NL	34	0.26 [oo]	1.81 - 3.95 [ooo]	Wibowo et al., 1982
I	268	0.07 [oo]	0.01 - 0.32	Alessio et al., 1982

[o] Quoted by Friberg et al., 1971

[oo] Geometric mean

[ooo] 67% range

In smokers a modest but significant correlation has been found between CdB and CdU, no correlation in non smokers (Alessio et al., 1982).
Cadmium blood concentrations are not influenced by age. No differences have been reported by sex (Kowal et al., 1979; Alessio et al., 1982).

In occupationally exposed subjects cadmium levels in blood are considerably higher than in the general population (Lauwerys, 1978). Higher CdB values have been found in workers with elevated exposure against to workers with lower exposure (Piscator, 1974; Lauwerys et al., 1976). According to Harada et al. (1979) CdB undergoes a relatively linear increase in proportion to the airborne cadmium concentration.
After the start of cadmium exposure CdB increases straight up to 120 days and then levels off (Lauwerys et al., 1979a). Kjellstroem (1979), following 17 newly employed workers for 1 year, exposed to an average Cd concentration of 50 μg/m³, found nearly the same behaviour for CdB, but no significant increase of CdU was noticed. This is shown for one worker in Fig. 4; also all the other workers had a similar pattern.
No correlation has been found between CdB and length of exposure, independently of the exposure degree (Friberg et al., 1974; Lauwerys et al., 1976).
After cessation of exposure, CdB - though diminishing - may remain above normal values for a long period (Tsushiya, 1969; Friberg et al., 1971). But as previously referred, it has a more rapid decrease than CdU (Harada et al., 1979).

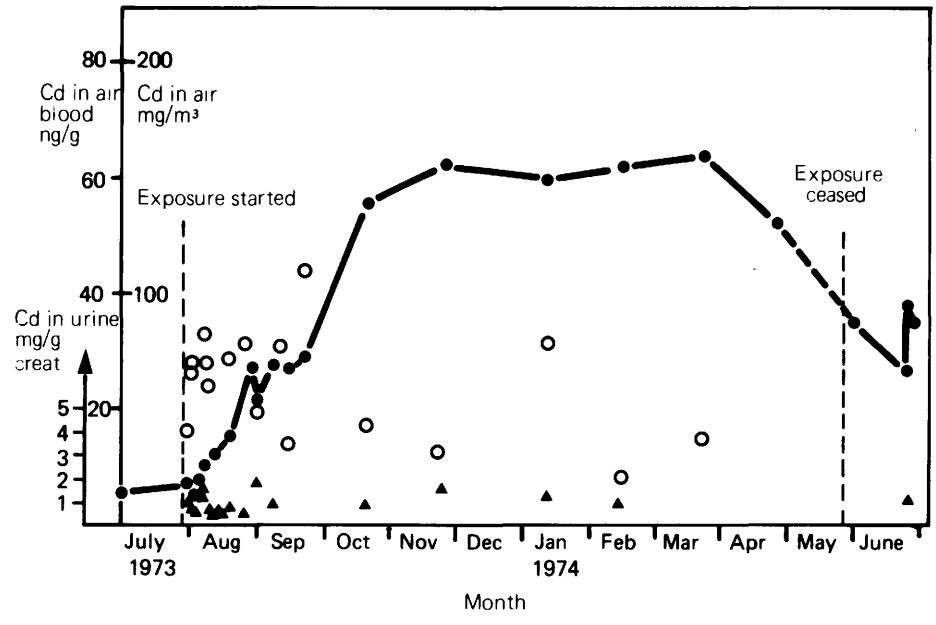

Figure 4 - Cadmium concentration in air (o), blood (●), and urine (▲) of a worker during the first year of exposure (from Kjellstroem, 1979)

The observation that in currently exposed workers CdB appears to be unrelated with the level of cadmium in the organs (Friberg et al., 1971; Nordberg, 1976) seems to be supported by recent results obtained with the new atomic absorption technique; examining 72 cadmium exposed workers, a rather weak correlation has been found between CdB and cadmium concentration in kidney cortex (Lauwerys et al., 1979b).
Finally, according to Piscator (1979) it can be suggested that CdB should be mainly considered as an indicator of recent exposure.

Indicators of effect - Proteinuria

As previously mentioned, the most typical feature of long-term exposure to cadmium is proteinuria (Friberg, 1950).
Proteinuria is mainly represented by low molecular weight proteins and therefore called "tubular proteinuria" (Butler and Flynn, 1958; Piscator, 1962).

Qualitative-quantitative determinations

Because of the prevalence of low molecular weight proteins, tubular proteinuria is not detected with the classic boiling or citric acid tests (Baader, 1951). A qualitative determination is possible using the TCA test or the SSA test, but these methods are not sensitive enough and permit only the detection of a fully developed proteinuria (Friberg, 1979).
Also, the quantitative determination of urinary proteins with turbidimetric tests or with the Tsushiya-biuret test is not always sufficient, since the excretion of some low molecular weight proteins might be increased considerably, while the total proteinuria is still within the normal range (Piscator, 1972).
Using the above methods, a correlation between proteinuria and length of exposure has been reported (Tsushiya, 1967).
Once established, proteinuria persists after cessation of exposure (Piscator, 1962); it can also appear many years after exposure has ceased (Bonnell et al., 1959; Friberg et al., 1971). On the other hand, it has been reported that proteinuria can be reversible in workers no longer exposed to cadmium (Tsushiya, 1976).
The onset of proteinuria depends on the degree of exposure. Kjellstroem (1976) analysing the studies on proteinuria among cadmium exposed subjects published up to 1976, has concluded that tubular proteinuria may develop after 10-20 years of exposure to less than 50 $\mu g/m^3$ CdA.

Low molecular weight proteins - tubular proteinuria

Quantitative determinations of low molecular weight proteins have been performed in cadmium exposed subjects.

For routine controls of cadmium exposed subjects ***beta$_2$-microglobulin***, among the urinary low modular weight proteins, is at present the most widely used.

Beta$_2$-MG (PM 11,800) was first isolated in urine by Berggard and Bearn in 1968.
The urinary excretion increases markedly in cases of tubular disfunction, i.e. not only in cadmium poisoning, but also in Franconi's syndrome, Wilson's disease, Balkan nephropathy, and in general in any disease involving the immunitary system. In the past, beta$_2$-MG was determined with immunodiffusion, recently a more sensitive radio-immunoassay has been developed (Evrin et al., 1971). As to analysis, it has to be noticed, that if urine pH is below 5.5, beta$_2$-MG may be degraded (Evrin and Wibell, 1972).
Average values of beta$_2$-MG in urine in non-exposed subjects are usually below 100 µg/l, but discrepancies exist between the reported "upper normal values" (Table III). This indicates an opportunity of establishing preliminarly a reference group, before studying exposed subjects.

Table III - Some examples of urinary beta$_2$ microglobulin concentration, determined by RIA, in not occupationally exposed subjects

Country	No.	Age	Geometric mean	"UPPER LIMIT" 95% tolerance interval	Reference
S	87 ♂	44.1 °	84 µg/l p.s. 1023	290	Kjellstroem et al., 1977a
J	40 ♀	51 - 60	61.9 µg/g creat	690	Kjellstroem et al., 1977b
J	93 35 ♂ 58 ♀	50 - 69	86 µg/l	700	Kojima et al., 1977
B	87 ♂	20 - 65	71 µg/g creat °°	200	Buchet et al., 1980
GB	542 ♂+♀	Adults	57 µg/l °°	167	Strehlow and Baltrop, 1981
GB	203 ♂	18 - 55	76 µg/l	441	Stewart et al., 1981
NL	34 ♂	20 - 60	79 µg/g creat	161 °°°	Wibowo et al., 1982
I	102 ♂+♀	38.3 °°	67 µg/g creat 86.2 µg/l	250 300	Alessio et al., 1982

° Geometric mean

°° Arithmetic mean

°°° Upper 67% range

In non occupationally exposed subjects the increase of beta$_2$-MG in urine is associated with age (Kitamura, 1976; Tsushiya et al., 1979). Data are too scarcely available to draw any conclusion on the influence of sex and tobacco smoke on the urinary excretion of beta$_2$-MG in non-exposed individuals. Elevated levels have been reported after physical exercise (Kitamura, 1979).

In cadmium exposed subjects urinary beta$_2$-MG have a higher sensitivity for detecting tubular proteinuria in comparison to the qualitative and quantitative methods for total proteinuria and even to some electrophoretic techniques (Shirohishi et al., 1977). Cadmium exposed workers, as well as Itai-Itai disease patients, often have 100 to 1000 times higher urinary beta$_2$-MG levels above the normal range (Kjellstroem et al., 1977a).
Kjellstroem et al. (1977b) have found that in 186 workers of a battery factory, exposed to an average CdA concentration of 50 µg/m^3, beta$_2$-MG in urine increase with length of

employment (Fig. 5). In these subjects after only 6-12 years employment a major prevalence (19%) of increased urinary $beta_2$-MG, referred to an upper limit of 290 µg/l, was present as against the reference group (3.4%). Smokers had a higher prevalence rate than non smokers.
Similarly, in subjects living in a cadmium polluted area, an increasing prevalence of high urinary $beta_2$-MG levels has been found with increasing residence time (Kjellstroem et al., 1977a; Kojima et al., 1977).
Analysing a group of cadmium workers Smith et al. (1980) have reported an association between time-weighted cumulative exposure and $beta_2$-Hg in urine.

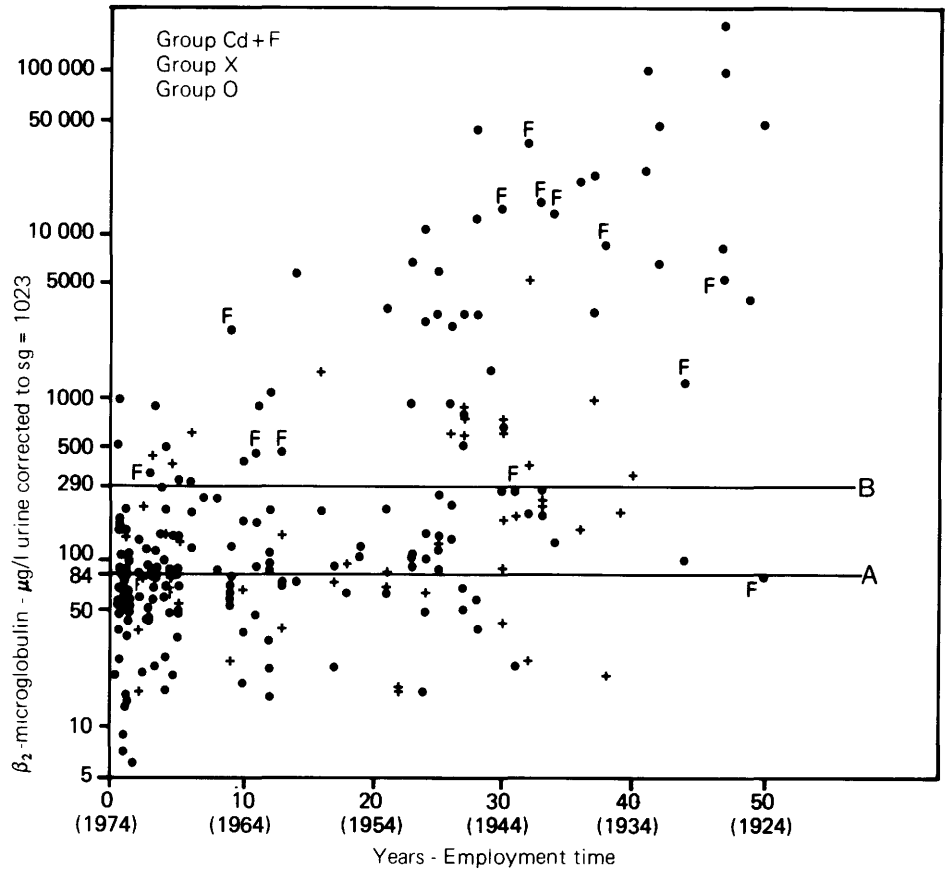

Figure 5 - Relationship between urinary $beta_2$-microglobulin concentrations and employment time
A = geom. aver. in the reference group
B = upper 95 % tolerance level in the reference group (from Kjellstroem et al., 1977 b)

In non occupationally exposed subjects urinary $beta_2$-MG is significantly correlated with both CdU and CdB (Kjellstroem et al., 1977a).
Japanese authors are of the opinion that, in cadmium exposure the rise of urinary $beta_2$-MG may be not due to tubular impairment, but to an increase of serum $beta_2$-MG stimulated by cadmium (Tsushiya et al. 1979; Harada et al., 1979).
On the other hand, Swedish authors affirmed that the increase of $beta_2$-MG in serum is determined by a reduction of the glomerular filtration rate (Kjellstroem and Piscator, 1979; Piscator, 1981).
In workers who have been retired for many years from cadmium exposure, high urinary $beta_2$-MG levels have still been reported (Stewart and Hughes, 1981). Also Tsushiya (1976) referred the persistence of moderately increased urinary $beta_2$-MG levels in workers with past exposure to cadmium, though accompanied by the disappearance of proteinuria. However, more data are required concerning the behaviour of urinary $beta_2$-MG in subjects with past cadmium exposure.

Also the urinary excretion of another low molecular weight protein, the ***retinol-binding protein*** (PM 21,000), increases in cadmium exposure (Kanai et al., 1971). RBP follows the same pattern of excretion than $beta_2$-MG, but offers the advantage of being stable in acid urine (Bernard and Lauwerys, 1981). The development of a simple non isotopic latex immunoassay for the analysis of RBP in urine will probably permit in future a greater diffusion of this test (Bernard et al., 1982).

The main question concerning both $beta_2$-MG as well as RBP is to see if they enable detection of a still reversible renal disfunction. More information in this regard will probably come by coupling the determinations of $beta_2$-MG and RBP with neutron activation measurements of cadmium in the kidney.
Also lysozyme (PM 14,400) and ribonuclease (PM 13,700) undergo a marked urinary excretion in workers with chronic cadmium exposure (Piscator, 1966; Adams et al., 1969).
The appearance of aminoaciduria is considered a late effect of long-term cadmium exposure together with glycosuria and phosphaturia (Piscator, 1966).

As mentioned before, ***metallothionein*** is the main binding protein for cadmium, and therefore has a role in cadmium toxicity. However, until some years ago no reasonably sensitive techniques were available for its determination. Recently a radioimmunoassay with a sufficiently low detection limit has been developed for the analysis of MT in human biological fluids (Chang et al., 1980). Only a few studies have been performed using this test. In 40 subjects with occupational exposure to cadmium of different degree, urinary excretion of MT was augmented in the subjects with higher exposure levels. A good correlation was found both between urinary MT and CdU, urinary MT and CdB. MT concentration in urine was similar in individuals with and without renal damage (Chang et al., 1980).
In subjects with Itai-Itai disease higher urinary MT levels have been found than in control subjects (Tohyama et al., 1981).
The same authors report a significant correlation between urinary MT and liver and kidney cadmium levels determined by neutron activation in workers of a cadmium producing plant.
These data seem to indicate that urinary MT has rather to be considered an indicator of dose than an indicator of effect. But for a correct interpretation of MT in urine more information is necessary, especially in regard to different exposure conditions.

High molecular weight proteins - glomerular proteinuria

Electrophoresis of urinary proteins has to be considered a useful test in cadmium subjects first, because it enables detecting of early renal disturbances, and second, because it permits distinguishing between tubular and glomerular disfunctions.
The most frequent electrophoretic pattern in chronic cadmium poisoning is of tubular type (Piscator, 1962; Adams et al., 1969). However, Belgian authors have found patterns of both tubular and glomerular type; quantitative determinations have demonstrated a significant increase in the urinary excretion of single high molecular weight proteins like albumin and transferrin (Bernard et al., 1976). The tubular and the glomerular alterations can appear associated or independently, but without chronological order (Bernard et al., 1979).

Conclusions

From the foregoing, it is possible to draw some considerations on the significance of the tests at present available for the biological monitoring of cadmium-exposed workers.

The indicators of internal dose — CdU and CdB — are influenced to a different degree by exposure and body burden.
CdU mainly reflects body burden in low exposure conditions and in absence of signs of renal damage. At high exposures CdU is principally affected by current exposure.
CdB may be considered mainly as an indicator of recent exposure than of body burden. However, more information in this regard would derive from the distinction between erythrocyte and serum cadmium.

Considering the indicators of effect, qualitative and quantitative determinations of total proteinuria permit only the demonstration of an already developed renal disfunction.
The determinations of urinary $beta_2$-microglobulin and retinol-binding protein seem to be, until now the most sensitive test for the detection of an early tubular damage. However, uncertainties exist concerning their specificity and their capability of detecting renal impairment in a reversible stage.
The glomerular component of cadmium proteinuria, revealed by electrophoresis of urinary proteins, can be quantified by the determination of single high molecular weight proteins like albumin and transferrin.
The few available data on the urinary excretion of metallothionein in cadmium-exposed subjects seem to indicate that urinary MT has to be regarded as an indicator of dose, but more data are needed.

Considering the relationship between CdU and the critical concentration in the kidney cortex — generally regarded as 200 μg/g — a "limit value" for CdU of 10 μg/g creat. can be suggested. For CdB and the different indicators of effect sufficient information is not yet available to permit the indication of "limit values".

Finally it has to be remarked, that the persistent uncertainty regarding carcinogenic activity of cadmium in man, represents a considerable limitation in the proposal of biological monitoring criteria.

Research Needs

Though much progress has been achieved in the last years, especially performing in vivo measurements of cadmium contents in liver and kidneys, many topics need further investigation for a correct approach to the problem of biological monitoring, namely:

- standardization of analytical techniques for both internal dose and effect indicators
- relationships between external exposure and indicators of dose and effect in different exposure conditions
- relationship between CdB and CdU
- relationship between indicators of internal dose and indicators of effect
- relationship between cadmium concentration in liver and kidneys and indicators of dose and effect in order to establish critical levels for the biological indicators
- research on the reversibility of the proteinuria detected with currently available indicators of effect, especially urinary $beta_2$-MG and urinary RBP
- more information concerning the critical level of cadmium in kidney cortex
- studies on the mechanism of cadmium toxicity in the kidney
- studies defining the tubular or glomerular pattern of cadmium induced proteinuria
- information on absorption, distribution, retention and excretion of cadmium in man
- investigations on the role of metallothionein in cadmium toxicity, and on the possible use of urinary metallothionein as a biological indicator
- finally, studies to answer the most important question concerning the carcinogenic effect of cadmium in man.

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Human biological monitoring of industrial chemicals series

Chlorinated Hydrocarbon Solvents

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Table of contents

Introduction

This monograph is based upon a review submitted by the Coronel Laboratory, Faculty of Medicine, University of Amsterdam, The Netherlands, to CEC in 1976; this has recently been updated to the state of the art of early 1982.

The monograph reviews biological monitoring in occupational exposure to *volatile halogenated hydrocarbons,* i.e. several widely used chlorinated solvents and a few agents applied as refrigerant and blowing agent, pesticide, anaesthetic or plastic monomer. In this respect this monograph differs from most other monographs in this series which usually review biological monitoring methods for one agent only. Only those agents for which at least a few tentative data are available, have been reviewed.

Each review is built up as follows, if feasible:

1. Chemical and physical properties
2. Effects on humans
3. Metabolism
4. Biological indicators
5. Conclusions
6. Research needs

As far as possible the quantitative data on biological monitoring have been presented for three points of time, which are most feasible in practice:

- at $^1/_2$-1 h after exposure: at the end of the working day
- at 16-20 h after exposure: at the following morning before exposure starts
- at 60-64 h after exposure: after a weekend at Monday morning before exposure starts.

These points of time are particularly feasible for biological monitoring of fat-soluble volatile agents. It will be shown that the points of time are crucial for the estimation of total exposure.

This review does not contain all specified information, which is required for adequate biological monitoring. Even for widely studied compounds definite conclusions cannot yet be drawn, particularly because of the following reasons:

- various human volunteer studies applied different designs, measured different parameters, and this again at different points of time
- various industrial studies only measured a few parameters; they often were deficient in exposure data
- differences in analytical techniques lead to different results; particularly the recent application of gaschromatographic methods resulted in more sensitive and reliable data than older analytical methods
- in previous years biological monitoring almost exclusively relied upon analysis of metabolites in urine, whereas levels of agent and/or metabolites in exhaled air or blood may present more reliable biological indicators.

To a large extent there exists a great comparability in analytical gaschromatographic methods for measuring agents or metabolites in biological specimen. Moreover, the same metabolites may occur in exposure to different chlorinated hydrocarbons, e.g. trichloroethanol and trichloroacetic acid, in blood and/or urine in exposure to trichloroethene, tetrachloroethene and 1,1,1-trichloroethane. On the other hand these agents widely differ in the percentage metabolized, e.g. a large procentual biotransformation in the case of trichloroethene and a minor biotransformation in the case of the other two. Dichloromethane (methylene chloride) even is metabolized into the previously unexpected metabolite carbon monoxide. Therefore, in the case of

dichloromethane also the forthcoming monograph on carbon monoxide should be consulted. In the case of monochloromethane a minority of subjects appear to have a poor capability for biotransformation, which may increase their susceptibility.

All agents reviewed act on the nervous system as a narcotic; some solvents have even been widely applied as anaesthetic (particularly trichloroethene). Some agents are exclusively applied as such (halothane, methoxyflurane). Moreover, all liquid compounds have a defattening effect on the skin.

Some agents also exert specific effects. The saturated chlorinated hydrocarbons trichloromethane and tetrachloromethane may exert severe hepatotoxic and nephrotoxic effects. Chloromethane and bromomethane may lead to severely disabling neurologic sequelae. Because of its specific biotransformation dichloromethane acts both as narcotic agent and as precurser of carbonmonoxide (decreased transport and utilisation of oxygen: hypoxia).

A few agents have proved to be an animal carcinogen: trichloromethane and tetrachloromethane, and even a human carcinogen: monochloroethene (vinylchloride).

The gaps in knowledge sometimes are serious; there still is need for research to establish even tentative data. Moreover, in most cases the data available only allow application of the biological exposure data as group average values. There is a great need to establish individual biological exposure data which reliably estimate individual total exposure through respiration and/or dermal absorption, and which also take into account individual variability particularly in fatty mass. In obese subjects the levels of the agent in blood may increase less and more slowly than in lean subjects during exposure; the total amount taken up however, will be larger and consequently after exposure the levels in exhaled air and blood will be higher than in lean subjects, particularly when measured at 16-20 h or at 60-64 h after the end of exposure. Because females usually have twice the relative fatty mass than males, females will follow the trends of obese males. However, data from exposed workers, according to sex and to obesity, are hardly available.

Summary

This monograph reviews data on biological assessment of occupational exposure to the following volatile halogenated hydrocarbons: monochloromethane (methylchloride), monobromomethane (methylbromide), dichloromethane (methylene chloride), trichloromethane (chloroform), tetrachloromethane (carbon tetrachloride), 1,2-dichloroethane (ethylene dichloride), 1,1,1-trichloroethane (methylchloroform), trichloroethene (trichloroethylene), tetrachloroethene (perchloroethylene), monochloroethene (vinylchloride) and two anaesthetics (halothane and methoxyflurane). Most agents are widely used as solvents.

All agents exert non-specific narcotic effects and are defattening the skin; irritation of mucosae is usually weak. Some agents also exert specific hepatotoxic, nephrotoxic or neurotoxic effects. A few agents are established animal carcinogens, and vinylchloride is even an established human carcinogen.

All agents are liposoluble, and most of them undergo biotransformation into metabolites; this allows biological assessment of exposure by measurement of agent and/or metabolites in exhaled air, blood and/or urine. The points of time of sampling are critical for quantitative assessment of total exposure.

Although for the above mentional agents many data are available for only a few substances it is possible to suggest practicable methods for biological assessment of occupational exposure, and only for group average levels. This is particularly true for trichloroethene. For dichloromethane, 1,1,1-trichloroethane and tetrachloroethene tentative group average biological exposure data in relation to exposure levels as occur in occupational practice are suggested. For the other agents too little information is available to suggest more than a possible biological monitoring approach. There is a great need for more human volunteer studies and occupational exposure studies to improve biological assessment of occupational exposure.

Abbreviations

MC	methylchloroform (1,1,1-trichloroethane)
TRI	trichloroethylene (trichloroethene)
PERC	perchloroethylene (tetrachloroethene)
TCE	trichloroethanol
TCA	trichloroacetic acid
TTC	total trichlorocompounds (TCE + TCA)
VC	vinylchloride (monochloro ethene)
COHb	carboxyhaemoglobin
Ci	concentration in inhaled air
Ce	concentration in exhaled (alveolar) air
R	retention $\left(\frac{Ci-Ce}{Ci}\right)$
LC	lungclearance: volume of inspired air cleared from vapour in one minute (minute volume x retention)
ppm	parts per million (10^{-6}) volume/volume
bw	body weight
h	hour
BSP	bromsulphtalein test
CPK	creatinephosphokinase
γ-GT	γ-glutamyl-transpeptidase
LDH	lactic dehydrogenase
SGOT	serum glutamic-oxalacetic transaminase (aspartate aminotransferase)
SGPT	serum glutamic-pyruvate transaminase (alanine aminotransferase)

1. Monochloromethane (Methylchloride)

Chemical and physical properties

physical state	:	colorless gas
molecular weight	:	50.49
melting point	:	—97.7 °C
boiling point	:	—23.7 °C
vapour density	:	1.78
molecular formule	:	CH_3Cl
structural formule	:	H H C H Cl

1 mg/m^3 = 0.48 ppm; 1 ppm = 2.09 mg/m^3 at 20 °C and 760 mm Hg.

Effects on humans

Methylchloride has three physical properties that increase its potential for hazard to workers' health: it is a gas at roomtemperature, it is colorless, and its odor is undetectable at concentrations in air that may already be injurious to health. The nervous system appears to be the critical target system. Symptoms observed are ataxis, staggering gait, weakness, tremor and vertigo; these symptoms may occur after a latency time of 2 to 3 h.

Repko et al (1976) studied the behavioral and neurological effects in 122 workers in several industrial plants of a company using methylchloride as a blowing agent for foam manufacture; 49 non-exposed subjects served as controls. The overall mean concentration was 34 ppm, between departments ranging from mean 8 to 60 ppm. Exposure to methylchloride adversely affected performance in cognitive timesharing tasks and it increased the finger tremor. No relationship was established between exposure and various psychological effects and personality tests employed; no exposure related neurological effects were observed.

Stewart et al (1977) and Hake et al (1977) exposed 10 adult males and 9 adult females to 20, 100 and 150 ppm for periods of 1, 3 and 7.5 h. Physical, neurological, behavioral, clinical and medical studies revealed no deleterious effects of methylchloride exposure.

Putz-Anderson et al (1981) exposed male or female adults to 100 (n = 8) or 200 (n = 24) ppm for 3 h; in addition they received a diazepam dose or a placebo. Diazepam produced a significant 10% impairment in task performance, whereas the effect of 200 ppm methylchloride was only marginally significant (average impairment of 4.5%).

Metabolism

Inhaled methylchloride is easily absorbed and rapidly metabolized. After exposure of volunteers to concentrations up to 150 ppm the mean breath concentrations dropped very rapidly (Stewart et al 1977); methanol was not found in the urine of the volunteers.

In urine samples obtained from persons occupationally exposed to methylchloride

(30-90 ppm) Van Doorn et al (1980) identified S-methylcysteine as a metabolite of methylchloride. No significant increase was found for thioethers. The excretion patterns of S-methylcysteine indicated a relatively long biological half-life for this metabolite (or its percursors). The authors calculated that almost all the retained methylchloride was excreted in urine as S-methylcysteine in 4 of 6 workers; the other two excreted less than 10%.

Stewart et al (1977) and Putz-Anderson et al (1981) also distinguished two groups in exposure of human volunteers to methylchloride (see 3.2.0.). A majority had methylchloride blood and breath concentrations which were two to six times lower than in three of ten males and one of nine females (Stewart et al 1977), and in three of twentyfour subject, respectively (Putz-Anderson et al 1981). These findings strongly suggest the existence of two populations: a minority of « poor converters » with a high body burden of methylchloride and a low excretion of S-methylcysteine and a majority of « converters » with a lower methylchloride body burden and a high percentage of methylchloride excreted as S-methylcysteine. It is not clear whether this difference in metabolism indicates a difference in susceptibility to methylchloride.

Biological indicators of exposure

In order to develop a practical « biologic » test to estimate the magnitude of an industrial exposure volunteers of both sexes were exposed repetitively on a daily basis to methylchloride in concentrations of 20, 100 and 150 ppm for periods of 1, 3 and 7.5 h. (Stewart et al 1977). Blood and breath analysis revealed that the subjects could be divided into two groups: a minority of subjects (type B) with two to six times higher concentration in blood and exhaled air than the majority of the subjects (type A) (see section 3.3.0.). This phenomenon should encourage the use of breath monitoring.

In table 1 the result of the breath analysis are summarized for type A subjects. The alveolar concentrations in type B subjects were 60 to 110% higher than the mean values in type A subjects at zero time, and were three to six fold higher at one h post exposure. For type A subjects one cannot differentiate between 1, 3 and/or 7.5 h exposure from post exposure decay curves. The breath concentrations of the B type subjects were much higher and did not overlap with the values from the type A subjects at 15, 30 and 60 min after exposure.

TABLE 1

Mean concentrations of methylchloride in alveolar air (Ce) after exposure as percentage of the inhaled concentration (Ci) (calculated from Stewart et al 1977).

exposure condition			mean Ce as % of Ci		
Ci	time		time after exposure		
ppm	h	number of times	$^1/_2$ h	1 h	2h
20	$7^1/_2$	4x	1.8	1.0	0.60
100	$7^1/_2$	5x male	2.0	1.2	0.50
100	$7^1/_2$	5x female	2.8	1.4	0.55
100 ± 40	$7^1/_2$	5x fluct., male	1.4	0.8	0.45
150	$7^1/_2$	2x male	2.0	0.9	0.50

The blood concentrations at the end of exposure in type B subjects were about 5 times higher than in type A subjects (about 10 mg/l methylchloride compared to about 2 mg/l after to 3 h exposure to 100 ppm). The results indicate that the higher post exposure breath levels of type B subjects are direct results of higher blood levels.

Repko et al (1976) also measured the concentration of methylchloride in the breath of the exposed workers immediately prior to the termination of the exposure. The mean concentration in breath was 13 ppm, while the mean exposure concentration was 34 ppm.

Use of urinary excretion of S-methylcysteine for assessment of the exposure magnitude is subject to the same problem of « converters » and « poor converters ».

Conclusions

Possibilities for biological monitoring of exposure to methylchloride exist: measurement of methylchloride in exhaled air or in blood, or its metabolite S-methylcysteine in urine. However, a minority of subjects is not or only poorly capable to convert methylchloride. This results in a high body burden of methylchloride in these subjects.

Which of the type of subjects is more susceptable to the toxic effect of exposure to methylchloride, is not clear. Additional studies should be carried out to solve this problem.

Research needs

More studies have to be carried out in exposed workers to establish a relationship between respiratory exposure (concentration and duration), biological indicators of exposure and health effects.

2. Monobromomethane (methylbromide)

Chemical and physical properties

physical state	:	colorless gas
molecular weight	:	94.95
specific gravity	:	1.73 0°/0°C
melting point	:	—93.6 °C
boiling point	:	4.6 °C
vapour density	:	3.27 (air = 1)
molecular formule	:	CH_3Br
structural formule	:	

1 mg/m^3 = 0.257 ppm; 1 ppm = 3.89 mg/m^3 at 25 °C and 760 mm Hg.

Effects on humans

Toxis effects mainly refer to the central nervous system. Van den Oever (1979) reviewed the clinical symptoms: in intensive exposure coma and convulsions may occur, followed by neurological sequelae and pulmonary oedema. In less intensive exposure there usually exists a latency period of one to several hours, before prodromal symptoms develop: headache, nausea, vomiting, drunkenness, drowsiness. This may develop into a neurological syndrome: myoclonic contractions (particularly in hands and face muscles) followed by convulsions, disorientation and maybe coma. Typical cerebellar disturbances may cause adiodochokeinesis, myoclonic contractions. In acute intoxications also hyperthermia, pulmonary oedema and sometimes hepatorenal damage may occur. Dermal contact may cause dermatitis and blistering; exposure of the eyes causes conjunctivitis and keratitis. In intensive exposure death may occur within 24 to 48 h; in survival neurological sequelae may persist. In two fatal cases 92 and 83 mg bromide/l blood have been reported; one case that survived had 69 mg/l (Clarke et al 1945), but 400 mg/l has also been reported: Rathus and Landy, 1961 (survived) and Hine, 1969 (fatal). In 10 of 33 workers Verberk et al (1979) observed slight disturbances of the encephalogram and a small increase of serumtransaminases; the bromide in blood levels (neutronactivation) were 4 to 23 mg/l; in those with eeg-disturbances the geom. average (—SD geom. to + SD geom.) was 10.9 mg/l (6.2-19.2), in the others 8.2 mg/l (5.6-12.0).

Metabolism and biological indicators

Exposure mainly occurs by inhalation; limited dermal absorption may also take place. The whole molecule as such and not Br seems to be the causative agent for health effects; it interferes with energy metabolism and inhibits triosephosphate dehydrogenase; methylbromide also methylates SH-, amino- and carboxylgroups. Shapovalov (1974) observed in exposed workers a decreased concentration of

SH-groups in blood and increased levels of protein, decreased levels of albumin and increased levels of globulins, although Verberk et al (1979) did not observe any effect on the albumin/globulin quotient.

In the body methylbromide is metabolized into bromide; this results in increased bromide levels in blood. In non-exposed workers Hine (1969) found 0.5-2 mg bromide/l blood; in the Netherlands in 1300 non-exposed males (av. 19 yr) 0.2-1.5 mg bromide/l blood was measured (neutronactivation); Bowen (1974) reported 1.3-10 mg/l in blood and 1-14 mg/l in bloodserum. According to Gay (1962) the maximal acceptable bromide in blood level was 50 mg/l. Although a relationship between bromide in blood levels and the health hazard probably exists, the relationship is not yet clear, probably also due to analytical problems (see 4.4.0.) and different times of sampling; often the analytical methods have even not been reported.

The reported bromide in blood levels have not been corrected for exposure to inorganic bromide and, therefore, are as such not per se a good measure for exposure to methylbromide. Methylbromide levels in blood have not been measured.

Bromide levels in urine in non-exposed subjects have been reported to be 3-5 mg/l (Hauck 1968); there are no data on urinary levels in methylbromide exposed workers.

Analytical methods

Various methods exist for measurement of bromide in blood (Hessing 1980):
- goldchloride method; not accurate at levels below 100 mg/l (Rathus and Landy 1961; Greenberg 1971)
- Conway microdiffusion; applicable for levels below 50 mg/l (Rathus and Landy 1961)
- röntgenfluorescence; detection level in urine 0.2 mg/l (Hauck 1968)
- neutronactivation; coefficient of variation 6-12%, low detection level (De Goeiy et al 1976)
- spectrophotometry; its coefficient of variation is considerable at levels below 20 mg bromide/l (Hessing 1980)
- Heuser (1970) described a gaschromatographic method for simultaneous determination of bromide and methylbromide in vegetation.

Conclusion

Measurement of bromide, possibly methylbromide, in blood very probably offers the method of choice in biological monitoring. There is need to improve the quality of analytical methods for bromide and methylbromide in blood. Because insufficient knowledge exists on the relation between methylbromide in blood and health effects, it is not possible to propose a routine biological monitoring program. Simultaneous exposure to other Br-containing compounds, interalia drugs, may confuse interpretation of bromide in blood levels.

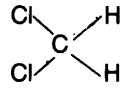
It may be prudent to keep levels below about 15 mg bromide/l blood.

Research needs

There is need to study
- methods to measure bromide and methylbromide in blood
- the halftime of bromide and methylbromide in blood
- the relation between bromide and methylbromide in blood and external exposure
- the relation between bromide and methylbromide in blood and health effects.

3. Dichloromethane (methylene chloride)

Chemical and physical properties

physical state	:	colorless liquid
molecular weight	:	84.94
specific gravity	:	1.32 20°/4°C
melting point	:	—96.7°C
boiling point	:	+ 40.1°C
vapour density	:	2.93 (air = 1)
vapour pressure	:	440 mm Hg at 25°C
molecular formule	:	CH_2Cl_2
structural formule	:	

1 mg/m^3 = 0.288 ppm; 1 ppm = 3.48 mg/m^3 at 25°C and 760 mm Hg.

Effects on humans

Methylene chloride has the wellknown narcotic effects and is slightly irritative for mucous membranes and skin. Particularly in exposure for more than 3 to 4 h endogeneously (from methylene chloride) produced carbon monoxide (CO) may exert in own effects. In a human volunteer study (12 males) Putz et al (1978) studied psychomotor performance in exposure to 76 ppm CO - 4 h in rest or to 195 ppm methylene chloride - 4 h in rest; the % COHb increased from 1.4 to 4.85 respectively 5.1: in various tests performance decreased, in one of these more in exposure to methylene chloride than in exposure to CO. This suggests a synergistic effect of methylene chloride and CO together. In tests with exposure to methylene chloride for less than 4 h this extra effect of CO is not observed (Stewart et al. 1972, Winneke 1974, Gamberale et al 1975).

In animal tests (mice 5000 ppm, 24 h per day, 1 to 7 days) moderate degenerative changes were observed in liver and kidney; however, much less than in exposure to e.g. carbon tetrachloride. Combined exposure to ethanol had a potentiating effect. No teratogenic and/or reproductive effects have been observed.

In epidemiological studies in workers with exposure to 50-150 ppm (Friedlander et al 1978) no increased mortality (e.g. cardiovascular disease, cancer) was observed. In exposure of male (and not of female) rats (3500 ppm - 6 h/d- 5 d/wk - 24 mths) an increased incidence of sarcomata in and next to the parotid gland were observed; however, there appears to be no reason to consider this as indicating human carcinogenicity. In mutagenicity tests methylene chloride is weakly positive.

In industry combined exposure to other solvents (e.g. dichloroethane, dichloroethene) often occurs; this makes it difficult to ascribe observed health effects to methylene chloride itself.

Metabolism

Methylene choride is readily taken up through inhalation, and is also absorbed considerably through the skin. The agent itself does not accumulate significantly in repeated exposure over 5 days (Stewart et al 1976). The partition coefficient of methylene chloride between blood and air at 37° C is about 8-10 (Sato and Nakajima 1979, Lindqvist 1978) and between fat/air about 150-160 (Droz and Fernandez 1977, Sato and Nakajima 1979).

Production of carbon monoxide (CO) after exposure to methylene chloride was first observed by Stewart et al (1972). Evidence that CO is a biotransformation product of methylene chloride has been obtained by use of isotope tagged methylene chloride (Carlsson and Hultengren 1975). About 25% of the methylene chloride absorbed was ultimately excreted as CO and the post exposure excretion of methylene chloride by exhalation was less than 5% of the amount absorbed (Di Vincenzo and Kaplan 1981a).

In animal experiments it has been shown that increased COHb-levels only occur in exposure to di- and trihalogen-methyl compounds; production of CO increases with atomic weight of the halogen (Cl → Br → J) (Fodor et al 1973). Ciuchta et al. (1979) observed inhibition of biotransformation into CO in combined exposure to various alcohols or toluene.

The biological half life of COHb after exposure to halogenated hydrocarbons is about 10-12 h, i.e. double the $T^1/_2$ of COHb-levels after CO-exposure. This can be explained by the still continueing formation of CO from methylene chloride after exposure.

Biological indicators

Human volunteer studies

Concentrations of methylene chloride in exhaled air (Ce)

Table 2 presents data from human volunteer studies. The following conclusions may be drawn:

- the retention (R) seems to decrease with increasing exposure concentration (Ci) and with increasing duration of exposure (T);
- for similar T the decay curves of Ce for different Ci's run parallel; Ce itselft increases with a larger factor than Ci;
- for similar Ci-levels the early post exposure Ce-levels are about the same, but the Ce-levels decrease faster as T becomes shorter; after about 4 h post exposure Ce-levels are more or less proportional to T;
- fluctuation of Ci does not or hardly affect Ce;
- determination of Ce at 1-2 h after exposure presents the best means to estimate previous exposure; measurement of a series of samples over a few hours highly increases the predictive power (Stewart et al 1976);
- female subjects have about the same Ce-levels as males during the first hours after exposure; at 16 h females tend to have higher Ce-levels (Stewart et al. 1976); this difference could be due to a higher amount of fatty tissues in women. This is also found by Engström and Bjurström (1977). During the first 2 h after exposure the concentration in alveolar air tended to be lower and declined more rapidly in obese subjects than in slim ones. Thereafter concentrations dropped more slowly in the obese group. During the later phase of elimination, the obese subjects tended to have a higher concentration in alveolar air;
- there is no cumulation in the body in repeated exposure (Stewart et al 1976, Di Vincenzo et al 1981a);
- physical workload increases Ce-levels over the whole post exposure period.

Åstrand et al 1975 studied also the effect of physical exercise (50-150 W) on the uptake; the total duration of exposure was always 2 h, but broken up in 4 periods of 30 min. with difference in Ci and in physical activity. The results may be summarized as follows:

- at rest about 55% of the amount inhaled is taken up
- physical exercise (50 Watt) increases uptake with about a factor 2 (45% of the amount inhaled; with continuous 50 Watt workload the percentage ultimately becomes somewhat smaller: 30-35%)
- with increasing workload (100-150 Watt) the alveolar ventilation increases 5 to 7 fold of the rest value, but the uptake is not considerably higher than in the case of 50 Watt workload.

Di Vincenzo et al (1981b) reported similar results: the percentage of methylene chloride absorbed at rest, light, moderate, and heavy work intensities was 72, 58, 53 and 47% respectively. Exercise was accompanied by an increased pulmonary excretion of CO during exposure which undoubtedly contributed to the lower than expected COHb values encountered during heavy workloads.

Stewart and Dodd (1964) examined absorption through the skin of 4 subjects, by thumb immersion for 30 min; mean alveolar Ce after exposure was 3.1 ppm, at 2 h 0.69 ppm; these levels were considerably higher than for tri-, tetrachloroethylene, carbon tetrachloride and 1,1,1-trichloroethane and are comparable with a respiratory exposure to 50-100 ppm methylene chloride for 1 h, see table 1.

TABLE 2: Mean concentration of methylene chloride in alveolar air (Ce) after exposure as percentage of the inhaled concentration (Ci), the retention (R) at the end of exposure and COHb concentration.

reference	conc. Ci (ppm)	time (h)	R alv. end exp.	mean Ce as % of Ci time after exposure ½ h	1 h	2 h	16-20 h	max COHb
Stewart et al (1976)	50	1		3.6	2.0	0.8		
	50	5×1	0.76	2.8	1.2	0.6		
	100	1		3.7	2.0	0.9		
	100	5×1	0.64	5.0	3.0	0.9		
	250	1		4.8	2.7	0.7		
	250	5×1	0.66	6.1	3.7	1.0		Δ 1
	250 fl	1		—	2.0	0.7	0.08	
	250 fl	5×1		4.9	2.7	1.0	0.40	
	250 fem	1		7.2	4.3	1.1	1.10	
	250 fem	5×1		7.2	4.5	1.3	0.76	
	500	1	0.57	7.6	4.0	0.9	0.08	
	500	2×1		7.9	4.3	1.6	0.08	
Engström et al (1977)	750	1 wl		15	7.7	3.8		
	100	2		16	11	2.7		
Riley et al (1966)	100	2		16	13	11	2.7	
	90	5.2	3.7	1.8				
Di Vincenzo et al (1971)	210	2		5.8	3.2	2.2		
Di Vincenzo et al (1972)	100	2		5.4	4.0	1.3		
	200	2		6.7	3.4	1.5		
	100	2 wl		10.8	6.1	3.4		
Åstrand et al (1975)	250/500	2 wl		16	9	4.4	0.15	4.5
	250	2 wl		17	8.8	4.3	0.03	3.2
Stewart et al (1976)	500	2 wl		20	13	4.6	0.17	4.0
	50	3		4.2	1.8	(2.0)		
	50	5×3	0.72	3.8	2.2	1.8		
	100			5.0	3.7	1.6		
	100	5×3	0.63	6.1	3.8	1.9		
	250	3		6.6	3.8	1.5	0.16	
	250	5×3	0.59	8.9	4.3	1.9	0.20	Δ 3
	250 fl	3		5.4	3.7	1.8	0.20	
	250 fl	5×3		8.2	4.6	2.0	0.16	
	250 fem	3		7.0	5.5	0.5	0.60	
	250 fem	5×3		7.6	5.9	1.4	0.72	
	500	3		16	8.9	3.9	0.10	
	500	2×3	0.50	17.7	9.5	3.4	0.08	
Di Vincenzo et al (1972)	100	4		5	3	2		
Putz et al. (1978)	195	4						5.1
Stewart et al (1976)	½		7.2	2.8	1.8			
	50	5×7½	0.70	7.2	3.6	2.2		Δ 1.8
	100	7½		6.1	4.0	3.7		
	100	5α7½	0.67	7.9	4.5	3.2		Δ 3.5
	250	7½		13.2	5.9	2.3	0.16	
	250	5α7½	0.52	8.7	5.1	3.6	0.32	Δ 7
	250 fl	7½		12.9	7.8	3.5	0.28	
	250 fl	5×7½		9.7	6.8	3.7	0.40	
	250 fem	7½		12.3	8.8	2.8	1.40	
	250 fem	5×7½		13.3	9.3	2.8	0.92	
	500	7½	0.45	17.8	12.1	5.0	0.26	
	500	2×7½		21.2	13.2	5.1	0.28	Δ 10
Di Vincenzo et al (1981a)	50	7½	~ 0.70	5	4			1.9
	100	7½	~ 0.60	8	5			3.4
	150	7½	~ 0.65	7	4			5.3
	200	7½	~ 0.60	9	5			6.8

fl = fluctuating
fem = female
wl = workload
Δ = increase during exposure

Concentration of methylene chloride in blood

Di Vincenzo et al (1971 and 1972) exposed 11 subjects to 100 and 200 ppm for 2 and 4 h; venous blood was taken at different points of time. In exposure to 200 ppm the blood level was twice that in exposure to 100 ppm, but doubling of exposure time from 2 to 4 h did not double the blood level. Two hour exposure to 200 ppm resulted in about 2.2 mg/l blood at the end of exposure. Serial breath and blood die-away curves were very similar in form in the post exposure phase; however during and after exposure blood concentrations did not show such a rapid increase and decrease as the concentration in exhaled air. In 1981 Di Vincenzo et al (1981a) reported similar results with exposure of 14 subjects to 50, 100, 150, or 200 ppm methylene chloride for 7.5 h.

Åstrand et al. (1975) measured in subjects exposed to 250 or 500 ppm methylene chloride in arterial and (peripheral) venous blood. The relationship between concentrations in arterial blood and in alveolar air was linear at the end of each exposure period: [concentration in blood (mg/kg)] = —1.059 + 0.0124 [concentration in alveolar air (mg/m^3)]. The venous concentration followed the arterial concentration; at higher levels of uptake (physical activity) the arterio-venous difference increased considerably. The correlation between the arterial blood levels and amount of methylene chloride taken up was rather poor.

Concentrations of methylene chloride in adipose tissue

Engström and Bjurström (1977) exposed 12 subjects to a concentration of 750 ppm for 1 h while performing work at an intensity of 50 W. The amount of methylene chloride absorbed was highly correlated with the degree of obesity and with body weight. Needle biopsy specimens of subcutaneous adipose tissue were taken before exposure and 0,1,2,3 and 4 h after exposure. The mean concentration in the adipose tissue was 10.2 mg kg at 1 h after exposure and 8.4 mg/kg after 4 h. In six slim subjects the concentration in adipose tissue at 4 h after exposure was on the average twice that in six more obese subjects. On the other hand, in spite of the lower concentration the obese subjects had a greater calculated amount of methylene chloride in the total fat depots of the body. Two subjects were studied about 22 h after exposure, the concentrations in subcutaneous adipose tissue were 1.6 and 1.7 mg/kg respectively.

More studies have to be carried out to provide data on concentrations in adipose tissue in relation to various exposure levels.

Concentration of methylene chloride in urine

The overall excretion with urine is negligible in comparison with elimination through exhalation (Di Vincenzo et al 1971, 1972, 1981b); it did not appear to be related to work intensity (Di Vincenzo et al 1981b). There is no reason to propose biological monitoring based upon urine sampling.

Concentrations of carboxyhemoglobine in blood

Table 1 also presents data on % COHb. In short-terms exposure to < 250 ppm for 3 h, the % COHb probably does not exceed 5%, but it may do so in repeated exposure for 7 to 8 h/day. The % COHb can be indirectly estimated by measuring CO in exhaled air (see document on Carbon Monoxide).

It should be emphasized that monitoring for CO in exhaled air or COHb in blood can only be applied for biological monitoring of exposure to methylene chloride in non-smoking subjects. Moreover, during monitoring the concentration in inhaled air (environment) should not exceed 9 ppm CO.

Åstrand et al (1975) also measured COHb levels in blood. In sharp contrast with methylene chloride levels, COHb levels increased in the postexposure phase, even up to 2-4 h. The venous COHb did not differ systematically from the arterial levels. The COHb exceeded 5% when subjects were exposed to 500 ppm for 2 h with workload. In the case of 8 h exposure to < 100 ppm total COHb levels (inclusive pre-exposure level of 1-1.5%) will probably not exceed 5% in non-smokers.

In human volunteers (10 males, 9 females) exposed to 50-500 ppm for 1, 3 or 7.5 h/day at rest for up to five successive days Peterson (1978) derived the following equation:

$$\Delta\ \%\ \text{COHb} = \frac{0.0842(D.Ci)^{0.72}}{(T + 220)^{0.775}}\ \text{, in which}$$

D = exposure duration in minutes
Ci = concentration methylene chloride in ppm
T = time after the end of exposure in min

This equation is only valid for exposure to 50-500 for D= 1-7.5 h, in rest.

Di Vincenzo and Kaplan (1981a) observed in a human volunteer study (see table 2) that exposure for 7.5 h 5 days to 50, 100, 150 or 200 ppm resulted in peak COHb-levels of 1.9, 3.4, 5.3 or 6.8%. Alveolar air and blood levels of CO were directly proportional to the magnitude of exposure; at 100 ppm 8 h a COHb-level of 3% could be expected. In a second study by Di Vincenzo and Kaplan (1981b), in which the effect of exercise and of smoking was examined in 3 volunteers exposed for $7^1/_2$ h to 100 ppm, it was observed that exercise did not increase COHb-levels, probably because of the increased respiratory excretion of CO; when smoking during or after exposure to methylene chloride there appeared to be an additive effect on % COHb.

Concentration of carbon monoxide in exhaled air

In the human volunteer study carried out by Stewart et al (1976) CO was present in alveolar air at all conditions; the CO level in alveolar air reached its maximum at 1-2 h *after* exposure, and was directly related to magnitude of exposure. The portion of CO due to methylene chloride in combined exposure can be calculated by taking into account the difference in biological half life of COHb due to methylene chloride and of COHb due to e.g. smoking. It should be noted that simultaneous exposure to other solvents may increase biological half life of COHb (triple in case of methanol). In the experiments of Di Vincenzo and Kaplan (1981a) the concentration of CO in expired air was also directly proportional to the magnitude of the exposure both during and after the exposure.

Industrial exposure studies

Ratney et al (1974) examined a group of non-smoking workers (n = 7) exposed to 160-200 ppm (mean 180 ppm) at day of sampling, but already exposed for several years; in addition there was exposure to 25-36 ppm (mean 31 ppm) chloroform. The pre-exposure average CO level in alveolar air was 29 ppm, which increased during exposure to about 50 ppm; before next exposure the CO level was 23 ppm; these levels correspond to COHb (calculated) 4.9% (3.3-5.3), 8.3% (5.7-12.0) and 3.9% (3.6-4.9). The biological half life of COHb was 13 h. From these data it became clear that — although methylene chloride itself is not cumulative in repeated exposure — HbCO-levels are; the « morning after » pre-exposure levels were almost 5%.

Benzon et al (1978) examined a worker after he had accidentally been overcome by methylene chloride vapours; the COHb-levels had increased up to 19%; another worker with a history of ischemic heart disease had been exposed concurrently with the other patient; the following day his COHb level was 6%.

Perbellini et al (1977) examined methylene chloride levels in alveolar air and in blood of shoe sole factory workers. At 21 ± 8 ppm methylene chloride the alveolar concentration was average 14 ppm and in blood average 40.41 mg/l; at 35 ± 12 ppm the average levels were 20 ppm and 0.99 mg/l; at 96 ± 75 ppm the levels were average 65 ppm and 3.07 mg/l (groups of 4 or 5 workers). In this factory the methylene chloride exposure was rather variable (high S.D.). The data are too limited to allow valid extrapolation.

Conclusions

Biological monitoring of methylene chloride exposure can be based upon measuring the agent itself in exhaled air or blood, however, because methylene chloride is not cumulative, and because in exposure for more than 3 to 4 h per day production of CO appears to be the limiting factor as regards health risk and the half life of COHb is longer than in the case of exposure to CO, biological monitoring based upon either exhaled air analysis of CO or blood analysis of COHb is to be preferred. However, this can only be applied in non-smoking workers. Sampling should be done at about 2 h post exposure, or after 16 h (following morning). Post exposure COHb levels (at 2 h) are not expected to exceed 2-3% and at 16 h post-exposure 1% in the case of exposure for 8 h to < 100 ppm methylene chloride in non-smokers.

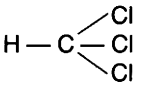
Methods for analysis of methylene chloride in exhaled air and blood have been described by Di Vincenzo et al. (1971), Stewart et al (1976) and Åstrand et al. (1975). For methods of analysis of COHb and CO see document on biological monitoring of CO-exposure.

Research needs

There particularly is need of studies of workers: measurement of methylene chloride in inhaled air by means of personal sampling, and of methylene chloride and carbon monoxide levels in exhaled air and in blood, at the end of a work day at 1 to 2 h, at 16 h (following morning) and at 64 h after exposure (Monday morning), separately for non-smoking and for smoking workers. Such studies should lead to valid biological monitoring indices, which allow to estimate individual uptake of methylene chloride.

4. Trichloromethane (chloroform)

Chemical and physical properties

physical state	:	colorless liquid
molecular weight	:	119.39
specific gravity	:	1.49 15°/4°C
freezing point	:	—63.5°C
boiling point	:	61.2 °C
vapour density	:	5.3 (air = 1)
vapour pressure	:	200 mm Hg at 25°C
molecular formule	:	$CHCl_3$
structural formule	:	

1 mg/m^3 = 10.206 ppm;
1 ppm = 4.89 mg/m^3 at 25°C and 760 mm Hg.

Effects on humans

Chloroform exerts non-specific narcotic effects and specific effects on liver and kidney; this determines the permissible level of exposure. In animal experiments liver hepatomata have been observed, with possible carcinogenity, however at high dosages. According to IARC (1979) chloroform produced hepatomas and hepatocellular carcinomas in mice, malignant kidney tumours in male rats and tumours of the thyreoid in female rats. In humans no case reports or epidemiological studies are available which point to human carcinogenicity. However, IARC concluded that it is reasonable for practical purposes to regard chloroform as if it presented a carcinogenic risk in humans.

According to Challen et al (1958) the following tentative relationships for occupational exposure could be given: in average exposure to 77-237 ppm there were complaints of nausea, loss of appetite, frequent and burning micturition, lack of mental concentration, depression, irritability; no evidence of liver injury was observed 3-4 yr after exposure. Among workers exposed for 10-24 months to 22-71 ppm (with a few short term peak concentrations) dryness of mucosae and lassitude were observed; no evidence of liverfunction impairment. Bomski et al (1967) studied workers exposed to 2-205 ppm; there was an increased incidence of viral hepatitis, enlarged liver and spleen. Gambini and Farina (1973) observed an enlarged liver without evidence of disturbed function in 29% of 64 workers exposed to 17-28 ppm (peaks up to 360 ppm) in 12-37 months. Alcohol intake increases the hepatotoxicity.

Metabolism

Hardly no data exist on metabolism in healthy workers; most data refer to studies in anaesthesized patients. Chloroform is absorbed through inhalation and eliminated by exhalation. Because of its liposolubility the agent will be deposited in fatty tissues (e.g. brain). The partition coefficient of chloroform between blood and air at 37°C is about 10-12 (Sato and Nakajima 1979, and Lindqvist 1978) and between fat/air about 400-425 (Droz and Fernandez 1977, Sato and Nakajima 1979). No pertinent data are available on metabolism.

Bergman (1979) studied metabolism by means of autoradiography after respiratory exposure of mice. Chloroform showed a high affinity for nervous and adipose tissue, with a specific long-time retention in the cerebellar cortex, meninges and spinal nerves. Biotransformation particularly took place in liver and kidney, with an accumulation of non-volatile radioactivity; metabolites were also found in the bronchi, and non-volatile metabolites in the testicular interstitium. In the nervous or fatty tissues no non-volatile metabolites were observed. Metabolites were excreted via urine (9%) and bile; CO_2 (30%) was found as metabolite in exhaled air and about 30% was exhaled as chloroform during 8 h after exposure.

Lehmann et al (1910) found 60-70% retention in two subjects exposed for 20 min to 4000-5000 ppm. Fry et al. (1972) recovered 18-67% of the dose in expired air as chloroform within 8 h after oral administration of 0.5 g chloroform to humans. The lower

percentages were obtained with obese persons. Of a dose of 0.5 g ^{13}C-chloroform 48 and 51% respectively was recovered as $^{13}CO_2$ in expired air of two human subjects. There was a linear relationship between the rate of pulmonary excretion of chloroform and its concentration in blood. With smaller dose the percentage of chloroform in exhaled air was also smaller. The same dose-dependency has also been demonstrated in animal experiments.

Pohl et al (1977) observed in vitro (rat liver microsomes) biotransformation to trichloromethanol, which spontaneously dechlorinates to phosgene. This was confirmed in vivo by Pohl et al (1979) in phenobarbital pretreated rats. Reitz et al (1979) observed that after correction for metabolic activation in rats and mice a correct estimate for the cancer risk could be made, in contrast to estimation from the exposure levels as such. This, moreover, resulted in a much lower risk estimate for humans than when extrapolated from the inhaled concentrations.

Biological indicators

According to NIOSH (1974b) only Lehmann et al (1910) studied levels of chloroform in exhaled air after exposure to concentrations in air, however not relevant for present day practice (4400 ppm (21500 mg/m^3) - 30 min; 7200 ppm (35300 mg/m^3) - 15 min); at about 20 min after exposure the concentrations in exhaled air were 970 and 1040 mg/m^3 respectively. Other authors measured the agent in blood in anaesthesized patients. These data are inadequate for proposing an appropriate method for biological monitoring in workers.

Conclusions

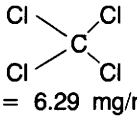
At the present state of the art not even tentative conclusions can be presented for biological monitoring methods in case of occupational exposure.

Research needs

There hardly exist any valid data on biological assessment of exposure. There is need for studies in human volunteers and in workers, particularly on the relationship between chloroform concentrations in inhaled air and in exhaled air and/or blood.

5. Tetrachloromethane (Carbon tetrachloride)

Chemical and physical properties

physical state	:	colorless liquid
molecular weight	:	153.8
specific gravity	:	1.59 25°/4°C
freezing point	:	—22.6°C
boiling point	:	76.8°C
vapour density	:	5.3 (air = 1)
vapour pressure	:	91 m Hg at. 20°C
molecular formule	:	CCl_4
structural formule	:	

1mg/m^3 = 0.159 ppm; 1 ppm = 6.29 mg/m^3 at 25°C and 760 mm Hg

Effects on humans

In addition to non-specific narcotic effects on the central nervous system (the narcotic properties are weaker than for trichloroethylene and chloroform) mild to moderate irritative effects on skin and mucous membranes, non-specific effects (e.g. nausea, colic, diarrhoea) on the gastro-intestinal tract may occur. The most important more or less specific effects are those on liver and kidney function. Liver damage may result

from either short term high level or longterm low level exposure: necrosis and cirrhosis. Renal impairment is a common feature; it may exist in the absence of demonstrated liver effects. The liver and kidney impairment is enhanced by ingestion of ethanol and other aliphatic alcohols.

Liver and renal function tests are regarded to be the most sensitive and practical methods to detect early health impairment. In acute exposure both liver and kidney function may be most critical, in chronic exposure liver effects are predominant.

Stewart and Witts (1944, quoted by WHO 1979) noted a high incidence of gastro-intestinal symptoms in workers exposed under wartime conditions: severe nausea, vomiting, colic, diarrhoea, with relief during brief episodes of absence. Similar findings were reported by Kazantkis et al (1960) in workers exposed to concentrations ranging from 48 to about 100 ppm.

According to WHO (1979) measurement of serum enzym activities provides the most useful practical indicator of early changes in liver function, although according to Stewart et al (1961) urinary urobilinogen and serum iron might be a more sensitive indicator. Ornithine carbamyl transferase (OCT) is considered to be the most specific enzyme for the liver, but in practice application meets difficulties. Lactic dehydrogenase isoenzymes (LDH_4 and LDH_5) and sorbitol dehydrogenase (SDH) appear to be also reasonably specific. The less specific cytoplasmic enzymes SGPT and SGOT have also shown a good sensitivity for liver changes; SGPT appears to be preferred for monitoring early effects in long term low-level exposure; SGOT is more transient because of a shorter half life (WHO 1979).

IARC (1979) reviewed the carcinogenic properties of carbon tetrachloride. In several animal experiments liver tumours, including hepatocellular carcinomas have been induced. There exists sufficient evidence that carbon tetrachloride is carcinogenic in experimental animals. No epidemiological studies are available to assess human carcinogenicity; however, a few case reports described appearance of liver tumours associated with cirrhosis, following rather short term exposure to carbontetrachloride. IARC concluded that it is reasonable to regard carbon tetrachloride as if it presented a carcinogenic risk to humans.

Metabolism

There only exist few studies on metabolism. Carbon tetrachloride is readily absorbed by the lungs and the gastro-intestinal tract. The blood/air and fat/air partition coefficient at 37°C are about 10-12 (Sato and Nakajima 1979; Lindqvist 1978) and about 400 (Sato and Nakajima, 1979; Droz and Fernandez 1977) respectively.

Stewart and Dodd (1964) detected carbon tetrachloride in exhaled air of 3 subjects within 10 min following immersion of the thumb for 30 min in the solvent; the concentration in exhaled air increased during exposure, continuing to rise in the 10-30 min interval following exposure. The concentration was followed till 5 h after exposure. The concentration in exhaled air would be equivalent to a 30 min vapour exposure to 2.5-12 ppm. The uptake through topical application to both hands for 30 min was estimated to be equivalent to respiratory exposure to about 10 ppm for 3 h.

Studies with repeated respiratory or dermal exposure in volunteer studies or in worke have not been carried out.

When carbontetrachloride is administered together with fat, the intestinal absorption and pulmonary elimination increase, ethanol also increases slightly absorption.

Bergman (1979) studied the distribution and elimination of carbon tetrachloride in mice by means of whole body autoradiography. Carbon tetrachloride had a high affinity for nervous and adipose tissues; carbon tetrachloride was retained for a longer period of time than any other chlorinated solvent studied (methylene chloride, chloroform, carbon tetrachloride). Biotransformation mainly takes place in liver and kidney; accumulation of nonvolatile radioactivity takes place in these organs. Metabolites were also localized in the bronchi, non-volatile metabolites were noted in testicular interstium; $^{14}CO_2$ was identified as a metabolite in exhaled air. Within 8 after exposure about 70% of the dose was unchanged in exhaled air and about 4.5% as CO_2.

Monkeys breathing a concentration of 46 ppm carbontetrachloride for 139-349 minutes retained 30.9% of the inhaled amount. One monkey inhaling 46 ppm ^{14}C-carbon tetrachloride for 344 minutes exhaled 40% and 11% of the absorbed amount as unchanged ^{14}C-carbon tetrachloride and $^{14}CO_2$ respectively during 1800 hrs after exposure (McCollister et al 1951).

Metabolism of carbon tetrachloride is almost unknown. The formation of CCl_3 — radicals is assumed to be the initial step in the biotransformation, the radicals

subsequently bind irreversibly to cellular macromoleculars and initiate lipid peroxidation. This formation of the radicals is supported indirectly by the appearance of small amounts of chloroform and hexachloroethane (a dimerization product of CCl_3-radicals) in tissue of rabbits after administration of carbon tetrachloride (Fouler, 1969).

Biological indicators of exposure

In exposure of 6 healthy subjects to two times 10 ppm for 3 h (Stewart et al 1961) the concentration in mixed exhaled air was 2-3 ppm at the end of exposure, 0.7 ppm at 1 h and about 0.25-0.3 ppm at 5 h after exposure. In a group of 6 subjects exposed to 49 ppm for 70 min the levels in mixed exhaled air were 14 ppm at o h, 1.7 ppm at 1 h and 0.3 ppm at 5 h after exposure. In both conditions the limit of detection (0.2 ppm) was achieved at 6 h after exposure. The agent was not detected in blood or urine (detection limit 5 mg/l).

Stewart et al (1963) examined a case of ingestion of carbon tetrachloride and methanol; in exhaled air about 300 ppm was found 3-5 h after ingestion, $2^1/_2$ week later the concentration was about 0.3 ppm.

Very probably measurement of carbon tetrachloride in exhaled air will be the method of choice. However, the data available do not allow to propose a reliable relationship between concentration and duration of exposure and the level in exhaled air. There does not seem to be any possibility to base biological assessment of exposure by measurement of metabolites in urine or carbon tetrachloride in blood.

Methods of measurement carbon tetrachloride are presented by Stewart et al (1961). Because of the rather slow elimination in exhaled air one should not take samples immediately after exposure; serial analysis over a period of time will be most promising.

Conclusions

At the present state of knowledge, no reliable indicators for biological monitoring of workers exposed to carbon tetrachloride can be proposed. Very probably serial analysis of the concentration in exhaled air will prove to be the method of choice.

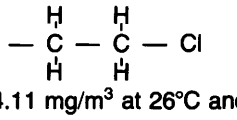
Research needs

There is need to study:

- the relationship between concentrations in air/duration of exposure and serial concentrations in exhaled air;
- rather specific early indicators of impairment of liver- and kidney functions;
- the effect of factors not related to carbon tetrachloride exposure on the biological indicators, e.g. intake of ethanol and drugs, sex, age.

6. 1,2-Dichloroethane (ethylene chloride, ethylene dichloride)

Chemical and physical properties

physical state	:	colorless oily liquid
molecular weight	:	98.9
specific gravity	:	1.25 (20°C)
melting point	:	—35.4°C
boiling point	:	83.5°C
vapour density	:	3.42 (air = 1)
molecular formule	:	$C_2H_4Cl_2$
structural formule	:	

1 mg/m^3 = 0.24 ppm; 1 ppm = 4.11 mg/m^3 at 26°C and 760 mm Hg.

Effects on humans

Only few quantitative data on effects on humans are available. Reviews are published by NIOSH (1976) and IARC (1979).

1,2-Dichloroethane may exert a variety of effects; a specific critical effector organ cannot be given. Exposure may affect the circulatory, respiratory and nervous systems, liver, kidney, skin and mucous membranes. Acute exposure has often resulted in death; in fatal cases the signs and symptoms usually start with headache, dizziness, nausea, vomiting, anorexia, tenderness or pain in the epigastrium, tachycardia, leading to cyanosis and coma, and finally to respiratory, circulatory or kidney failure. In autopsy pulmonary oedema, congestion of visceral organs and haemorrhages into most organs were observed. Fatalities have also occurred without preceding narcosis.

According to Byers (1943, quoted by NOISH 1976) the worst symptoms may occur with a certain delay in the evening after a day's work in case of exposure to about 100 ppm. Rosenbaum (1947, quoted by NIOSH 1976) did not observe any changes in blood or internal organs, but increased lability of the vegetative nervous system, and frequent symptoms of fatigability, irritability and sleeplenessness were reported in 100 workers exposed for 6 months to 5 years to levels below 25 ppm.

Shchepotin and Bondarenko (1978) examined 248 patients with acute poisoning; they distinguished four different clinical syndromes: toxis encephalopathy (63.7%) acute gastro-enteritis (84.6%), hepatopathy (35.4%) and acute circulatory insufficiency (57.7%). Akimov et al (1978) described in 14 fatal cases cerebral oedema, haemorrhagy, ischemia and myeline degeneration.

IARC (1979) concluded that sufficient evidence exists that 1,2-dichloroethane is carcinogenic in mice and rats (oral administration by gavage): malignant tumour in lung, liver, mamma, uterus, stomach and haemangiosarcoma. In the absence of adequate data in humans, it appeared to be reasonable, for practical purposes, to regard 1,2-dichloroethane as if it presented a carcinogenic risk to humans. However, IARC did not yet take into account negative findings in rats and mice with respiratory exposure for 2 years to 5, 10, 50 or 250 ppm, reported by Maltoni (1977) and absence of skin tumours after topical application for over one year in mice by Goldsmidt (1978).

Metabolism

In rats exposed to 220 or 800 ppm Sopikov and Gorshunova (1979) measured after 3 h 20 and 56 mg dichloroethane/L in blood respectively. The pulmonary retention decreased during exposure from 70% at 15 min to 10% at 3 h. According to Sato and Nakajima (1979) the partition coefficient of dichloroethane between blood-air is 19.5 and between blood-fat 447. Sopikov and Gorshunova (1979) found a similar partition coefficient for blood-air. They also studied the procentic relationship between the concentrations in blood (as 100%) and that in various organs, e.g. liver 80%, kindney 44%, spinal nervous system 70%, medulla 57%, brain 15-20%. After oral dosage in rats the biological half-life of the dichloroethane level in blood was 88 min and in exhaled air 76 min.

Hofmann et al (1971) and Yllner (1971) established that biotransformation takes place in liver microsomes as follows: 1,2-dichloroethane → chloroethanol → chloroacetaldehyde → monochloroacetic acid → glycolic avid → oxalic acid. In mice receiving i.p. 50-170 mg/kg b.w. labelled dichloroethane, 10-42% was exhaled unchanged and 12-4.2% as CO_2, depending upon the dosage; most of the remainder was excreted as chloroacetic acid, S-carboxymethylcysteine and thiodiacetic acid (Yllner, 1971).

In two cows, which received 1.75-17.5 mg/kg b.w. in their food for 22 days Sykes and Klein (1957, quoted by NIOSH 1976) detected the agent in the milk (0.13-0.35 mg/l); there was no clear gradient with dosage or duration of exposure.

Urusova (1953, quotes by NIOSH 1976) analysed the 1,2-dichloroethane concentration in milk and in exhaled air of women occupationally exposed to 15.5 ppm for an unspecified period of time; near to the end of the workday the concentration in exhaled air was reported to be 14.5 ppm, and in milk 5.4.-6.4. mg/l, at 18 h after work the levels were respectively 2-4 ppm and 1.95-6.3 mg/l.

Biological indicators of exposure

Very few studies have been performed in humans. On the basis of limited animal experiments one may expect that measurement of 1,2-dichloroethane and/or its metabolites in exhaled air, blood and/or urine offer possibilities for biological monitoring.

Conclusions

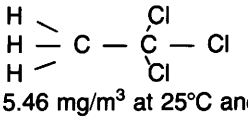
Possibilities for biological monitoring of exposure to 1,2-dichloroethane exist: measurement of the agent in exhaled air or in blood, or of its metabolites in blood or urine. However, reliable studies in humans do not exist.

Research needs

There is need to study the toxicokinetics in humans, with the objective to evaluate the relationships between concentrations in air/duration of exposure and biological indicators of exposure, ánd this for exposure up to about 20 ppm.

7. 1,1,1-Trichloroethane (methylchloroform, MC)

Chemical and physical properties

physical state	:	colourless liquid
molecular weight	:	133.4
specific gravity	:	1.34 25°/4°C
freezing point	:	—30.4°C
boiling point	:	74.1°C
vapour density	:	4.6. (air = 1)
vapour pressure	:	127 mm Hg at 25°C
molecular formule	:	CH_3CCl_3
structural formule	:	

mg/m^3 = 0.183 ppm; 1 ppm = 5.46 mg/m^3 at 25°C and 760 mm Hg

Effects on humans

1,1,1-Trichloroethane has non-specific narcotic, irritative and degreasing porperties, common to all chlorinated hydrocarbon-solvents.

Stewart et al (1961) exposed groups of 6 to 7 subjects to 500 ppm — 78 min: only sight transient irritation of eyes was observed; to 496 ppm — 186 min: no effect on well-being, equilibrium, coordination; to 900-955 ppm for 73 min (3 subjects), 35 min (2 subjects), 20 min (2 subjects): evidence of disturbed equilibrium was present in a few subjects. Salvini et al (1971) exposed 6 subjects to 400-500 ppm for 8 h (1.5 h interruption): only in the first 30 min a few complaints of dizziness and slight excitation were mentioned; in various performance tests only a decreased tachistoscopic perception carried out under mental stress was observed; in exposure to 350 ppm no effects were seen. Gamberale and Hultengren (1973) exposed 12 subjects to subsequently (30 min periods) 250, 350, 450 and 550 ppm, total 120 min; they examined reaction time, perception speed and manual dexterity. At 350 ppm (at $^1/_2$-1 h) a transient decrease in performance was observed, but not at 250 ppm (at 0-$^1/_2$ h). Stewart et al (1975) exposed 10 male subjects in an extensive testing programme: 0 100 350 and 500 ppm for 1,3 and 75 h 5 d/wk; and 10 females only to 0 and 350 ppm for 1,3 and 7.5 h, 5 d/wk. Only in exposure to 500 ppm, 5 d/wk, changes in the EEG were observed at day 5. In exposure to ⩾ 350 ppm a moderate to strong odour was noted, decreasing during exposure. There were no effects on haematological and biochemical parameters, no neurological effects, no effects on lungfuncion (only slight decrease in diffusion), no effects in various performance tests, and only slight increase in some subjective symptoms was observed. The authors concluded that in exposure of males to 500 ppm, 7.5

h/day, 5 d/wk in rest no evidence of health impairment was observed, except some changes in the EEG.

In various studies hardly any effects on liver — and kidney functions were observed.

In studies in workers Seki et al (1975), Maroni et al (1977) and Kramer et al (1978) observed no exposure dependent effects on liver function.

There is no evidence of carcinogenicity. IARC (1979) concluded that the available data did not permit an evaluation of the carcinogenicity of 1,1,1-trichloroethane to be made.

Metabolism

Uptake

By the lungs. The retention and the uptake (lungclearance, LC) decrease quickly in the course of exposure at rest (Table 3). Because the solubility in blood of MC is small, the uptake decreases quickly; the release to metabolism is small. The uptake during the first exposure hour was twice as high as during the fourth exposure hour (Monster et al 1979b).

TABLE 3: Uptake by the lungs of MC at rest

		time after start of exposure					
		min				h	
Reference		0	10	30	60	4	8
Humbert and Fernandez 1977	alveolar retention (R)			0.42	0.37	0.28	0.24
Monster et al 1979b	alveolar retention (R)	0.75	0.51	0.47	0.39	0.30	
Monster et al 1979b	lungclearance (LC,l/min)	6.0	3.7	3.0	2.5	1.9	

The blood/air and fat/air partitioncoefficients at 37°C are 3-6 and about 360, respectively (Åstrand 1975, Lindqvist 1978, Monster 1979, Sato and Nakajima 1979, Droz and Fernandez 1977).

Workload during exposure increases the uptake (Table 4); the retention decreases, but the minute volume increases faster. The uptake during 100 Watt workload was 2-2.5 times that at rest, but during the first workload (after 1 h exposure) the uptake was 30% higher than the uptake during the second workload (after 3 h exposure): LC was 6.0 and 4.5 l/min, respectively (Monster et al 1979b).

TABLE 4: Uptake by the lungs of MC during workload (Astrand et al 1973)

	workload (30 min exposure)			
test	rest	50 W	100 W	150 W
alveolar retention (R)	0.80	0.62	0.50	0.40
alveolar minute volume (l/min)	6.6	22	36	55
lungclearance (LC, l/min)	5.3	13.6	18	22

Through the skin. Immersion of one hand in liquid MC over $^1/_2$ h resulted in a concentration of MC in alveolar air, comparable with the concentration after a respiratory exposure to 50-250 ppm (275-1365 mg/m^3) for $^1/_2$ h. Immersion of one thumb in liquid MC resulted in 1/20 of the concentration of alveolar air obtained after immersion of one hand in liquid MC. About the same concentrations in alveolar air were obtained after repeated immersions of one hand in liquid MC for 60 time in $^1/_2$ h (Stewart and Dodd 1964). On the basis of the alveolar concentration Fukabori et al (1976, 1977) came to the conclusion that application of liquid MC over 2 h to 12.5 cm^2

of forearm skin or immersion of both hands in liquid MC 11 times for 10 min would be equivalent to a 2 h exposure to 10-20 ppm (55-110 mg/m^3) MC of vapour. Despite the large range in absorption rate between the experiments, we can conclude that absorption through the skin during normal industrial work will be small compared to that in respiratory exposure.

When subjects, dressed in pyjamas, socks and a full face piece respiratory, were exposed to MC vapor the amount of MC expired postexposure was only 0.1% of the amount that should be expired in exposure to the same concentration without a respirator (Riihimäki and Pfäffli 1978).

Biotransformation

We assume that a small amount of MC absorbed into the body is biotransformed by hydroxylation to trichloroethanol (TCE); subsequently, partial oxidation of TCE to trichloroacetic acid (TCA) will take place.

The half-life of MC in blood and expired air depends on the length of exposure and on the time of sampling after exposure. The concentration follows a multi-exponential curve. The toxicokinetic behavior of TCE and TCA is similar after exposure to trichloroethylene, although the concentrations are much lower. TCE reaches the maximum concentration in blood and urine almost directly after exposure and decreases with a half-life of about 10-15 h. The concentration of TCA in blood and urine increases up to 20-40 h after single exposure. Thereafter the concentration decreases with a half-life of about 70-100 h (Monster et al 1979b).

Recovery

The percentage of the uptake, excreted as MC in expired air and as TCE and TCA in urine are presented in Table 5. We may conclude that MC is almost totally exerted by expiration; only small amounts were excreted in urine as TCE (2% to 5%) and TCA (1% to 2%).

TABLE 5 : Percentage of the amount taken up excreted as MC in expired air and as trichloroethanol (TCE) and trichloroacetic acid (TCA) in urine

	expired air (%)	urine (%)	
Reference	MC	TCE	TCA
Humbert and Fernandez 1977	97 (88 - 106)	4.6	1.7
Monster et al 1979b	60 — 80	2	1.5

Biological indicators

Human volunteer studies

Concentrations of trichloroethane in exhaled air

Table 6 summarizes data from various studies; the concentrations after exposure in alveolar air (Ce) are expressed as a percentage of the inhaled concentration (Ci) and arranged according to the length of exposure.

The following conclusions may be drawn from this table:

- The concentration in alveolar air decreases quickly in the first $^1/_2$ h after exposure from 60% to 80% (end of exposure, Table 3) to 10% to 20% of Ci.
- As duration of exposure increases, the relative concentration in alveolar air is higher, more pronounced when the sampling after exposure occurs later. After exposure at rest for 6-8 h the mean concentration in alveolar air is about 19% ($^1/_2$ h), 15% (1 h), 2.4% (16 h) and 0.6% (64 h), respectively. After exposure while resting for 6-8 h, over 5 days, the mean concentration is about 30% ($^1/_2$ h), 20% (1 h), 4 (16 h), and 1 (64 h) of Ci, respectively.
- Workload during exposure increases the concentrations in alveolar air over the whole postexposure period.

TABLE 6: Concentration of MC in alveolar air (Ce) after exposure as percentage of the inhaled concentration (Ci)

Reference	Number of subjects	Ci (ppm)	Time (h)	Mean Ce as % of Ci					
				1/2 h	1 h	2 h	16 h	64 h	120 h
Stewart et al (1975)	4	350	5x1	9	6	3	0.4	0.2	
Fukabori (1970)	4	195	2	11	5	3.5			
"	4	376	2	9	6	3.2			
"	4	558	2	12	7	4.8			
"	4	831	2	9	5	3.3			
Åstrand et al. (1973)	4	250+WL[a]	2	16	12	6	0.8		
"	5	250/350+WL[b]	2	17	13	3	0.6		
Salvini et al (1971a)	6	600	3		13	10	1.5	0.25	0.08
Stewart et al (1969)	11	507	3	10			0.8		
Stewart et al (1961b)	3	496	3	13	6	4.4	0.7		
Stewart et al (1975)	4	350	5x3	17	11	7	1.0	0.4	
Monster et al (1979b)	6	70	4	15	10	7	0.58	0.07	0.03
"	6	140	4	14	11	6	0.58	0.10	0.03
"	6	140+WL[c]	4	16	12	8	0.66	0.09	0.03
Stewart et al (1969)	6	482	6 1/2-7	14.5			2.1	0.40	
Humbert and Fernandez (1977)	3	72	8	22	14	11	2.8	0.63	0.17
"	2	213	8	20	16	12	2.8	0.66	0.16
Stewart et al. (1969)	5	507	5x6 1/2-7	20			4.5	1.2	0.55
Rowe et al. (1963)	4	370	5x7		15		3.8	2.2	
Stewart et al. (1975)	4	100	5x7 1/2	35	28	20	4.5	0.7	
"	4	350	5x 1/2	26	20	13	3	0.6	
"	4	350 fem.	5x7 1/2	23	14	7	2	1.0	
"	4	350 fluc.	5x7 1/2	29	23	16	3	0.9	
"	4	500	5x7 1/2	40	30	17	2.4		

a 1/2 h rest, 1/2 h 50 Watt, 1/2 h 100 Watt, 1/2 h 150 Watt
b 1/2 h 250 ppm, 1/2 h 350 ppm, 1/2 h 250 ppm + 50 Watt, 1/2 h 350 ppm + 50 Watt
c During exposure 2x 1/2 h 100 Watt workload
fem = female
fluc = wide fluctuation (± 150).

Trichloroethanol in expired air

As in exposure to trichloroethylene, TCE can be measured in expired air (Monster et al 1979b); the concentration of TCE, however, is much smaller than in exposure to trichloroethylene under comparable conditions of exposure.

Excretion of metabolites (trichloroethanol and trichloroacetic acid) in urine

In two investigations (Humbert and Fernández 1977, Monster et al 1979b) the amounts of TCE and TCA excreted in urine after a single exposure were measured. The authors came to the same conclusion: about 75% of TCE was excreted within 48 h, while about 75% of TCA was excreted within 5 days, in concordance to the differences in half-life. The amounts of TCE and TCA, however, were much smaller than after exposure to trichloroethylene.

With repeated exposures (n=5) to 500 ppm (2730 mg/m^3) of MC, 7 h/day, over 5 days, a slow increase was found in the amounts of TCE excreted in 24 h urine: the first day about 20 mg, the fourth day about 50 mg, and 5 days after the last exposure still about 7 mg was found (Stewart et al 1969). There was hardly an increase in the TCA excretion; the amount of TCA excreted in urine before exposure, however, was high: 15 mg in 24 h. With repeated exposures (n =2) to 420 ppm (2300 mg/m^3) of MC 2 h/day, over 3 days, a maximum excretion of 7 mg TCA per 24 h was found on the fifth day after the start of the exposure (Tada 1969).

Concentrations of MC, trichloroethanol and trichloroacetic acid in blood

In a serie of experiments in which volunteers were exposed subsequently to various conditions (250 or 350 ppm (1365-1900 mg/m^3)) at rest or with physical activity of 50, 100 or 150 Watt the arterial and venous blood concentrations of MC were measured (Åstrand et al 1973). During exposure there was a linear relation between the concentration in alveolar air and in arterial blood. The concentration in blood was about 5 times the concentration in alveolar air. The concentration in venous blood was somewhat lower than in arterial blood. The difference in concentration between arterial and venous blood was the smallest at the highest physical activity (150 Watt).

In a serie of experiments in which volunteers were exposed to 70 or 140 ppm (380-764 mg/m^3) of MC for 4 h, the concentrations in blood of MC, TCE and TCA were measu-

red (Monster et al 1979b). The blood concentrations of MC in the postexposure period (up to 64 h) ran parallel to those in alveolar air. The concentration in blood was 5 times higher than in alveolar air. The kinetic behaviour of TCE and TCA in blood was the same as in exposure to trichloroethylene; the concentrations, however, were much lower.

In the experiments in which volunteers were exposed to 100, 350 or 500 ppm of MC for 1,3 or $7^1/_2$ h. Stewart et al (1975) measured concentrations of MC in blood. In exposure to 350 ppm for $7^1/_2$ h/day, 5 days/week the blood concentrations were 0.27 mg/l (before next exposure), 6.06 mg/2 (4 h), 6.44 mg/2 (pre exit) and 5.06 mg/l (15 min post exposure) respectively.

Conclusions from experiments

MC in alveolar air and blood in the first hours after exposure indicate recent exposure concentrations; the concentrations next morning indicate the time weighted average (TWA) concentration over the preceding day(s) and the concentrations after a weekend probably indicate the TWA over the preceding week. TCE in exhaled air, blood, and urine in the first hours after exposure and the next morning indicate the TWA over the preceding day(s). For TCA in blood and urine the time of sampling is not so important; it indicates the TWA over the preceding week.

Extrapolation from human volunteer studies to industrial exposure

As in exposure to trichloroethylene the results of the volunteer studies can be used for extrapolation to concentrations to be expected in occupational (weekly) exposure.

The results of the single exposure experiment — 70 ppm (380 mg/m^3) exposure to 50 ppm (275 mg/m^3)or MC 4 h (Monster et a l 1979 b) were used for extrapolation to a TWA, 8 h/day, 5 days/week. The estimation of TCE and TCA urine concentrations at the end of the workshift (at the latter part of the week) is shown in Table 7, together with the concentrations in urine extrapolated from the repeated exposure experiments to 500 ppm (2750 mg/m^3) of MC, 7 h, 5 days (Stewart et al 1969).

The MC concentrations in alveolar air to be expected in exposure to a TWA exposure to 50 ppm (275 mg/m^3) can be estimated from the mean concentrations measured in the volunteer studies after exposure at rest for 6-8 h, 5 days (Table 6), and are about 15 ppm (80 mg/m^3) at $^1/_2$ h after exposure, about 2 ppm (11 mg/m^3) at 16 h, and about 0.5 ppm (3 mg/m^3) at 64 h after exposure.

Caperos et al (1982) simulated absorption and excretion of MC as well as the formation and elimination of TCE and TCA by a mathematical model. They concluded that theoretically the most suitable method to estimate the exposure is by two determinations, before and after a work shift. Following this procedure, analysis of TCE in urine is more sensitive than determination of MC in breath. TCA is not sensitive enough to provide an estimate for the average exposure during one day.

TABLE 7: Estimated trichloroethanol (TCE) and trichloroacetic acid (TCA) concentrations in urine at the end of the workday in subjects exposed to 50 ppm (275 mg/m^3) of MC 8 h/day, 5 days/week

Reference	TCE mg/g creatinine	TCA mg/g creatinine
From experiments		
Monster et al 1979b	~ 6	~ 2
Stewart et al 1969	~ 4	~ 1.3
From industrial studies		
Seki et al 1979	9.9	3.6
Tada 1969		5 mg/l

Industrial exposure studies

Seki et al (1975) measured the urinary metabolites (in mg/l) in the latter part of the workweek in workers from 3 printing plants.

The workers were exposed to MC for $5^1/_2$ days/week, 8 h/day. There was a linear relationship between the mean MC concentration in air and the mean metabolite concentration in urine. Although the concentrations of TCE and TCA were given in mg/l, almost the same values are to be expected in mg/g creatinine, because for TCE + TCA the values in mg/l and in mg/g creatinine were almost the same. In Table 7 the concentrations in urine are shown for 10 workers exposed to 53 ppm (290 mg/m^3) of MC.

Tada (1969) collected urine from 15 workers in a printing plant exposed to 37 ppm (200 mg/m^3) of MC, 7 h/day. The mean TCA exretion was 3.4 mg/l; recalculation to an exposure to 50 ppm (275 mg/m^3) of MC resulted in an excretion of about 5 mg/l (Table 7).

Nakaaki et al (1978) descibed an investigation in a printing plant. They compared during exposure the inhaled MC concentration with the expired concentration. After 3 h exposure the MC concentration in expired air was about half the inhaled concentration. After 6 h exposure the expired MC concentration was almost equal to the inhaled concentration.

Recently Monster et al (in press) investigated exposure to MC in 4 workshops. About 8 workers were followed up in each workshop. During a whole normal workweek exposure to MC was measured with personal air samplers. The average exposure during the whole week per subject was between 2 and 70 ppm (11-380 mg/m^3). The day to day variation was large: in one third of the subjects the highest daily average was 5 times or more greater than the lowest daily average.

During that week biological specimens were collected (blood, alveolar air, urine) on Monday morning, Wednesday morning and evening, Friday morning and evening, and again on Monday morning. From the relations between the exposure magnitude (as TWA concentration) and the concentrations in biological specimens, the mean concentrations in the biological specimens can be estimated for a TWA exposure to 50 ppm (275 mg/m^3) of MC 8 h/day, 5 days/week (Table 8).

TABLE 8: Mean concentrations of MC, trichloroethanol (TCE) and trichloroacetic acid (TCA) in blood, alveolar air and urine in subjects exposed to a TWA concentration of 50 ppm (275 mg/m^3) of MC, 8 h/day, 5 days/week

Test	Time of sampling after exposure			
	end of exposure	5 - 15 min	16 h	64 h
Blood (mg/l)				
- MC		0.9		0.07
- TCE		0.16		—
- TCA		2.3		1.6
Alveolar air				
- MC, mg/m^3 (ppm)		210(39)	13(2.4)	8(1.5)
- TCE mg/m^3		0.014	0.007	—
Urine (mg/g creatinine)				
- TCE	4.9		2.5	0.9
- TCA	2.5		1.8	1.4

The results indicate that on Monday morning TCA and MC in blood and TCE in urine seem to be relatively good parameters for estimation of the mean weekly exposure. TCA in blood on Friday evening also seems to be a reasonable parameter for estimation of the mean weekly exposure. TCE in urine, blood and alveolar air at the end of the workday and the next morning are usable for estimation of the TWA exposure of the preceding day. The variation between individuals became much smaller when the TWA exposure was estimated over the preceding 2 days. MC in blood and alveolar air at the end of the workday and the next morning seems to represent only a rough estimation of the exposure (large variation between individuals with about the same TWA exposure). The concentrations of TCE and TCA in urine at the end of exposure are somewhat lower than those found by Seki et al (1975) and Tada (1969). This can partly be explained by differences in analysis: Monster et al used a specific gaschromatographic method, while Seki et al and Tada used a nonspecific spectrophotometric method (Fujiwara reaction).

The mean concentration of MC in alveolar air 5-15 min after exposure was only somewhat lower than the mean inhaled concentration. The concentrations 16 h and 64 h after exposure were somewhat higher those measured in the volunteer studies after exposure for 6-8 h over 5 days. This difference probably can be explained by a higher accumulation of MC in (adipose) tissue with weekly exposure compared to that with only 5 days exposure.

Analytical methods

Methods for measuring MC in air and blood have been given by Åstrand et al 1973, Fukabori et al 1976, Riihimäki and Phäffli 1978.

Methods for measuring TCE and TCA in blood and urine are discussed in the subchapter trichloroethylene.

Conclusions

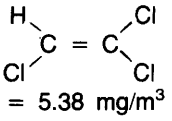
The existing information on MC exposures is limited. *Tentative* group average levels of 1,1,1-trichloroethane, trichloroethanol and trichloroacetic acid in blood, alveolar air and/or urine in occupational exposure to 50 ppm, however, could be presented.

Research needs

More data, particularly from repeated exposure studies and from studies in industry should be available before an adequate proposal for biological monitoring can be presented.

8. Trichloroethene (trichloroethylene, TRI)

Chemical and physical properties

physical state	:	colourless liquid
molecular weight	:	131.4
specific gravity	:	1.45 25°/4°C
melting point	:	—86.8°C
boiling point	:	+86°C
vapour density	:	4.54 (air = 1)
vapour pressure	:	77 mm Hg (25°C)
molecular formule	:	$ClCH=CCl_2$
structural formule	:	

1 mg/m^3 = 0.186 ppm; 1 ppm = 5.38 mg/m^3 at 25°C and 760 mm Hg

Effects on humans

The principal effects of TRI are on the central nervous system: narcotic effects, as induced by organic solvents as such. In addition there exists slight to moderate irritation of mucosae of eyes and airways. There probably are no or hardly any specific effects. When TRI exposure is followed by alcohol consumption, « degreasers'flush » may occur: red blotchs on face, neck, shoulders, due to vasodilatation of skin vessels.

Overexposure leads to confusion, headache, vertigo, dizziness, tremor, nausea, sleeplessness or insomnia, feeling of drunkenness etc. Cardiac failure may occur, particularly in association with mild exercise following exposure to high concentrations. Epidemiological studies in workers have in general not found functional changes of liver and kidney, except in case of preexistent pathology. The odour threshold is above 19 ppm.

Many research groups have studied early effects on psychophysiological performance. Stewart et al (1970) exposed five human subjects to 200 ppm - 7 h for 5 consecutive

days; this induced in 2 subjects a slightly positive Romberg test, and feelings of fatigue at day 4 and 5. Salvini et al (1971) observed in 6 volunteers and in 6 TRI-workers, exposed for 8 h to about 100 ppm, transient irritation of the eyes and decreased performance; other studies at these exposure levels did not show effects on the nervous system at exposure for 2 to 3 h, but in exposure of 15 volunteers to about 300 ppm - $2^1/_4$ h there was a trend to decreased performance in the Bourdon-Wiersma test (Ettema et al 1975). In surveys in industry (NIOSH 1978) vague complaints were registered from workers exposed to average 25 ppm (15-50 ppm). WHO (1981) suggested that subjective symptoms were not expected to occur at exposures not exceeding 25 ppm as TWA.

In animal experiments some evidence of carcinogenicity has been observed (Weissburger 1977); per oral (gavage) administration of 500 or 1000 mg/kg b.w. (TRI in corn oil) in rats, 1200 or 2400 mg/kg b.w. in male mice and 900 or 1800 mg/kg b.w. in female mice 5 d/wk for 78 wk induced hepatocellular carcinomata in mice, but not in rats (only a slightly increased incidence in female rats). Henscher et al. (1980) exposed mice, rats and Syrian hamsters to 100 or 500 ppm pure (not: technical) TRI-6 h/d-5 d/wk-18 months: only in female mice an increased incidence of tumors was observed; there was no dose-response relationship. TRI has weakly mutagenic properties in various tests. Page (1979) concluded that TRI should be regarded at most as a weak carcinogen because of: only liver tumors in one species, weak mutagenicity, only after metabolic activation (into an epoxide), no evidence of human carcinogenicity. In workers Axelson et al (1978), Tola et al (1980) could not find any evidence of human carcinogenicity; Novotna et al (1979) did not find an increased prevalence of TRI-exposure amongst 63 cases of liver carcinoma.

Metabolism

Uptake

By the lungs. The influence of the duration of exposure on the uptake is small. After an initial decrease in retention in 5-15 min the retention remains at the same level (Monster et al 1976, 1979a, Fernandez et al 1975), due to release of TRI from blood by metabolic processes. In alveolar air the retention is about 0.75 (Fernandez et al 1975), but in total (mixed) exhaled air (including physiological deadspace) only about 0.45 (Monster et al 1976, 1979a). The resulting lung clearance (LC) however, is the same: 4.5. l/min. The LC, suggested by Van Rees (1974), indicates which volume of inspired air/min is cleaned from vapour: it is the retention multiplied with the minute volume. This product should be preferred as an indicator of the uptake instead of the retention as such.

Workload during exposure increases the uptake (Monster et al. 1976, Åstrand et al 1976); the retention decreases, but the minute volume increases faster (Table 9). During 100 Watt workload the uptake was 2.5-3.0 times the comparable uptake at rest (Monster et al. 1976), but during the first workload (after 1 h exposure) the uptake was 15% higher than during the second workload (after 3 h exposure): LC 11.5 and 10.0 respectively.

TABLE 9: Uptake of TRI by the lungs during workload (calculated from Åstrand et al 1976)

Test	Condition (after 30 min)			
	rest	50 W	100 W	150 W
Alveolar retention (R)	0.80	0.62	0.50	0.40
Alveolar minute volume, l/min	6.6	22	36	55
Lungclearance (LC), l/min	5.3	13.6	18	22

Through the skin. Immersion of a thumb in liquid TRI during $^1/_2$ h resulted in concentrations of TRI in alveolar air comparable with those in respiratory exposure to about 20 ppm (110 mg/m^3) for $^1/_2$ h (Stewart and Dodd, 1964). There was, however, a considerable individual variation in the rate at which TRI penetrated the skin. The amount of metabolites excreted after immersion of one hand during $^1/_2$ h in liquid TRI was about

one third of the amounts excreted after respiratory exposure to 100 ppm C 535 mg/m^3) during 4 h (Sato et al 1978). The uptake of vapour through the skin is not known, but this will probably be small.

The blood/air and fat/air partition coefficients of trichloroethylene at 37°C are 9-15 and about 750, respectively (Åstrand 1975, Sherwood 1976, Lindqvist 1978, Sato and Nakajima 1979, Monster 1979, Droz and Fernandez 1977).

Biotransformation

Figure 1 illustrates the metabolic pathway. The first reaction product is trichloroethylene oxide. This is transformed into chloralhydrate. Chloralhydrate is partly reduced to trichloroethanol (TCE) and partly oxidized to trichloroacetic acid (TCA). After oral administration TCE is partly metabolized into TCA (Marshall and Owens 1954, Müller et al 1974). TCE is most often conjugated with glucuronic acid before being excreted in urine. Only small amounts of free TCE are excreted in urine and in exhaled air.

The half-life of TRI in exhaled air and blood depends upon the length of exposure and upon the time of sampling after exposure: the concentration follows a multi-exponential curve. After single exposure to TRI, TCE reaches its maximum concentration in blood and urine almost directly after exposure. Thereafter the concentration decreases with a half-life of about 10-15 h (Monster et al 1976, 1979a, Müller et al 1974, Vesterberg et al 1976). After single exposure to TRI the concentration of TCA in blood and urine increases up to 20-40 h after exposure. Thereafter the concentration decreases with a half-life of about 70-100 h (Monster et al 1979a, Müller et al 1974).

TCA as such (in oral administration) has a smaller half-life: about 50 h. The half-life is somewhat larger in oral administration of TCE and of chloralhydrate and about 85-99 h after repeated exposure to TRI, due to delayed formation of TCA from TRI and TCE still available from the tissues (Müller et al 1974).

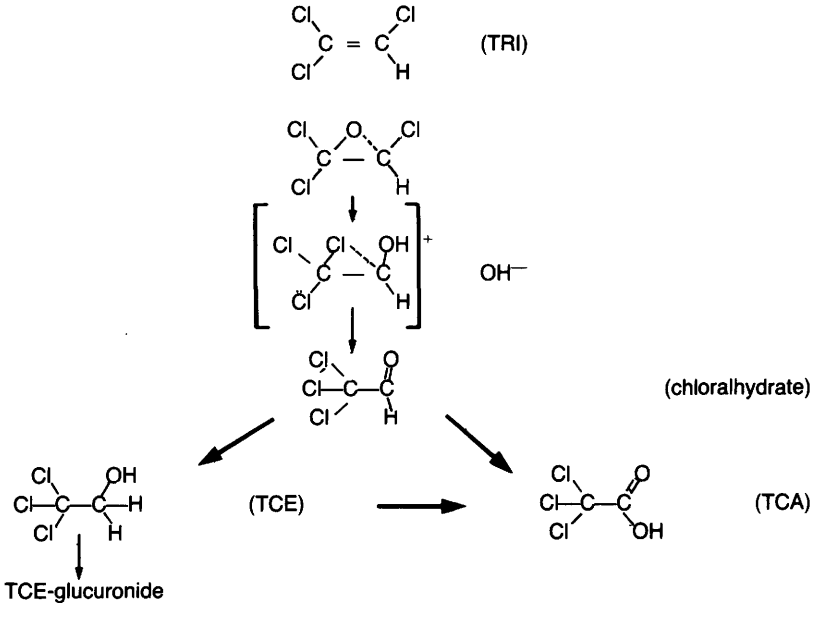


Figure 1. Biotransformation of trichloroethylene (TRI)

Recovery

After respiratory exposure about 10% of the amount taken up is expired unchanged, about 30% to 50% is excreted as TCE in urine, and about 10% to 30% as TCA also in urine (Table 10). Additional pathways of metabolism and elimination may be operative since in these experiments the total recovery accounts for only 50% to 75% of the amount absorbed.

TABLE 10: Percentage of the amount taken up excreted as TRI in expired air and as TCE and TCA in urine

Reference	Expired air %	urine %	
	TRI	TCE	TCA
Monster et al 1976	10	28-51	7-27
Monster et al 1979a	8	29-37	10-21
Souček and Vlachova 1960	—	32-59	10-30
Bartoniček	—	38-49	27-35

Biological indicators

Human volunteer studies

Concentrations of trichloroethylene in exhaled air

Table 11 summarizes data from various studies; the concentrations after exposure in alveolar air (Ce) are expressed as a percentage of the inhaled concentration (Ci) and arranged according to the length of exposure. The following conclusions may be drawn from Table 11:

- The concentration in alveolar air decreases fast in the first half hour after exposure: from about 25% at the end of exposure to about 5% of Ci.
- As the duration of exposure increases, the relative concentration in exhaled air is higher, more pronounced when the sampling after exposure occurs later. After exposure at rest for 6-8 h the average concentration in alveolar air is about 6% to 7% of Ci at $^1/_2$ h, about 4% at 1 h, about 0.6% to 0.7% at 16 h, and about 0.15% after 64 h.
- Fluctuating concentrations influence the early postexposure concentrations; these reflect the concentrations at the end of exposure.
  At 16 h postexposure the alveolar air concentrations are generally in agreement with the TWA exposure (Stewart et al 1974).
- Workload during exposure increases the concentrations in alveolar air over the whole postexposure period.
- Alcohol consumption during exposure increases the concentration in alveolar air.
- The concentration in alveolar air appears to be somewhat lower in women than in men, probably due to higher fatty mass in women.
- In exposure for 5 days compared to 1 day the results are conflicting. The results of Kimmerle and Eben (1973b) show a slight increase of TRI in the early postexposure period at consecutive days compared to the first day. In the study of Monster et al (1979a) only an increase of the alveolar concentration was found in the concentration 16 h after each exposure at consecutive days, higher in subjects with more adipose tissue. The results of Stewart et al (1974) do not show an accumulation at all in alveolar air during the exposure week.

TABLE 11 : Mean concentration of TRI in alveolar air (Ce) after exposure as percentage of the inhaled concentration (Ci)

	Number of subjects[a]	Average Ci (ppm)	Time (h)	Mean Ce as % of Ci ½ h	1 h	2 h	16 h	64 h
Åstrand et al 1976	5 m	100/200+WL[b]	2	6.5	3.7	2.9	0.2	
Åstrand et al 1976	5 m	100+WL[c]	2	9.4	5.2	3.0	0.4	
Åstrand et al 1976	5 m	200+WL[d]	2	9.7	6.4	4.2	0.4	
Nomiyama et al 1971	5 m	320	$2^2/_3$	8.1	6.9	4.8		
Nomiyama et al 1971	5 f	320	$2^2/_3$	5.1	5.2	3.6		
Stewart et al 1974	3 m	20	5x3	3.5	2.5	1.3	0.35	
Stewart et al 1974	3 m	100	5x3	3.5	2.0	0.9	0.3	
Stewart et al 1974	3 m	100 fluct. last. c. 200	5x3	1.9	2.2	1.2	0.34	
Stewart et al 1974	3 f	100	5x3	3.0	1.2	1.1	0.28	
Stewart et al 1974	3 m	200	5x3	3.1	2.1	1.2	0.4	
Stewart et al 1970	2 m	200	$3^1/_4$			2.0	0.45	
Kimmerle et al 1973b	4 m	42	4	4.3	2.9	1.6		
Kimmerle et al 1973b	4 f	42	4	3.6	2.1	1.2		
Kimmerle et al 1973b	4 (3m+1f)	48 1st day	4	2.9	1.4	0.6		
		48 5th day	5x4	2.9	1.7	0.9		
Monster et al 1976	4 m	70	4	4.5	3.4	2.2	0.27	0.033
Monster et al 1976	4 m	70+WL[e]	4	6.0	4.7	3.4	0.34	0.044
Monster et al 1979a	5 m	70 1st day	4	5.2	3.0	2.1	0.17	
Monster et al 1979a	5 m	70 5th day	5x4	4.3	3.4	2.5	0.26	0.039
Stewart et al 1974	2 m	100	4			3.6	0.4	
Stewart et al 1974		100 fluct. last. c.200	4			5	0.3	
Stewart et al 1974		100 fluct. last. c.50	4			2	0.45	
Schäcke et al 1973	12 (10m+2f)	106	4		30	16	1.3	
Monster et al 1976	4 m	140	4	3.1	2.3	1.7	0.18	0.021
Monster et al 1976	4 m	140+WL[e]	4	4.8	4.6	2.5	0.30	0.030
Stewart et al 1974	2 m	200	4			3.5	0.35	
Sato et al 1978	4 m	100	4	4.8	3.0	1.8		
Nomiyama et al 1977	3 m	201	4	5.0	4.0	2.5		
Nomiyama et al 1977	3 m	81	4	4.0	4.0	3.7		
Nomiyama et al 1977	3 m	27	4	4.4	4.8	4.4		
Müller et al 1974	5 m	100	6			4.0	0.20	0.05
Stewart et al 1970	6 m	200	5x7	5.0	4.1	3.5	0.8	0.15
Stewart et al 1974	4 m	20	$5x^1/_2$	6.5	3.5	2.2	0.85	
Stewart et al 1974	4 m	100	$5x7^1/_2$	7.1	3.9	2.7	0.7	
Stewart et al 1974	4 m	100 fluct. last c.100	$5x7^1/_2$	7.2	4.0	2.5	0.92	
Stewart et al 1974	4 f	100	$5x7^1/_2$	5.3	4.0	2.7	0.38	
Stewart et al 1974	4 m	200	$5x7^1/_2$	7.1	5.0	2.7	0.8	0.23
Stewart et al 1974	4 m	200+alc.	$7^1/_2$	15	6.8	3.2	0.9	
Fernández et al 1977		160/135/ 97/56/54	8		5.0	4.0	0.6	0.20

[a] male (m) or female (f)
[b] ½ h 100 ppm, ½ h 200 ppm, ½ h 100 ppm+50 W, ½ h 200 ppm+50 W.
[c] ½ h rest, $1^1/_2$ h + 50 W.
[d] ½ h rest, ½ h 50 W, ½ h 100 W, ½ h 150 W.
[e] during exposure $2x^1/_2$ h 100 W.

Trichloroethanol in exhaled air

Trichloroethanol (TCE) can also be measured in exhaled air with exposure to TRI. A close relation (r = 0.98) was found over a wide range between the concentration of TCE in blood and in exhaled air (Monster et al 1975, 1976, 1979a). The concentration of TCE in alveolar air is about 10,000 times lower than in blood, but it permits prediction of the concentration in blood without blood sampling and does not require the absence of TRI in inhaled air.

Excretion of metabolites in urine

Single exposure. In most investigations the amounts of trichloroethanol (TCE) and trichloroacetic acid (TCA) excreted are expressed in amount par 24 h. In Table 12 the amount excreted in urine in the experiments are arranged according to the exposure magnitude (time x concentration). This enables the following conclusions:

- The major portion of TCE is excreted in the first 24 h after start of exposure.
- The major portion of TCA is excreted in the second and third 24 h after exposure.

The excretion of TCA continues a long time, according the half-life of about 70-100 h. Note that some of the experiments (Stewart et al 1970) seem to have started before the excretion of TCA of preceding exposure was minimal.

- Alcohol consumption during exposure decreases the excretion of TCE and TCA (Müller et al 1975).
- Workload during exposure increases the excretion of TCE and of TCA.
- Males seem to excrete more TCE in the first 24 h after exposure than females but, females seem to excrete more TCA than males; however, the ranges are large.
- The results are not consistent between the experiments. This is probably due to individual differences in metabolism, missing of voidings during daily urine collections, differences in alcohol consumption, and so on.

TABLE 12: Mean amounts of trichloroethanol (TCE) and trichloroacetic acid (TCA) excreted in urine per 24 h after single exposure to TRI

Reference	subjects[a]	Ci (ppm)	Time (h)	Ci time	TCE (mg/24 h) 1st	2nd	3rd	TCA (mg/24 h) 1st	2nd	3rd
Kimmerle et al 1973b	4 m	42	4	168	60	12	5	5	7	10
Kimmerle et al 1973b	4 f	42	4	168	55	22	10	10	13	13
Monster et al 1976	4 m	70	4	280	126	40	12	7	14	12
Monster et al 1976	4 m	70+WL[b]	4	280	162	55	20	9	19	13
Schäcke et al 1973	12 (10 m + 2 f)	106	4	424	130	80	50	4	10	12
Stewart et al 1970	2 m	100	4	400	163	25	4	69	49	66
Stewart et al 1970	2 m	100	4	400	101	13	9	52	57	42
Ogata et al 1973	4 m	170	3	510	max 22 mg/h			max 4 mg/h 70 h after exposure		
Ogata et al 1973	5 m	170	7	1190	max 40 mg/h			max 8 mg/h 50 h after exposure		
Müller et al 1974	5 m	100	5	500	250	50	20	40	40	40
Müller et al 1975	5 m	100	6	600	max 17 mg/h 2-4 h after exposure			max 2.0 mg/h 4 h after exposure		
Müller et al 1975	5 m	100+alc.	6	600	max 7 mg/h			max 0.2 mg/h		
Monster et al 1976	4 m	140	4	560	242	84	17	11	25	20
Monster et al 1976	4 m	140+WL[b]	4	560	272	93	20	16	36	28
Nomiyama et al 1971	5 m	320	$2^2/_3$	854	220	85	50	21	60	55
Nomiyama et al 1971	5 f	320	$2^2/_3$	854	140	90	50	55	80	70
Stewart et al 1970	2 m	200	$3^1/_2$	700	135	57	21	72	63	54
Stewart et al 1970	5 m	200	7	1400	147	71	94	31	48	33
Stewart et al. 1970	2 m	200	7	1400	219	143	100	43	62	104

[a] male (m) or female (f)
[b] during exposure $2x^1/_2$ h 100 W workload

Repeated exposure. In table 13 the amounts of TCE and TCA excreted per 24 h on the first and last day of exposure in the experiments are arranged according to the exposure magnitude (time x concentration).

The following conclusions may be drawn from this table:

- Cumulation of TCE during the week seems to be small, about 50% to 80%.
- Amounts of TCA excreted in urine per 24 h at the fifth day of exposure are 5-10 times higher than at the first day.
- Fluctuating concentrations in air seem to have only small influences on the amounts excreted per 24 h.
- Alcohol consumption during exposure decreases the amounts of TCE and TCA excreted in urine.
- Differences in metabolite excretion between males and females are not marked; there is a tendency towards higher excretion of TCE in male subjects.
- After exposure to 50 ppm (270 mg/m^3) for 8 h, each day, the amounts excreted per 24 h on the fifth day can be calculated from the regression lines: TCE: 220 mg/24 h; TCA: 130 mg/24 h.

TABLE 13: Mean excretion of trichloroethanol (TCE) and trichloroacetic acid (TCA) in urine after repeated exposure to TRI on the first and fifth day of exposure

Reference	sub-jects[a]	Ci (ppm)	time (h)	Ci time	TCE (mg/24 h) day 1	TCE (mg/24 h) day 5	TCA (mg/24 h) day 1	TCA (mg/24 h) day 5
Stewart et al 1974	3 m	20	3	60	24	26	< 33	< 31
Stewart et al 1974	4 m	20	7 1/2	150	37	43	< 22	< 40
Kimmerle et al 1973b	3m+1f	48	4	200	75	100	5	70
Monster et al 1979a	5 m	70	4	280	140	220	10	83
Müller et al 1975	5 m	50	6	300	100	160	20	90
Müller et al 1975	5 m	50+alc.	6	300	70	110	5	65
Ertle et al 1972	5 m	50	6	300	120	220	20	100
Ertle et al 1972	5 m	50 fluc	6	300	130	210	10	110
Stewart et al 1974	3 m	100	3	300	105	174	< 29	111
Stewart et al 1974	3 m	100 fluc	3	300	159	298	64[b]	110
Stewart et al 1974	3 f	100	3	300	118	113	< 25	84
Ertle et al 1972	5 m	100	6	600	180	260	20	250
Stewart et al 1974	3 m	200	3	600	362	429	98[b]	180
Triebig et al 1976	4m+3f	100	6	600	135	155	32	230
Stewart et al 1974	4 m	100	7 1/2	750	135	523	52	253
Stewart et al 1974	4 m	100fluc	7 1/2	750	198	220	165[b]	197
Stewart et al 1974	4 f	100	7 1/2	750	211	296	28	241
Stewart et al 1970	5 m	200	7	1400	308	405	51	391
Stewart et al 1970	4 m	200	7 1/2	1500	450	895	175[b]	390

[a] male (m) or female (f)
[b] carryover from preceding exposure
Note. The regression lines of all data on the 5th day of exposure for TCE and TCA are:
TCE (mg/24 h) = 0.45 (h x ppm) + 39, r = 0.85;
TCA (mg/24 h) = 0.27 (h x ppm) + 20, r = 0.97.

Concentrations in blood of trichloroethylene, trichloroethanol and trichloroacetic acid

Single exposure. In table 14 the concentrations of TRI, TCE and TCA in blood in experiments with human volunteers are arranged according to the exposure magnitude (time x concentration).

TABLE 14: Mean concentrations of trichloroethylene (TRI) trichloroethanol (TCE) and trichloroacetic acid (TCA) in blood after single exposure to TRI

Reference	sub-ject[a]	Ci (ppm)	time (h)	Blood levels TRI (mg/l) end experiment 2 h	TRI (mg/l) 16 h	TCE (mg/l) end experiment 16 h	TCE (mg/l)	TCA (mg/l) end experiment 16 h	TCA (mg/l)
Kimmerle et al 1973b	4 f	40	4	0.30	0.1	1.4	0.53		
Kimmerle et al 1973b	4 m	40	4	0.27	0.09	1.0	0.4		
Vesterbeg et al 1976	5m	100	2+Wl[b]	2.2	0.4	2.8	0.8(20h)	3.0	13
Vesterberg et al 1976	5 m	100+200	2+WL[c]	3.3	0.5	3.0	1.0(20h)	2.8	13.5
Vesterberg et al 1976	5 m	200	2+WL[d]	7.4	0.9	3.9	1.6(20h)	3.9	18
Monster et al 1976	4 m	70	4	1.3	0.16	0.011	3.2	0.67	3.3	9.0
Monster et al 1976	4 m	70	4+WL[e]	—	0.21	0.020	4.0	1.2	3.6	9.7
Konietzko et al 1975	20 m	95	4				3.0			
Sato et al 1978	4 m	100	4	1.7	0.17					
Müller et al 1974	5 m	100	5(5 1/2)	1.2	0.34	0.07	6.0[f]	1.7[f]	20[g]	50(20h)[g]
Monster et al 1976	4 m	140	4	—	0.27	0.020	5.2	1.8	4.0	12
Monster et al 1975	4 m	140	4+WL[e]	—	0.38	0.036	5.8	2.2	4.5	16
Müller et al 1975	5 m	100	6	1.3	0.4	(0.1)	4.5[f]	1.2[f]	12[g]	28[g]
Müller et al 1975	5 m	100	6+alc	2.7	0.9	(0.2)	2.0[f]	1.0[f]	0[g]	4[g]

[a] male (m) or female (f)
[b] 1/2 h, rest, 1 1/2 h 50 Watt workload
[c] 1/2 h 100 ppm, 1/2 h 200 ppm, 1/2 h 100 ppm + 50 Watt, 1/2 h 200 ppm + 50 Watt
[d] 1/2 h rest, 1/2 h 50 Watt, 1/2 h 100 Watt, 1/2 h 150 Watt
[e] during exposure 2 x 1/2 h 100 Watt workload
[f] nonglucouronidized fraction of TCE
[g] TCA in plasma

The following conclusions may be drawn from this table:

- TRI concentration in blood rises sharply after the start of exposure, but thereafter it levels off; at the end of exposure TRI concentration in blood decreases sharply, but not as sharply as in exhaled air (Åstrand et al 1976).
- TCE concentration in blood rises sharply during exposure and reaches a maximum in the first hour after exposure; thereafter the concentration declines rapidly and exponentially with a half-life of 10-15 h.
- TCA concentration in blood continues to rise even after the end of exposure; the increase persists up to 20-40 h after exposure; thereafter the concentration decreases exponentially with a half-life of 70-100 h.
- In blood two fractions of TCE exist: free TCE and TCE comjugated with glucuronic acid.
- Concentrations of TCA in plasma are higher by about a factor of 2 than in total blood, due to the high binding of TCA to plasma proteins.
- Workload during exposure increases the concentrations of TRI, TCE and TCA in blood.
- Alcohol consumption during exposure increases the concentration of TRI and decreases the concentrations of TCE and TCA in blood.
- Differences in absolute values between comparable experiments may be due to differences in analysis or to interindividual differences.

Repeated exposure. In table 15 the concentrations of TRI, TCE, and TCA in blood in repeated exposure experiments are arranged according to the exposure magnitude (time x concentration).

The following conclusions may be drawn:

- With each further exposure the values of TRI in blood recorded before starting the next exposure show a progressive daily increase (after the fifth day 2 x higher than at second day).
- With each further exposure both the maximum value of TCE (end exposure) and the minimum value (before starting next exposure) show a progressive daily increase (after the fifth exposure day 20% to 50% higher than after the first exposure day).
- TCA cumulates progressively from day to day and reaches a maximum concentration at the end of the fifth exposure day.
- Alcohol consumption during exposure decreases both TCE and TCA concentrations in blood.
- Fluctuating concentrations hardly affect the TCE and TCA concentration in blood.
- Differences in absolute values between comparable experiments may be due to differences in analysis or to interindividual differences.

TABLE 15: Mean concentrations of trichloroethylene (TRI), trichloroethanol (TCE) and trichloroacetic acid (TCA) in blood after repeated exposure to TRI

Reference experiment	subjects	Cl (ppm) 16 h	time (h)	day	TRI (mg/l) end experiment	TRI (mg/l) 2 h	TRI (mg/l) 16 h	TCE (mg/l) end experiment	TCE (mg/l) 16 h	TCA (mg/l) end experiment	TCA (mg/l) 16 h
Kimmerle et al 1973b	4	48	4(x5)	1	0.3	0.18		2.0	1.0		
				5	0.7			2.4	0.9		
Monster et al 1976	5	70	4(x5)	1	1.30	0.22	0.009	3.4	0.9	3	8
				5	1.32	0.22	0.018	4.2	1.2	31	30
Müller et al 1972	5	50	6(x5)	1				1.6	0.4	15	18
				5				2.2	0.7	52	49
Müller et al 1975	5	50	6(x5)	1				1.5	0.45	15	18
				5				2.2	0.6	52	49
Müller et al 1975	5	50	6(x5)	1				1.2	0.4	4	6
			+alc	5				1.5	0.5	25	31
Ertle et al 1972	5	50	6(x5)	1				1.7	0.5		
				5				2.0	0.8		
Ertle et al 1972	5	50[b]	6(x5)	1				1.6	0.5		
				5				2.3	0.7		
Ertle et al 1972	5	100	6(x5)	1				3.2	1.0		
				5				4.9	1.5		
Triebig et al 1976	7	100	6(x5)	1	1.3		1.2	6.5	4	11	32
				5	1.5			10	(6)	66	

[a] nonglucuronidized fraction of TCE and TCA in plasma
[b] mean 50 ppm, fluct. 250 ppm 12 min/h

Conclusions from volunteer experiments

TRI levels in alveolar air and in blood in the first hours after exposure indicate recent exposure concentrations. The concentration next morning before exposure indicates the TWA concentration over the preceding day(s) and the concentration after a weekend probably indicates the TWA over the preceding week. TCE levels in exhaled air, blood, and urine in the first hours after exposure and the next morning indicate the TWA over the preceding day(s). For TCA in blood and urine the time of sampling is not so important; it indicates the TWA over the preceding week.

Industrial exposure studies

Several researchers (Ogata et al 1971, Tanaka and Ikeda 1968, Ikeda et al 1972) have recommended measuring both metabolites (TTC = TCA + TCE) to estimate TRI exposure. This approach may also take into account individual differences in metabolism. However, since the biological half-lives of both metabolites are different, the time and duration of urine sampling is quite critical. Furthermore, during industrial operation where the magnitude of the exposure fluctuates during the day and from day to day, it is difficult to correlate the TTC value with the exposure on the preceding day in view of the fact that the major portion of TCE formed appears the same day in urine, whereas only a comparatively small portion of the TCA is excreted during the same period.

Ogata et al (1971) examined 10 men exposed to 10-40 ppm (54-214 mg/m^3) daily. The concentration was determined with Kitagawa tubes near the nose of each worker every 20 min. The average was taken as environmental concentration. The time of urine sampling was from 12.00-17.00 h on Wednesday. The concentrations were corrected to a urine density of 1.024. Ikeda et al (1972) examined 51 male workers in 10 workshops, in which the atmospheric concentrations of the solvent were relatively constant. The concentration in each workshop was determined at least 5 times with Kitagawa tubes. The average, 3-175 ppm (16-940 mg/m^3), was taken to represent the environmental concentration. Urine samples were collected in the latter half of the week around 15.00 h after urine was excreted at about 13.00 h. From the relations given by these authors the concentrations of TCE and TCA per gram creatinine can be calculated for an exposure to 50 ppm (270 mg/m^3) TRI (Table 16). Thus an exposure to 50 ppm (270 mg/m^3) TRI would correspond to an excretion of about 180 mg TCE/g creatinine and to 100 mg TCA/g creatinine in urine collected at the end of the work period.

Only a few data exist on the blood concentrations during occupational exposure. Pfäffli and Backman (1972) measured TRI concentrations in blood and expired air during and after exposure. Particularly in mixed exposure 1,1,1-trichloroethane seems to delay the excretion of TRI. Lindner (1973) measured the TCE concentration in blood before the exposure (0.5-2.7 mg/l) and in the middle of the worktime (1.2-4.0 mg/L). The exposure concentration was about 40 ppm (215 mg/m^3) TRI.

TABLE 16: Estimated concentrations of trichloroethanol (TCE) and trichloracetic acid (TCA) in urine at the end of the workday in subjects exposed to 50 ppm (270 mg/m^3) TRI, 8 h/day, 5 day/week

Reference	TCE (mg/g creatinine)	TCA (mg/g creatinine)
From industrial studies:		
Ogata et al (1971)	~150	~ 90
Ikeda et al (1972)	~215	~105
From experiments		
Repeated exposure experiments (Table 13)	160	100
Monster et al (1979a)	220	100
Gubéran (1977)	155	100

Extrapolation from human volunteer studies to industrial exposure

The results of the volunteer studies can be used for extrapolation to concentrations to be expected in occupational (weekly) exposure. The amounts of TCE and TCA in 24 h urine can be recalculate to amounts per gram creatinine assuming an excretion of 1.8 g creatinine/day. One has to keep in mind that at the end of the work period the

excretion rates of the metabolites are maximal. The excretion rate of TCE at the end of exposure is about 20% to 40% higher than over the whole 24 h. The excretion rate of TCA during the daytime is about 10% higher than over the whole 24 h and for TCA there is also a carryover of 20% to 30% from the preceding exposure week.

In the repeated exposure experiments the mean amounts excreted in urine per 24 h (220 mg TCE, 130 mg TCA) in exposure to 50 ppm (270 mg/m^3), 8 h, 5 days can be calculated. These amounts were used for the estimation of the urine concentrations at the end of the workshift (in the latter part of the week) and are presented in Table 16. The amounts excreted in urine in the repeated exposure experiment of Monster et al (1979a) were also used for extrapolation. Gubéran (1977), using a mathematical model for the simulation of the pharmacokinetics of inhaled TRI, calculated biological values in exposure to 100 ppm (535 mg/m^3) TRI. Their values recalculated to 50 ppm (270 mg/m^3) and to mg/g creatinine are also presented in Table 16.

The concentrations in alveolar air to be expected in exposure to 50 ppm (270 mg/m^3) can be estimated from the mean concentrations measured in the volunteer studies after exposure at rest for 6-8 h, 5 days (Table II) and are about 3-4 ppm (16-21 mg/m^3) at $^1/_2$ h after exposure, about 0.3-0.4 ppm (1.6-2.1 mg/m^3) at 16 h and about 0.1 ppm (0.54 mg/m^3) at 64 h after exposure.

Droz and Fernández (1978) used a mathematical model to study the influence of hourly variations and daily variations in exposure concentration on the alveolar TRI concentrations and on the excretion of TCE and TCA in urine. They calculated that two analyses, one before and one after exposure (morning urine), theoretically are sufficient to estimate the degree of exposure whatever day of the week is considered and whatever the daily variations are in exposure concentrations. Theoretically the analysis of TCE in urine appears to be better than analysis of TRI in exhaled air with regard to sensitivity. The excretion of TCA is not directly and simply connected with the degree of exposure; it can be used for qualitative evaluation of the preceding day's exposure. The authors note that blood analyses could in practice be preferable to analysis of urine, because of the smaller individual variations generally observed with the former. Indeed in experiments with repeated exposure to constant concentrations the smallest interindividual variation was found for the concentrations in blood (Monster et al 1979a).

Analytical methods

Exhaled air

Various methods have been described for the determination of TRI in exhaled air. Most of them are based on collecting exhaled air (alveolar or mixed exhaled air) into glass tubes or bags (Saran, Tedlar). The determination can be done by infrared (Stewart et al 1970, Schäcke et al 1973) or by gaschromatography (Müller et al 1974, Stewart et al 1974, Monster and Boersma 1975, Kimmerle and Eben 1973a). Monster and Boersma (1975) also determined TCE in exhaled air.

Urine

Spectrophotometric methods have been described for the determination of TCA and TCE in urine (Tanaka and Ikeda 1968, Ogata et al 1970, Weichardt and Bardodej 1970, Ogata et al 1974). Basically these methods involve the hydrolysis of the glucuronide of TCE in urine, followed by its oxidation to TCA. TCA is then determined colorimetrically by the Fujiwara reaction. This reaction is not specific, and the natural presence of chromophore substances in biological fluids may interfere. Very sensitive and specific gaschromatographic techniques are also available for TCE and TCA in urine (Stewart et al 1974, Buchet et al 1974, Humbert and Fernández 1976, Breimer et al 1974, Ogata and Saeki 1974).

Blood

Various gaschromatographic methods have been described for the determination of TRI, TCE and TCA in blood (serum). Determinations of TRI are based on extraction or headspace analysis. Determinations of TCE are based on extraction or headspace analysis of free TCE and total TCE. Determinations of TCA are based on decarboxylation to chloroform or esterification to TCA-methyl ester. The methods are descibed for TRI, free TCE, and chloralhydrate (Kimmerle and Eben 1973a); for TCA in plasma (Müller et al 1972), for total TCE in plasma (Muller et al 1975); for TRI, TCA, free TCE,

and alcohol (Herboltsheimer and Funk, 1974; for chloralhydrate, free TCE, total TCE, TCA, and monochloroacetic acid (Ogata and Saeki, 1974); for free TCE, total TCE, chloralhydrate, and TCA (Breimer et al. 1974); for TRI, total TCE, and TCA (Monster and Boersma 1975); for TCE, chloralhydrate, and TCA (Garret and Lambert, 1966); for free TCE, total TCE, and TCA (Vesterberg et al. 1975). Most of these determinations are based on separate analysis of TRI, TCE and TCA and use time-consuming extractions and hydrolysis. Only Monster and Bœrsma (1975) described a method for simultaneous determination of TRI, total TCE and TCA.

Conclusions

Among the chlorinated hydrocarbon solvents TRI has been studied most fully. Nevertheless only suggestions can be made for average group parameters in relation to exposure.

In table 17 the concentrations to be expected in a TWA exposure to 50 ppm are summarized. These data are only approximate.

Research needs

There still is a need for well designed epidemiological studies. Moreover, these studies should also pay attention to interindividual differences in toxicokinetics, and to the factors responsible for these: anthropometric measures, sex, genetic make up, and use of alcohol and drugs. This will make it possible to estimate individual exposure.

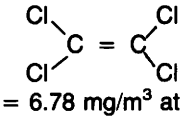
TABLE 17: Estimated concentrations of trichloroethylene (TRI), trichloroethanol (TCE) and trichloroacetic acid (TCA) in alveolar air, blood, and urine in subjects exposed to 50 ppm (270 mg/m^3) TRI, 8 h/days, 5 days/week

medium	End exposure	Time (h)		
		$^1/_2$	16	64
Alveolar air				
TRI (ppm)	10-15	3-4	0.3-0.4	0.1
TRI (mg/m^3)	50-80	16-21	1.6-2.1	0.5
TCE (mg/m^3)	0.4-0.6			
Blood (mg/l)				
TRI	0.9	0.2	0.02	0.006
TCE	4-6		1-2	?
TCA	60-70		60-70	35-50?
Urine (mg/g creatinine)				
TCE	180		120	15?
TCA	100		80	50?

? = uncertain, doubtful

9. Tetrachloroethene (tetrachloroethylene, perchloroethylene, perc.)

Chemical and physical properties

physical state	:	colorless liquid
molecular weight	:	165.9
specific gravity	:	1.62 24°/4°C
melting point	:	—23.3°C
boiling point	:	121.2°C
vapour density	:	5.7 (air = 1)
vapour pressure	:	19 mm Hg at 25°C
molecular formule	:	$CCl_2 = CCl_2$
structural formule	:	

1 mg/m^3 = 0.147 ppm; 1 ppm = 6.78 mg/m^3 at 25°C and 760 mm Hg

Effects on humans

Tetrachloroethylene has narcotica and irritative properties and is degreasing for the skin: these non-specific effects are comparable to those from the other chlorinated-hydrocarbon-solvents. In addition it may exert toxic effects in liver and kidneys, more so than trichloroethylene.

Tetrachloroethylene has had a wide spread use in the tropics as a drug in case of ancylostomiasis (dosage 11-206 mg/kg b.w.); only in a few cases serious effects have been observed with a dosage of 26 mg/kg b.w.

In exposure of 6 subjects for 2 h to 200 ppm irritation of mucosae, drowsiness and dizzeness occurred (Stewart et al 1961); in exposure of 16 subjects 1 to 5 days for 7 h to 100 ppm again slight irritation, disturbed equilibrium and light headedness was observed (Stewart et al 1970). In 32 subjects 20 to 54 times exposed to 25-50 100 or 150 ppm for 1, 3, $5^1/_2$ or $7^1/_2$ h/day performance in various tests altered, no potentiation of effects in additional intake of diazepam or ethanol was observed (Stewart et al 1976a).

Chmielewski et al (1976) examined 9 workers (A) exposed to levels in air below 30 ppm and 16 workers (B) exposed to 60-440 ppm for 2 months to 27 years. A slight increase of albumin and decrease of α-globulin fractions in serum and slight excretion of homovalic acid was observed in group A; in group B 4 workers showed EEG-abnormalities, increased activity of alanine-and aspartate-aminotransferase, decreased activity of acetylcholinesterase and increased α-lipoprotein-fraction in serum. Essing (1973 and 1975) examined 112 workers exposed for average 11.5 year to tetrachloroethylene: 74% of air samples were 0-61 ppm, 11% 61-116 ppm, 14% 116-190 ppm; 120 subjects served as controls. Drowsiness, nausea, anorexia, perspiration, impotence, tremor and decreased sensibility in the fingers were reported; the serum levels of SGOT, SGPT, bilirubin and alkalic phosphatase were lower than in the control group, i.e. no evidence of live dysfunction. The serum-creatinine level was increased in the exposed group; no increased levels of ureum in urine, specific weight or decreased phenolred-excretion was observed, i.e. no evidence of renal dysfunction.

Blair et al (1979) reported in the USA an increased proportional mortality for malignant tumours in drycleaning workers, exposed to tetrachloroethylene and also to carbon tetrachloride, trichloroethylene and petroleumsolvents. These preliminary findings were based upon a limited study. Katz and Jowett (1981) studied the proportional mortality pattern of 671 female laudry and drycleaning workers for the period 1963-1977 in Wisconsin, USA; they could not find any overall increase in malignant tumours, although indications for an elevated risk was observed for cancers of kidney and genitals, along with smaller excess of bladder and skin cancer and lymphosarcoma. This study could not find any specific relation with exposure to tetrachloroethylene, but it might serve as a signal for further study.

According to IARC (1979) there is only limited evidence that tetrachloroethylene is carcinogenic in mice. The epidemiological data discussed above were not yet evaluated by IARC.

Metabolism

Uptake

By the lungs. The retention and the uptake (lung clearance, LC) decrease in the course of exposure at rest (Table 18), but not as fast as in exposure to 1,1,1-trichloroethane (methylchloroform, MC). The solubility of PERC in blood and adipose tissue is higher than of MC (higher partition coefficients for blood/air and fat/air); therefore the capacity of the body to absorb PERC is higher than for MC. The blood/air and fat/air partition coefficients of PERC at 37°C are about 15 and 2000 respectively (Sato and Nakajima 1979, Monster 1979, Droz and Fernandez 1977).

Workload during exposure increases the uptake. The uptake during 100 Watt workload was 2.5-3.5 times the comparable uptake at rest, but during the first workload (after 1 h exposure) the uptake was about 20% higher than the uptake the second workload (after 3 h exposure): lungclearance 12.6 and 10.3 l/min respectively (Monster et al 1979c).

Through the skin. Immersion of one thumb in liquid PERC during 40 min resulted in a concentration of PERC in alveolar air comparable with the concentrations after a respiratory exposure to 10-15 ppm (70-105 mg/m^3) for 40 min (Stewart and Dodd 1964).

Subjects, dressed in pyjamas, socks and a full facepiece respirator, were exposed to PERC vapour (Riihimäki and Pfäffli 1978). The amount of PERC exhaled after exposure was about 1% of the amount that should be exhaled in exposure to the same concentration without a respirator.

Biotransformation

A small amount of PERC absorbed in the body is probably transformed by oxidation to perchloroethylene oxide and subsequently, by rearrangement, to trichloroacetyl chloride, which is hydrolyzed into trichloroacetic acid (TCA) (Yllner 1961, Daniel 1963).

The half-life of PERC in blood and expired air depends on the length of exposure and on the time of sampling after exposure. The concentration follows a multiexponential curve.

The toxicokinetic behavior of TCA after exposure to PERC is similar to its behaviour after exposure to trichloroethylene and to 1,1,1-trichloroethane.

The concentration of TCA in blood increases up to 20 h after single exposure; thereafter the concentration decreases with a half-life of about 80 h (Monster et al 1979c). Tetrachloroethylene is almost totally (80% to 100%) excreted by exhalation; only small amounts are excreted in urine as TCA (2%) (Fernandez et al 1976, Monster et al 1979c).

TABLE 18: Uptake by the lungs of PERC at rest

Reference		time after start of exposure					
		minutes				hours	
		0	10	30	60	4	8
Fernandez et al. (1976)	alveolar retention			0.75	0.70	0.60	0.47
Monster et al. (1979c)	alveolar retention	~0.90	0.80	0.74	0.69	0.60	
Monster et al. (1979c)	lungclearance (LC) (l/min)	6.7	5.0	4.5	4.2	3.8	

Biological indicators

Human volunteers

Concentrations of tetrachloroethylene in exhaled air

Table 19 summarizes data from various studies. The concentrations after exposure in alveolar air (Ce) are expressed as a percentage of the inhaled concentrations (Ci) and arranged according to the length of exposure.

The following conclusions may be drawn from this table:

- The concentration in alveolar air decreases in the first $^1/_2$ h after exposure from 40% — 50% (end exposure, Table 18) to 15% — 30% of Ci.
- As the duration of exposure increases, the relative concentration in alveolar air is higher, more pronounced when the sampling after exposure occurs later. After exposure at rest for 6-8 h, the mean concentration is about 23% ($^1/_2$ h), 18% (1 h), 4% (16 h), and 2% (64 h), respectively.
  After exposure at rest for 6-8 h, 5 days, the mean concentration is about 35% ($^1/_2$ h), 30% (1 h), 8% (16 h), and 5% (64 h), respectively.
- Workload during exposure increases the concentrations in alveolar air over the whole postexposure period.

Excretion of the metabolite trichloroacetic acid in urine

In table 20 the amounts of trichloroacetic acid (TCA) excreted per 24 h in the volunteer studies with single exposure are arranged according to the exposure magnitude (time x concentration).

TABLE 19: Mean concentrations of PERC in alveolar air (Ce) after exposure as percentage of the inhaled concentration (Ci)

Reference	number of subjects	Ci (ppm)	time (h)	mean Ce as % of Ci					
				$^1/_2$ h	1 h	2 h	16 h	64 h	120 h
Stewart et al 1961	6	194	3	13	9	6	1.1	0.44	0.26
"	6	101	3	12	8.5	6	1.0		
Essing et al 1972	5	69	3			7.2	1.2		
Stewart et al 1961	1	395	3.5	23	17	13	4.3	1.5	0.88
Fernandez et al 1976	1	100	4	18	12	9			
"	1	150	4	17	12	7			
"	1	200	4	19	13	8			
Monster et al 1979c	6	70	4	16	14	12	2.1	0.65	0.30
"	6	140	4	16	14	13	1.9	0.65	0.32
"	6	140+WL[a]	4	20	17	15	2.5	0.78	0.39
Hake and Stewart 1977	—	25+WL[b]	5.5	26					
"	—	100+WL[b]	5.5	18					
Fernandez et al 1976	1	150	6	19	15	10			
"	5	100	8	20	15	12	4	2	
"	4	150	8	27	21	16	4	2	
"	3	200	8	25	19	14	4	2	
Stewart et al 1970	15	100	7			20	3.5	2.0	1.3
"	5	100	5x7			30	8	5	3.0
Gubéran and Fernandez 1974	Model	100	5x8	35	31	25	7.5	5.0	
Hake and Stewart 1977	Model	100	5x7.5			25	8	4.5	

[a] during exposure 2 x 0.5 h 100 W workload
[b] during exposure 0.5 h 50 W workload

TABLE 20: Mean amounts of trichloroacetic acid (TCA) excreted in urine per 24 h after single exposure to PERC

Reference	number of subjects	Ci (ppm)	time (h)	Ci x time	TCA (mg)		
					0-24 h	24-48 h	48-72 h
Ogata et al 1971	4	87	3	261	2.4	1	0.7
Bolanowska and Golacka 1972	5	58	5	290	2-4	—	—
Monster et al 1979c	6	70	4	280	4.0	1.0	0.9
	6	140	4	560	6.8	2.3	2.1
	6	140+WL[a]	4	560	8.3	2.8	1.8
Fernandez et al 1976	2	150	8	1200	11-12	9-10	3.5-4

[a] 2 x 0.5 h 100 W workload.

In contrast to exposure to trichloroethylene and to 1,1,1-trichloroethane (MC) the amounts of TCA excreted in the first 24 h after start of exposure were higher than in the following 24 h. This difference can be explained by the difference in metabolism. The formation of TCA in exposure to PERC is mainly determined by the amount of PERC in the body, whereas in exposure to MC the formation is mainly determined by the amount of trichloroethanol (TCE). In exposure to trichloroethylene the formation of TCA has an intermediate position: a direct formation from chloralhydrate and indirectly from TCE (Monster et al 1979c). In repeated exposure experiments with exposure up to 150 ppm (1035 mg/m^3) for 7$^1/_2$ h/day only traces of TCA and no TCE could be found (Hake and Stewart 1977).

Concentrations of tetrachloroethylene and trichloroacetic acid in blood

Only few data on blood levels exist in volunteers exposed for at least 3-4 h. In a series of experiments in which volunteers were exposed to 70 or 140 ppm (480, 965 mg/m^3) for 4 h the concentrations in blood of PERC and TCA were measured (Monster et al 1979c). The concentrations of PERC in the postexposure period (up to 150 h) ran parallel to those in alveolar air. The concentration in blood was 16 times higher than the concentration in alveolar air (Monster 1979, Monster et al 1979c).

Hake and Stewart (1977) mentioned that the exposure concentration had a greater effect on the blood level than the length of exposure. The blood levels did not seem to increase much after 3 h of exposure; however, 30 min of moderate exercise (50 W) during exposure increased the venous blood levels about three to fourfold over that expected after an equivalent time of sedentary exposure. Alcohol consumption had no effect on the concentration of PERC in blood or in exhaled air during exposure.

Conclusions from experiments

The repeated exposure experiments indicate that PERC accumulates in the body; therefore, PERC in alveolar air and in blood in the first hours after exposure probably indicates more a time weighted average (TWA) exposure than the recent exposure concentration; the concentration next morning and after the weekend probably indicates the TWA exposure over the preceding days or weeks. For TCA in blood and urine the time of sampling is not so important; it indicates the TWA exposure over the preceding week.

Extrapolation from human volunteers studies to industrial exposure

The results of the single exposure experiments — 70 ppm (480 mg/m^3), 4 h — (Monster et al 1979c) were used for extrapolation to a TWA exposure of 50 ppm (345 mg/m^3) 8 h/day, 5 days/week.

The estimation of TCA in urine at the end of the workshift (at the latter part of the week) is shown in Table 21. The concentrations of PERC in alveolar air to be expected in a TWA exposure to 50 ppm (345 mg/m^3) can be estimated from the mean concentrations measured in the volunteer studies after exposure at rest for 6-8 h, 5 days (Table 19); they are about 18 ppm (120 mg/m^3) at $^1/_2$ h after exposure, about 4 ppm (28 mg/m^3) at 16 h, and about 2.5 ppm (17 mg/m^3) at about 64 h after exposure.

TABLE 21

Estimated concentrations of trichloroacetic acid (TCA) and trichloroethanol (TCE) in urine at the end of the workday in subjects exposed to 50 ppm (345 mg/m^3) PERC, 8 h/day 5, days/week

Reference	TCA	TCE
From experiment		
Monster et al 1979c	3.9 mg/g creatinine	0
From industrial studies		
Ikeda et al 1972a	45 mg/l	30 mg/l
Weichart et al 1975	~ 3 mg/l	~ 0.7 mg/l

Industrial exposure studies

Ikeda et al (1972a) measured TCA and TCE excretion in 34 workers during 6 days/week in 2 h samples at the end of the workday. They found about 45 mg TCA/l and 30 mg TCE/l at exposure levels of 50 ppm (345 mg/m^3), and about 50 mg TCA/l and 30 mg TCE/l at 100 ppm (690 mg/m^3). Despite the large variation they concluded that the urinary concentrations increased until the atmospheric concentrations reached 50-100 ppm, but little increase occurred at higher concentrations. In another study they measured 4-35 mg TCA/l in 4 workers exposed daily to maximal 20-70 ppm (140-180 mg/m^3) and 32-97 mg TCA/l in 66 workers exposed to maximal 200-400 ppm (1380-2760 mg/m^3). The TCE concentrations were at the same order of magnitude.

In urine of workers the same authors (Ikeda and Imamura 1973) measured a half-life for total trichlorocompounds (TCE + TCA) of about 144 h. The urine samples were obtained on Saturday, Sunday, and Monday morning.

Ikeda et al (1972a.b 1973) used a modification of the Fujiwara reaction. The Fujiwara reaction, however, is not specific for TCE and TCA. An increase was observed in coloration when the urine samples from tetrachloroethylene-exposed workers were oxidized before the Fujiwara reaction. This increase was tentatively attributed to TCE.

Weichart and Lindne (1975) examined workers in drycleaning shops; the average concentration was about 15 ppm (105 mg/m^3). They measured with a gas-chromatographic method PERC and TCA in blood, and TCA and small amounts of TCE in urine. The authors did not present any information on the time of sampling of blood and urine, so the data only provide limited information. The mean concentrations in urine extrapolated to 50 ppm (345 mg/m^3) are presented in Table 21.

Recently Monster et al (in press 1982b) investigated exposure to PERC in 3 drycleaning shops. About 8 persons were followed up in each workshop. During a whole normal workweek the exposure to PERC was measured with personal air samplers. The average exposure over the workweek varied from 1.5 ppm (10.5 mg/m^3) to 160 ppm (110 mg/m^3). During that week biological specimens were collected (blood, alveolar air, urine) on Monday morning, Wednesday and Friday evening, and again Monday morning.

From the relations between the exposure magnitude (as TWA) and the concentrations in biological specimens, the mean concentration in blood, alveolar air, and urine can be estimated in a TWA exposure to 50 ppm (345 mg/m^3), 8 h/day, 5 days/week (Table 22). The results indicate that PERC in blood on Friday after work seems to be a relatively good parameter for estimation of the TWA-week exposure. For PERC in alveolar air and TCA in blood the spread in the results was somewhat larger. TCE in urine was more related to the TWA exposure over the (two) preceding day(s) than to the TWA-week exposure.

TABLE 22: Mean concentrations of PERC, trichloroethanol (TCE) and trichloroacetic acid (TCA) in blood, alveolar air and/or in urine in subjects exposed to a TWA exposure of 50 ppm (345 mg/m^3) PERC 8 h/day, 5 days/week

	Time of sampling after exposure		
Test	end of exposure	5-15 min	64 h
Blood (mg/l)			
PERC		2.3	0.82
TCE		—	—
TCA		5.8	3.8
Alveolar air (PERC)			
mg/m^3		160	53
ppm		23	8
Urine (mg/g creatinine)			
TCA	9.7		4.9
TCE	0.5		—

The concentration of PERC in alveolar air in this study is higher (especially at 64 h after exposure) than estimated from the repeated exposure esperiments (Table 19).

The reason for this may be underestimation of the accumulation of PERC in the repeated exposure experiments compared to weekly exposure (accumulation in adipose tissue).

The concentration of TCA in urine in this study is also higher than estimated from the experiments, but much lower than measured by Ikeda et al (1972a). The last difference can partly be explained by differences in analysis: a specific gaschromatographic method versus an a specific spectrophotometric method.

In the industrial studies TCE was measured in urine, while no TCE could be detected in the experimental exposure studies. The reason for this difference may be that small amounts of other chlorinated hydrocarbons are present in PERC used in industry or that the metabolism of PERC changed after a certain period of exposure to PERC.

Analytical methods

Methods for measuring PERC in air and blood have been given by Riihimäki and Pfäffli 1978, Essing et al 1972, Stewart et al 1970.

Methods for measuring TCE and TCA in blood and urine have been discussed in the part trichloroethylene.

Conclusions

The data published are limited in scope and do not yet allow to propose sufficiently validated quantitative biological monitoring methods.

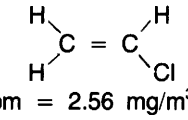
In Table 22 group average data on levels of tetrachloroethylene trichloroethanol and trichloroacetic acid in blood, alvelar air and/or urine in occupational exposure to 50 ppm have been presented.

Research needs

Because there apparently is a cumulation of PERC (and TCA) in the body when exposed for days and weeks, adequately controlled observations in industry in workers exposed for at least a few weeks are needed to establish valid biological monitoring parameters.

10. Monochloroethene (vinyl chloride, VC)

Chemical and physical properties

physical state	:	colorless gas
molecular weight	:	62.5
melting point	:	—153.7°C
boiling point	:	—13.9°C
vapour density	:	2.15 (air = 1)
vapour pressure	:	2580 mm Hg at 20°C and 760 mm Hg
molecular formule	:	$CH_2 = CHCl$
structural formule	:	

1 mg/m^3 = 0.391 ppm; 1 ppm = 2.56 mg/m^3 at 25°C and 760 mm Hg.

Effects on humans

According to Rowe (1974) vinylchloride has a rather low acute toxicity; it is narcotic in action (and actually used as such). Spirtas et al (1975) performed a questionnaire survey on a population of white vinylchloride polymerisation workers (n=298), compared with rubber workers (n=212) (response rate 67% and 42% respectively). There was a clear exposure response relationship for dizziness or light-headedness, nausea

or sickfeeling, headache, « pins and needles », general fatigue. These symptoms were most frequent in case of high exposure; most readings were 20-30 ppm, sometimes > 50 ppm, but in the past often > 200 ppm. Kramer et al (1972) studied 98 workers exposed up to 25 yr in a polymerisation plant; there was exposure to 10 ppm vinylchloride (as TWA with considerable variability) and less than 5 ppm to vinylidene chloride. Six parameters correlated significantly with cumulative TWA and with the product of TWA and time on the job (ppm-yr): systolic and diastolic blood pressure, BSP, icterus index, Hb, β-protein level. Most relevant probably are the parameters indicating disturbed liverfunction. Wyatt et al (1975) examined blood chemistry in 413 exposed workers and 469 controls: albumin levels (increased) and cholesterol level trends with age (smaller increase) were different; no such difference were found for total protein, Ca, P, creatinine, uric acid, bilirubin, alkaline phosphatase, LDH, SGOT, CPK. Lange et al (1976) observed increased coproporphyrin, sometimes also δ-aminolaevulinic acid in urine in patients with signs of liver damage. Generally speaking, liver function tests — as far as practicable in workers — do not provide early indication of potential liver disease in vinylchloride exposed workers (Falk et al 1976). It appears that detection of structural rather than functional changes is likely to be more rewarding for detection of early effects: grey-scale ultrasonography may be a promising practicable method for this (Duck 1976, Williams et al 1976). Lange et al (1974) observed decreased platelet counts. Ward (1976) reported immune complex disorders in 21 of 58 workers; the extent and severity of these abnormalities paralleled clinical features (Raynauds' disease) to a large extent, but in some individuals they were already present when clinical features were minimal or absent. Several research groups (inter alia Purchase et al 1976, Hansteen et al 1978) have also observed chromosomal defects in vinychloride workers.

EG (1978) requires for preventive medical examination at least in addition to clinical investigation, röntgen examination of hand skelet and EG (1978) suggest the following laboratory test: SGOT, SGPT, γGT, alkaline phosphatase, total bilirubin, platelet count, urine analysis, blood sedimentation rate and cryoglobulin.

These data are presented because they may offert the — as far as known — only means to detect early effects or early indications of late (irreversible) effects (Raynaud, acro-osteolysis, portal fibrosis, angiosarcoma, sclerodermia) in vinylchloride exposed workers.

Many studies in workers have shown that vinylchloride is also a human carcinogen. Infante (1981) recently reviewed various studies. In 4 of 8 epidemiological studies a significant excess of liver cancer among VC- workers was demonstrated; in 5 of 8 studies a significant excess of cancer of the central nervous system was observed. Moreover, there also are indications for increased risk of lung cancer and for lymphatic and haemopoetic system cancer. Some studies did not demonstrate an increased cancer risk; however, this may have been due to an insensitive study design.

Recently Maltoni et al (1981) reviewed the experimental evidence of carcinogenicity of vinylchloride (VC). The conclusions, based upon studies of respiratory exposure over a wide range of concentrations and many animal species, were as follows: (1) VC induces tumours in all animal systems tested; (2) VC is a multipotential carcinogen: different types of tumour in different sites; (3) liver angiosarcomas are observed in all animal species studied; (4) there is a clear-cut dose-respons relationship; (5) newborn animals are extremely responsive and easily develop hepato-carcinomas and angiosarcomas; (6) VC is a transplacental carcinogen; (7) carcinomas occur at exposure to 50 ppm VC and probably even at 10 ppm, but no carcinomas were observed in exposure to 5 ppm and 1 ppm. Radike et al (1981) observed an enhanced carcinogenic respons in combined exposure to ethanol and VC.

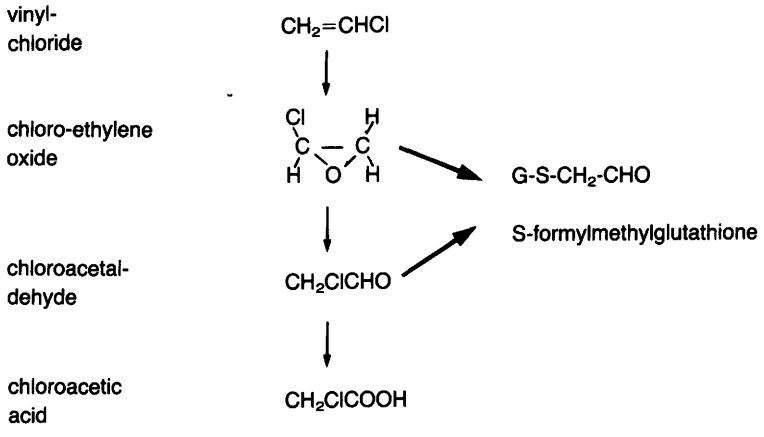
Metabolism

The most important route of exposure is through inhalation. At least in monkeys, exposed to 800 and 7000 ppm for 2.5 and 2.0 h (excluding the head) a small amount was absorbed through the skin: 0.02 to 0.03% of total vinylchloride available for absorption; the majority of absorbed agent was eliminated by the lungs (Hefner et al 1975).

Up to about 15 years ago vinylchloride was regarded as rather harmless, so little effort was devoted to study metabolism. After the discovery of carcinogenic effects, particularly liver angiosarcoma, much attention was directed to investigation of effects of long term low level exposure. After this discovery, there were ethical constraints in performing human volunteer studies. Older studies (quoted by Williams 1959) reported that vinylchloride is promptly exhaled; 82% is eliminated after discontinuation of exposure. The agent apparently is liposoluble, and so is readily taken up by the body, but also quickly eliminated. More recent data on humans are discussed in 12.4.0.

In rats (Stokinger 1976) two metabolites have been identified in the liver: N-acetyl-S-(2-hydroxy ethyl) cysteine and thiodiglycolic acid; they are thought to represent free available SH-groups that act as « scavengers » of the vinyl free radical, so removing its oncogenic properties. Müller et al (1975) found substances corresponding to S-carboxymethylcysteine and thiodiacetic acid in urine of exposed rats.

In 1978 Vainio reviewed metabolism, mutagenicity and carcinogenicity of vinychloride. The metabolism can be described as follows:



Biotransformation into the epoxide chloroethylene oxide involves microsomal mixed-function oxidase by a liver microsomal system; this epoxide rearranges with a half-life of 1.6 min spontaneously into the aldehyde; both can react with glutathione. Mutagenicity depends upon the metabolic activation to the epoxide, although also the aldehyde is mutagenic. Gehring et al (1978) demonstrated that in rats, exposed to 1.4-4600 ppm for 6 h the logarithmic probability incidence of angiosarcoma versus the amount of vinylchloride metabolized rather than the exposure concentration in air was linear. Assuming no threshold extrapolation of the data below the range of doses causing experimentally observable responses predicted an incidence of 0.01% hepatic angiosarcoma in rats exposed to 4.6 ppm vinylchloride. Theoretical extrapolation to man, exposed daily to 1 ppm suggested an incidence of 1.5 per 100,000,000, which is less than that expected to occur spontaneously. Bolt et al (1981) discussed the carcinogenic potential of peak exposure to vinylchloride in man on the basis of biotransformation in animals (monkeys) and humans. The model calculation suggested that, regardless of the exposure profile, the amount of reactive metabolites formed would solely be a function of the mean atmospheric vinylchloride over time, also for very short exposure peaks up to 200-300 ppm. In man, in contrast to rats, the capacity for a direct detoxication of the epoxide with glutathione, forming N-acetyl-S-hydroxyethylcysteine, is limited; this would indicate that low but constant exposure levels to vinylchloride could be less hazardous than marked peak exposures, which produce just the same integral amounts of the reactive metabolite.

Biological indicators

Human volunteer studies

Baretta et al (1969) exposed 4-7 subjects (total 13) to 50, 250 and 500 ppm vinylchloride for 7.5 h; mixed exhaled air was analysed (Table 23).

TABLE 23: Relationship between concentration in inhaled (Ci) and exhaled (Ce) air

Ci (ppm)	T (h)	Ce (ppm) at 2 h postexp.	Ce (ppm) at 16 h postexp.
50	7.5	0.6 — 0.9	0.08 — 0.12
250	7.5	4.8 — 6.0	0.45 — 0.65
500	7.5	10.0 — 15.0	0.8 — 1.3

It could be concluded that:

- the concentration in exhaled air increases about linearly with that in inhaled air
- there occurs an accumulation: vinylchloride still is detectable the following morning in exhaled air.

It should be noted however, that these experiments investigated concentration levels, which are not regarded acceptable any longer. Vinylchloride concentrations in blood and urine were not measured. Skin absorption has not been studied in humans.

Krajewski et al (1980) exposed 5 male volunteers, aged 26-31 yr, for 6 h to 3, 6, 12 or 24 ppm: the retention in mixed inhaled air was average 42% from 15 min onwards. The concentration in mixed exhaled air (Ce) in exposure to 6, 12 and 24 ppm (15.0, 30.0 and 60.0 mg/m^3) respectively was average 0.54, 1.30 and 2.84 mg/m^3 as mean value 30 min after exposure, i.e. 3.60, 4.30 and 4.73% of the concentration in inhaled air.

Industrial exposure studies

Baretta et al (1969) also studied on the job exposures in 10 workers (Table 24).

TABLE 24: Relationship between concentrations in inhaled (Ci) and exhaled (Ce) air in workers exposed to vinylchloride

Ci (ppm)	T (h)	Ce (ppm) at 2 h postexp.	Ce (ppm) at 16 h postexp.
25	8	0.32 — 0.60	0.02 — 0.05
50	8	0.70 — 1.20	0.06 — 0.12
100	8	1.60 — 2.50	0.15 — 0.28
250	8	3.90 — 7.80	0.5 — 0.9

These data agree reasonably with those observed in the human volunteer study.

Müller et al (1978) measured the excretion of thiodiglycolic acid in urine of 18 workers, exposed to 0.14 to 7.0 ppm (personal sampling) vinylchloride. The excretion of thiodiglycolic acid in 20 non-exposed workers was less than 1.5 mg/l; in workers the increase of metabolite excretion began shortly after starting exposure each day. The metabolite excretion exceeded the normal range, even after a mean exposure level of about 1.5 ppm, but not after exposure of less than 1.0 ppm. Müller et al (1979) described an analytical GC-MS method for thiodiglycolic acid in urine; in 34 non-exposed males 0.64 $\pm$ 0.32 mg/l was found, in 14 non-exposed females 0.51 $\pm$ 0.20 mg/l. Pharmaceutical drugs, other industrial agents and chloroacetylaldehyde precursors may also cause increased urinary levels; therefore, this method of biological monitoring is not wholly specific. The authors also suggested that measurement of hydroxyethylmercapturic acid might present possibilities for biological monitoring. Draminski and Trojanowska (1981) described another gaschromatographic method for masurement of thiodiglycolic acid; however, the detection limit was 10 mg/l.

Conclusions

There does not yet exist any valid routine biological monitoring method which can evaluate vinylchloride exposure at present day accepted levels $<$ 10 ppm. In view of the low Ce-levels at 2 h and 20 h after exposure to 25-50 ppm, a practicable technique still has to be developed.

Myers et al (1975) described methods to measure vinylchloride at sub-ppm level using a personal monitor; limit of detection $<$ 20 ppb. NIOSH (1974a) also described a gaschromatographic method after collection on charcoal tubes.

Particularly recent findings of increased urinary excretion of metabolites promise to become the method of choice, although more research is needed, and the metabolites are not fully specific for exposure to vinylchloride.

Research needs

Data on biological monitoring are still rather limited; there particularly is need to study the relationship between exposure to vinylchloride at concentrations below 10 ppm and the excretion of metabolites, on individual basis.

11. Anaesthetics

Introduction

In the past several of the halogenated hydrocarbons reviewed have been used as anaesthetics; trichloroethylene may still be used at a small scale. At present a few other compounds are largely applied as anaesthetics; personnel of the operation room (anaesthesists, nurses and technicians) may become exposed. This type of occupational exposure differs from that in industry: rather low level but irregularly shortterm peak exposures. The work may put heavy demands upon the central nervous functions and may involve emotional stress and irregular sometimes very prolonged working periods. Especially two agents merit discussion: *halothane* = fluothane (2-bromo-2-chloro-1,1,1-trifluoroethane) and *methoxyflurane* (2,2-dichloro-1,1-difluoroethyl methyl ether).

Effects on humans

Reviews on health effects have been published by inter alia NIOSH (1977), Chang (1977), Smith (1978), Ferstandig (1978), Vessey and Nunn (1980) and Edling (1980).

Although reported suggestions on health effects in operation room personnel, including effects on reproduction and offspring, cannot always be maintained, the evidence can neither be refuted. There exists suggestive evidence of increased risk of congenital abnormalities in the offspring, spontaneous abortion, liver and kidney disease, decrement in psychomotor performance, maybe of cancer. However, there always is a possibility that the health effects reported may be due to other work related factors, e.g. work stress, infections, radiation. In some studies there was an indication for decreased birth weight and in one study for increased infertility in fermale anaesthesists. It has also been reported that an increased risk of abortions or congenital malformations exists in the family of male anaesthesists, but the evidence is dubious (Edling 1980).

In a study of delivery ward personnel (see 13.4.0) Dahlgren (1979a) observed a relationship ($P < 0.001$) between exposure to *methoxyflurane* (MTF) and increase in serum uric acid, SGOT, SGPT and BUN levels. A small part of the increase was due to simultaneous exposure to nitrous oxide. This suggested an effect on hepatic and renal functions. In animal studies hepato - and nephrotoxic effects have also been demonstrated, although usually in high exposure intensity.

Gamberale et al (1974) studied effects of exposure to anaesthetic gases (halothane up to 10 ppm, nitrous oxide up to 3000 ppm, ethanol up to 94 ppm) in 20 anaesthetic nurses and in 20 intensive care nurses (controls); no measurable difference in impairment was observed in reaction time and in perceptical speed between both groups; however, the individual variability of the responses in some reaction tests was greater in the anaesthetic nurses at the end of the work day. Korttila et al (1978) examined 19 operation room (see 13.4.0.) nurses and 11 (younger) nurses working in the wards. No correlations were found between the concentration of halothane or nitrous oxide in end-tidal air and psychomotor or driving performance.

Metabolism

The anaesthetics are rapidly absorbed through inhalation; they are liposoluble and parly metabolized into various compounds. The biological half life is considerable, so accumulation probably takes place. The compounds and/or metabolites are measurable in exhaled air and/or urine. Holaday (1977) reviewed the metabolism of various anaesthetics. The percentage of anaesthetics metabolized was calculated with a model and were as follows (at 4 h duration of anaesthesia): *enflurane* 18%; *halothane* 26%; *methoxyflurane* 52%. The blood-gas and fat-gas partition coefficients were respectively for enflurane 1.9 and 70; for halothane 2.4 and 155; for methoxyflurane 11 and 670 (according to Dahlgren 1979b, 13.0 and 825). The liposolubility and the degree of biotransformation increase in the given order; this may explain the increasing drop of the concentrations in exhaled air within the first 24 h after anaesthesia in the reverse order.

According to Holaday (1977) excretion of metabolites occurs almost exclusively by urine; in urine sampled for up to 14 days 2.4%, 25% and 44% of the total amount taken up of *enflurane, halothane* and *methoxyflurane*, respectively was found. In the

case of *halothane* exposure *bromide* is a principal metabolite; this is excreted with a half life of 12-22 days. The plasma bromide level increases during 20-40 h after the end of anaesthesia. The biological half life of excretion of trifluoroacetic acid (another metabolite of halothane) in volunteers is 16 h; in patients longer half lifes (39-61 h) are reported, probably because biotransformation still occurs the first 2 or 3 days following anaesthesia. In addition to the biotransformation into the fraction excreted measured as fluoroacetic acid, bromide and chloride, a second fraction undergoes irreversible binding to microsomal proteins. Hepatotoxicity may be related to a third fraction: release of fluoride; this occurs under anaerobic conditions.

Studies in volunteers and in occupational exposure

Cascorbi et al (1970) performed studies with labelled *halothane* in volunteers and in exposed personnel; the agent was injected intraveneously. Exhaled air was measured over 5 min, urine collected up to 21 days. Urinary excretion of radioactivity was greater when the tracer was injected into unanesthesized subjects (n = 2) than when the same subjects were anaesthesized (N_2O-halothane). As a group pharmacists showed a much smaller variation in urinary metabolite excretion than anaesthesists. The amounts of labelled C exhaled in $4^1/_2$ h ranged from 10-45% of the injected dose; measurable amounts were still recovered as late as 144 h after injection. Four of the 5 anaesthesists excreted more radioactivity during the first 2 h after injection than the 4 pharmacists. This suggests enzyme induction in the regularly exposed anaesthesists; however, this was not the case in one of them: he had been least exposed during the two previous years.

In operating room personnel Hallen et al (1970) measured exhaled air (rebreathed) at the end of one or more operations and venous blood at the end of a days' period. The halothane concentration was 7.0 ppm, 29.0 ppm (median values) outside or within 25 cm of the outlet of gases; in expired air 4.0 ppm was found, in venous blood 0.13 mg/l (median values) (end of operation).

After observing *methoxyflurane* in exhaled breath of an anaesthesist up to 29 h after exposure to 2-10 ppm in his immediate work environment, Corbett (1973) studied levels in end-expired air in personnel at intervals following exposure: *halothane* was detected up to 64 h, and *methoxyflurane* up to 29 h after exposure. Corbett and Ball (1973) also measured breath decay curves in anaesthesists exposed to *halothane* for 70-390 min; the agent was detected in end-expired air for 26 h (70 min exposure) to 64 h (390 min exposure). Repeated exposure the next day increased the duration of exhalation. In surgeons with 70 min exposure (to lower levels than anaesthesists) halothane was detected for 9 h. The exposure concentrations were up to 10 ppm in the inhalation zone of anaesthesists, 0.14-0.25 ppm in the main hallway leading to the room, 0.02-0.04 ppm in the anaesthesia room, and 0.10-0.22 ppm in the recovery room.

Gostomzyk et al (1973) measured *halothane* levels in blood; the levels depended upon duration of exposure, size of operation room, halothane index (indicator of exposure). They found up to about 1000 µg halothane/l serum at the end of exposure in physicians, and up to about 800 µg/l in nurses; with lower halothane index and larger room-size the levels were much lower (up to 200 and 100 µg/l respectively). Even in the last case halothane was still present in blood the following morning.

Eichler et al (1975) examined *halothane* levels in exhaled air of anaesthesists at the end of an operation; they compared the effects of three narcosis-systems: open (n = 23), half closed intubation (n = 8) and half closed system, mask (n = 2). The levels in exhaled air were 1-243 ppm (duration narcosis 30-270 min), 2-14 ppm (120-270 min) and 45 ppm (180-240 min) respectively.

Gostomzyk et al (1975) measured *halothane* levels in blood in 17 anaesthesists and 17 nurses at the end of operation (3-4 h), and at 20 h after exposure. The levels were 40-770 µg/l serum and 15.2 µg/l respectively.

Dalhgren (1979a, 1979b) particularly studied exposure to *methoxyflurane* (MTF) in obstetrical analgesia. Urinary *fluoride* levels were measured. In 15 mothers exposed to MTF the concentration was 24.9 ± 19.6 mg/l at 2-12 h after delivery and 6.8 ± 6.0 mg/l at 60-72 h; in nitrous oxide (N_2O) analgesia the levels were 0.4 and 0.3 mg/l respectively. In addition 24 delivery ward personnel were studied. It had been shown that the urinary F-concentrations were maximal about 12 h after exposure. Urinary fluoride concentrations were measured at the beginning of each workday during 5 separate three-weeks periods; during 3 periods MTF/N_2O was employed as anaesthetic, during 2 periods only N_2O. The duration (in minutes) of exposure was recorded for each subject; this was related to the fluoride (F) concentration (in mg/l) the day after this exposure. In the MTF/N_2O-periods mean exposure time was 72 min (± 25), mean

F = 0.707 mg/l (± 0.582); in the N_2O-periods respectively 73 min (± 33) and 0.352 mg/l (± 0.256). In the general population the F-concentrations were 0.28-0.62 mg/l. The data showed a clear difference ($P < 0.001$) between the F-concentrations in MTF/N_2O-and N_2O-periods: the mean level was doubled, although the mean duration of exposure was similar. Previously it had not been possible to detect even traces of MTF in exhaled air in the same personnel of the same delivery wards. The mean MTF-concentrations in the delivery rooms were 0.5-0.8 ppm MTF and 300-540 ppm N_2O.

Conclusions

Occupational exposure of operation room personnel to halogenated hydrocarbons like *halothane* and *methoxyflurane* offers possibilities for biological monitoring.

Because of the irregular working conditions in the operations theatre, no adequate data on exposure (level, duration, respiratory minute volume) are available; methods of biological sampling of exhaled air, blood or urine show a large variability. Nevertheless these studies have shown that:

- biological monitoring of exposure by means of sampling exhaled air and blood for halothane is feasible;
- metabolites can be found in blood and urine: bromide, trifluoroacetic acid in case of halothane, fluoride in case of methoxyflurane;
- the biological half life may be rather long; cumulation in repeated exposure may take place, particulary of halothane;
- adequate data on relation between exposure (level, duration) and biological parameters are not yet available.

Research needs

Although various possibilities for biological monitoring have been demonstrated, there still is little insight in the relationship between exposure (concentrations-time weighted average, peaks; duration; irregualarity; repetitive exposure) and levels of the anaesthetic and/or metabolites in blood, exhaled air and/or urine. There is need for more systematic studies, and for comparative studies of analytical methods.

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Human biological monitoring of industrial chemicals series

Lead

L. Alessio, V. Foà

Summary

This document reviews inorganic lead as related to occupational exposure and the possibilities of the biological monitoring of exposure.

The main route of absorption in occupational exposure is the respiratory apparatus. Derangement in heme synthesis is currently considered the first adverse effect associated with increasing concentration of lead in the soft tissues.

A vaste number of tests which permit an evaluation of the degree of exposure, body burden, and toxic effect are available for monitoring lead workers.

For periodic monitoring of workers exposed to lead it is recommended that two tests be used simultaneously; one test should be designed to indicate internal dose and another to indicate effect. In general it is advisable to use blood lead levels as a measure of internal dose, and erythrocyte protoporphyrin as an indicator of effect.

For screening studies an inexpensive test which is easy to perform, sensitive, specific and precise should be used to indentify subjects with high exposure. Both protoporphyrin and delta-aminolevulinic acid dehydratase comply with these requirements.

For assessment on a group analysis basis of the environmental condition of a work place, the urinary tests may be used; blood tests, however, provide more accurate information.

Individual blood lead levels in male workers should not exceed 60 μg/100 ml, and in women workers of child-bearing age they should not be higher than 40 μg/100 ml because of the potential adverse effect of lead on the foetus.

Further investigations are required on the relationship between external and internal dose and standardization of the various biological tests.

Abbreviations

ALAD	δ-aminolevulinic acid dehydratase activity of erythrocytes
ALAU	δ-aminolevulinic acid in urine
CPU	urinary coproporphyrin
EP	erythrocyte protoporphyrin
PbA	lead in blood
PbU	urinary lead
PbUEDTA	amount of chelatable lead excreted with 24-h urine after administration of $CaNa_2$ EDTA (1 g intravenously)
ZPP	erythrocyte zinc protoporphyrin

Lead

Chemical and physical properties

Lead is a chemical element represented by the symbol Pb and with an atomic number of 82, atomic weight 207.21, specific weight 11.342; melting point at 327°C; boiling point at about 1740°C. Starting from temperatures of 550 - 600°C, there is considerable production of vapours which combine with oxygen in the air to form lead oxide. Lead is found in the natural state in mineral deposits. The most common and most widely used mineral for extraction is galena (PbS). The lead content in directly mined mineral varies from 3 to 10%.

Effects on humans

Derangement in heme synthesis is currently considered the first adverse effect (critical effect) associated with increasing concentration of lead in the soft tissues; in fact, lead can inhibit some enzymatic activities of heme biosynthesis (Chisolm, 1971; De Bruin, 1971; Baloh, 1974; Waldron and Stoefen, 1974). See Fig. 1 (Chisolm, 1971).

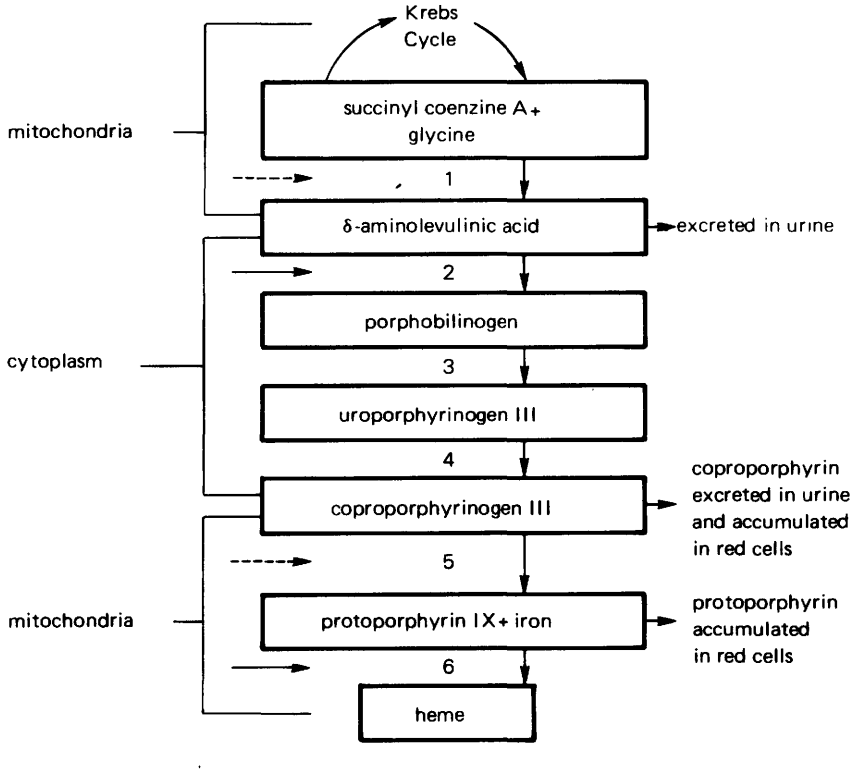

Figure 1 - Biosynthesis of heme is inhibited by lead, resulting in accumulation of intermediates in the synthetic pathway. Lead inhibits two steps (solid arrows) and may inhibit two others(broken arrows).

The inhibition by lead of ALAD and heme synthetase, which are enzymes containing SH groups, is well documented. Due to ALAD inhibition, an accumulation of ALA occurs in the serum and consequently in the urine; inhibition of heme synthase (iron chelatase) produces an accumulation of protophorphyrin IX in the erythrocytes. An increase in urinary coproporphyrins is an indirect evidence of coprogenase inhibition by lead.

The combination of decreased delta-aminolevulinic acid dehydratase activity in red blood cells, increased urinary delta-aminolevulinic acid, increased urinary coproporphyrin, and increased erythrocyte protoporphyrin is pathognomonic for lead, distinguishing it from all other disorders of pyrrole metabolism in man (Chisolm, 1975).

Also changes in nerve conduction velocity should be regarded as a critical effect (Zielhuis, 1977). These changes will not, however, be considered here because the investigation methods are rather time-consuming, difficult to perform in working environments, and are not always reliable since the alterations are unspecific. Such investigations can nevertheless be very useful for studies on groups of workers.

Metabolism

In working environments the main route of absorption is the respiratory apparatus. It is generally considered that 35-50% of the lead that reaches the lower respiration tract is absorbed into the blood stream.

The potential increase in the body burden of lead can be expressed as:

$$BB = L \times V \times R \times D \times 10^{-3}$$

where BB = potential increase in body burden in mg; L = air lead concentration in mg/m^3; V = pulmonary ventilation in m^3/day; R = fraction of inhaled lead retained; D = duration of exposure in days. R values vary according to the solubility and particle size of individual lead compounds.

The uptake of lead by the gastro-intestinal tract is less complete than by the lung. Not more than 5-10% of ingested lead is generally absorbed, the balance being excreted in the feces.

A potential gastro-intestinal absorption in industry should not be underestimated. Both because it can increase due to particular personal habits, e.g. smoking, eating in the workplace and because as much as 40% of inhaled lead of large diameter trapped in the upper respiratory tract may be swallowed (Kehoe, 1961; Knelson et al., 1973; Hamilton and Hardy, 1974; Waldron and Stoefen, 1974).

In a steady-state situation, lead intake equals output and the skeletal system contains about 80-90% of the total body burden of lead.

Figure 2 (Baloh, 1974) gives a schematic representation of the dynamic interchange of the body lead pool. Blood is the major factor in determining the steady state distribution of lead in body tissues. There is a dynamic equilibrium between red cell lead and plasma lead on the one hand and between extracellular lead and intracellular

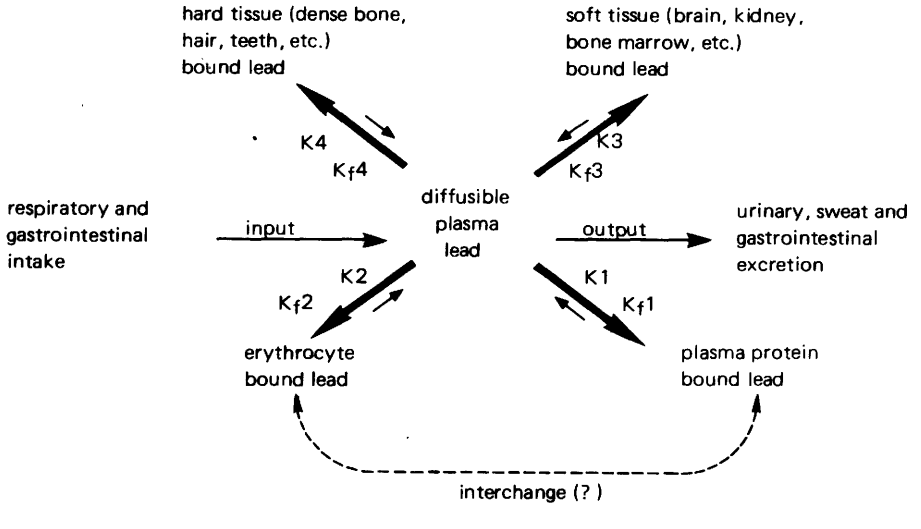

Figure 2 - The dynamic interchange of the body lead pool

lead on the other. It is likely to be the ionic fraction of the plasma lead which is transferred to the other body compartments. The equilibrium constants of the reactions probably rank in the following order: $K_4 > K_3 > K_2 > K_1$. However, since a state of equilibrium is rarely reached, the rate constants K_f also become important. The rate constants indicate the speed with which the state of equilibrium can be reached in any given reaction.

The rate constants in Fig. 2 are probably in the reverse order of the equilibrium constants, i.e. $K_{f1} > K_{f2} > K_{f3} > K_{f4}$, indicating that the bone takes more time to reach its final lead concentration than erythrocytes or proteins (Baloh, 1974; Waldron and Stoefen, 1974).

According to Pietrovsky (1970) the total body burden of lead can be roughly divided into: 1) rapid exchange pool in blood and soft tissues; 2) intermediate exchange pool in skin and muscles; 3) exchange pool in bone (intermediate exchange in bone marrow, trabeculae, and slow exchange in dense bone and teeth).

Biological Indicators

Indicators of internal lead dose

Dose should, ideally, be defined as "the amount or concentration of a given chemical at the site of its action", i.e. where its presence leads to a given effect. Since the determination of this amount is often impossible in practice, the dose may have to be estimated by various means and in most cases one can speak only in terms of these dosage estimates. Metal concentration in biological media can often be used as indicators (or indices) of exposure and of concentration in the critical organ (Nordberg, 1976).

Below are considered the biological tests which may be used as indicators of an internal lead dose.

Concentration in blood

The level of lead in blood (PbB) is a function of the quantity of lead absorbed from the environment minus the lead deposited in the bone cortex and soft tissues and the lead excreted with urine and feces (Waldron, 1971). PbB is about 2% of the total lead burden. Approximately 95% of blood lead is bound to erythrocytes and is not readily diffusible; plasma lead (0.2% of the total burden) is made up of two fractions: the plasma protein bound fraction and the diffusible fraction, the latter being probably the metabolically active centre of the body lead pool (see Fig. 2) (Baloh, 1974). Diffusible plasma lead probably gives the best approximation of the biologically effective lead burden, although at present it is not possible to measure it. It should be noted, however, that the plasmatic fraction of lead is not a constant function of the total blood lead concentration and therefore cannot be predicted by PbB (Waldron, 1974). However, for groups of subjects, PbB is probably a reasonable indication of plasmatic levels (Zielhuis, 1975a).

In practice PbB is the most reliable means of measuring the extent of exposure: it allows distinctions to be made between ***normal*** subjects, subjects with ***permissible*** absorption levels, and subjects with ***non-permissible*** absorption levels. It is, moreover, particularly useful in epidemiological studies. In fact, a good correlation exists between PbB and lead levels in the atmosphere (Williams et al., 1969).

Interpretation of blood lead levels must take into account the fact that they reflect only one point in time, and a dose which is steady, increasing or decreasing (Nordberg, 1976). However, although these levels allow a satisfactory evaluation of current exposure, they are not necessarily always correlated with the lead body burden. In fact, after cessation of exposure, PbB may reach "normal" values while a body burden persists. This is demonstrated by a high urinary lead excretion after chelating therapy (Prerovska and Teisinger, 1970), or when disorders of heme synthesis are still evident (Selander and Cramer, 1970; Alessio et al., 1976c). On the other hand, cases are known in literature of adults and children who showed clinical symptoms of intoxication but who had relatively low PbB values (Beritic, 1971; Moncrieff et al., 1964). This apparent "paradox" could be due to the fact that measurement occurred some time after cessation of exposure (Kehoe, 1972).

Studies on volunteers who received different quantities of lead also showed that the PbB levels reach a given ceiling even when the body lead burden increases

continuously during exposure (Kehoe, 1961). This was also observed in occupational exposure (Benson et al., 1976).

Factors exist which can influence PbB levels independently of exposure and body burden. For example, blood levels are greatly affected by the red blood cell mass (anemia, polycythaemia). There has been much discussion of whether these levels should be corrected to the haematocrit values, but there is disagreement on the biological validity of such correction (Lauwerys, 1975).

Further, the measurement of lead concentration in blood also presents difficulties (Berlin et al., 1974; WHO, 1977; NIOSH, 1978). A number of interlaboratory control programmes have revealed high rates of variation in the results, which are probably due to the fact that this parameter is measured with methods and instruments that differ considerably one from the other. In January 1978, an interlaboratory control programme for PbB was sponsored by the EEC (within the frame of the activities provided for in the EEC guidelines of 29.3.77). This programme is still under way and preliminary results indicate that the extractive methods followed by flame AAS always give lower results than the other techniques with atomic absorption. The flameless methods or the "Delves Cup" give similar results but it should be noted that the "Delves Cup" tends to overestimate the values.

Concentrations in urine

The kidney is presumed to excrete lead by two routes: glomerular filtration and transtubular flow or excretion (Vostal and Heller, 1968).

The relative importance of the two routes is uncertain, but the formation of lead containing inclusion bodies suggests that in subjects with heavy lead exposure transtubular flow may assume a greater importance (Cramer et al., 1974).

Since the analysis of lead in urine (PbU) does not require blood withdrawal, it is sometimes preferred to PbB determination (Lauwerys, 1975). The "normal" PbU concentration in adults usually oscillates between 10 and 80 μg/l, lower than 50 μg/g creatinine (Baloh, 1974; Lauwerys, 1975). In subjects under continuous exposure, a satisfactory correlation was found between atmospheric lead levels and PbU and between PbB and PbU (Williams et al., 1969). In the case of new lead exposure there is also a good correlation between PbB and PbU, but while PbB increases without any demonstrable time lag, the increase in PbU requires a latency period of about 2 weeks (Tola et al., 1973).

Many factors other than lead absorption such as fluid intake and specific gravity of the urine may influence the excretion of lead (Ellis, 1966). Patients with chronic nephritis frequently have PbU levels within "normal" limits in spite of the existence of high lead stores (Lilis et al., 1968). Prerovska and Teisinger (1970) have demonstrated that subjects with heavy lead exposure in the past can have normal urinary lead excretion even when excretion of chelatable lead remains high.

Concentration in feces

In the non-occupationally exposed general population the quantity of lead eliminated with the feces is clearly higher than that eliminated with the urine. In fact, the greater part of the metal present in the feces consists of ingested lead that has not been absorbed by the intestines (Kehoe, 1961).

The levels of lead in feces of "normal" subjects varies between 240 and 400 μg/24 h (Kehoe, 1961; Barry, 1975). During occupational exposure the values increase to 760 - 3800 μg/24 h, according to the data of Saita and Moreo (1958).

Measurement of lead in feces can be used to determine absorption by ingestion (accidental or intentional). Fecal lead excretion above 4 mg/100g, 4 weeks after occupational exposure has ceased, is a sure indication of ingestion (Zielhuis, 1972). The analysis is valid only when performed during the period of ingestion or in the days immediately following (Vigliani and Debernardi, 1934).

Chelatable lead

Chelatable lead is strictly dependent on the active deposit of the metal in the soft tissues of the body, including the trabecular bone (Teisinger et al., 1969), and as a result it provides a more direct measurement of the rapid exchange pool.

Chelatable lead can be measured by injections of $CaNa_2$ EDTA or by penicillamine per

os. The levels of PbU EDTA (mean + 2 SD) in 26 inhabitants of Milan who were not occupationally exposed to lead, were 630 μg/24 h (Alessio et al., 1976a).

Limited data on humans strongly suggest that the $CaNa_2$ EDTA mobilization test may be a better indication of the concentration of lead in affected organs of man (Nordberg, 1976).

Since $CaNa_2$ EDTA is capable of binding only with extracellular lead (Teisinger et al., 1958; Castellino e Aloj, 1965), it is likely that measurement of metal in urine after administration of this drug permits an indirect, though rough, evaluation of the levels of diffusible lead.

After administration of $CaNa_2$ EDTA, the reduction in levels of lead in the plasma creates a cells/plasma gradient which slowly disappeares. After the first few days of treatment in fact, urinary lead is greatly reduced and it is necessary to interrupt administration for a few days so that an equilibrium may be established in the distribution of lead in the cellular and extracellular compartments and so that a high excretion of the metal may once again be obtained (Saita, 1962).

In studies on children and adolescents (Chisolm et al., 1976), a statistically significant linear relationship was found between blood lead concentration and the logarithm of the quantity of lead excreted in the 24-hour period immediately following administration of $CaNa_2$ EDTA. In our laboratory, studies in progress have shown that a good correlation exists between PbB and PbU EDTA in adult subjects with current occupational exposure to lead. In subjects with past ocupational exposure, the correlation between the two parameters is definitely lower, although still statistically significant. Analysis of the regression curves shows that for corresponding values of chelatable lead, subjects with past exposure have lower blood lead levels than currently exposed subjects. The slopes of the regression lines are statistically different (Fig. 3).

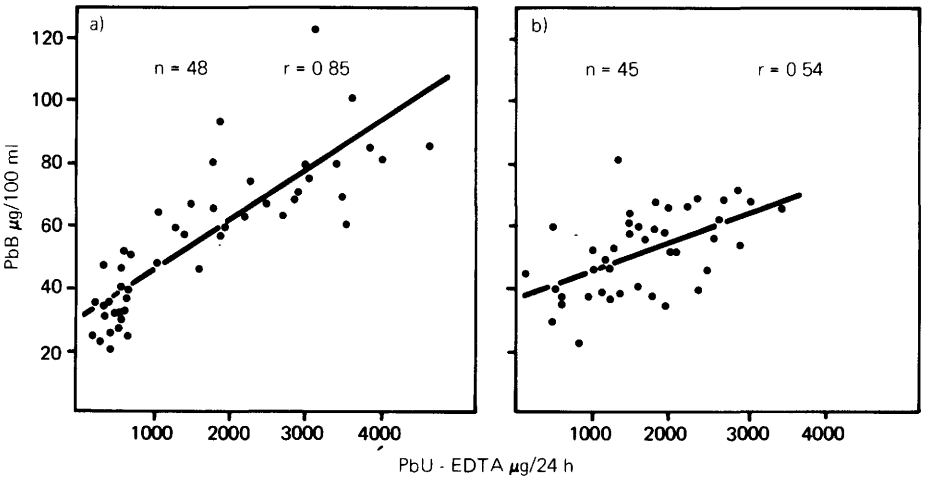

Figure 3 - Relationship between chelatable lead (PbU EDTA) and PbB in male subjects with current (a) and past exposure (b) to lead

Chelatable lead cannot be used in epidemiological studies because it necessitates administering a drug and also because 24-hour urine samples are difficult to obtain.

The induced urinary lead test is capable of detecting and evaluating the existence of lead absorption which occurred in the past. It can therefore be used to determine whether former acute manifestations or current chronic manifestations are attributable to lead intoxication, even when the other indicators of internal dose have returned to normal (Saita, 1962; Prerovska and Teisinger, 1970).

Teisinger (1971) maintains that in subjects with past exposure, a urinary lead excretion above 1 mg/24 h after administration of $CaNa_2$ EDTA (2 g i.v.) is indicative of a potentially dangerous body burden of the metal; for subjects still exposed, however, the author sets critical level at 2 mg/24 h.

Relationship between external lead exposure and indicators of internal dose

Many studies have demonstrated the existence of a correlation between PbB, PbU and the atmospheric lead levels in the working environment (PbA).

The relationship between PbA and PbB has a similar profile both when the atmospheric lead levels are low and when levels of 0.2 mg/m³ are reached (See Fig. 4) (Harada, 1976). Williams et al. (1969) found a close correlation between PbA and PbB ($r = 0.90$) and between PbA and PbU ($r = 0.82$) with high statistical significance ($p < 0.01$). Table I (Williams et al., 1969) gives the mean values and the 95% confidence limits of single determinations of PbB and PbU which correspond to 0.2 and 0.15 mg/m³ lead in air. The wide range of the confidence limits is evident from these data.

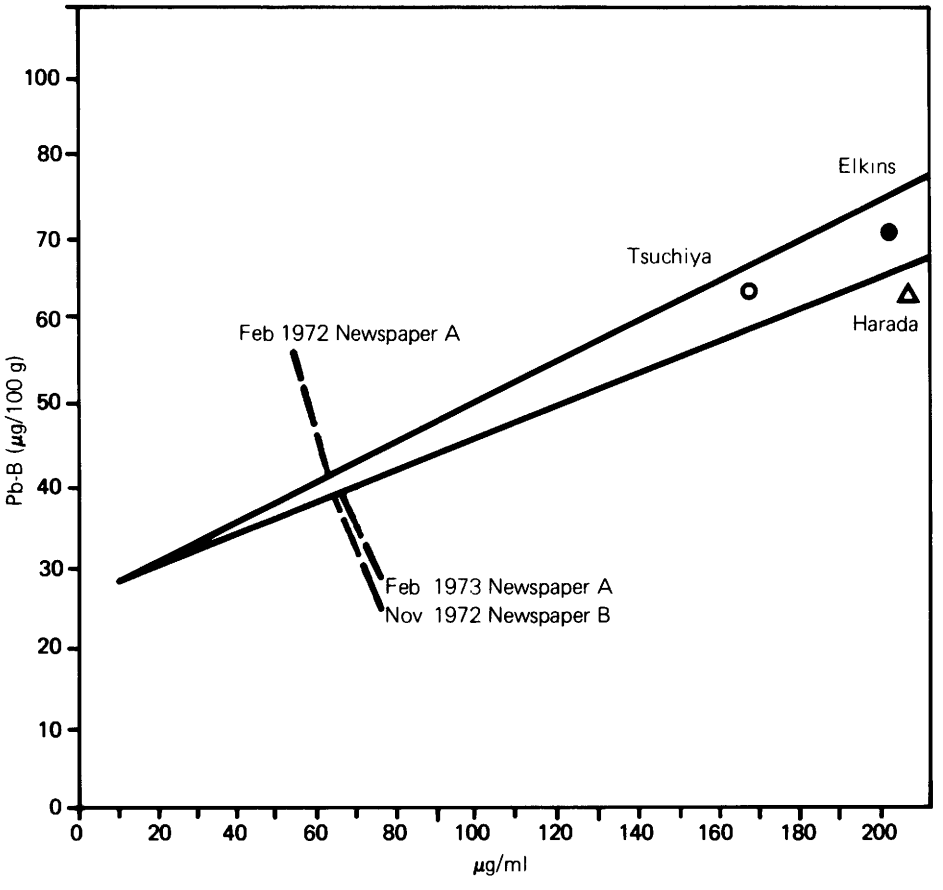

Figure 4 - Relationships between lead concentration in air and lead concentration in blood of lead workers. The curves are those obtained from a study of the PbA/PbB relationship in newspaper industries (Harada, 1976), extended to plot also the values of Elkins (1959), Harada et al. (1960) and Tsuchiya and Harashima (1965)

On the basis of Williams' data, Zielhuis and Verberk (1974) examined the validity of various PbB and PbU levels as indicators of "unacceptable" exposure; they assumed PbA = 0.12 mg/m³ to be the "acceptable" level (see Table II). In this sample for the cut-offs considered, PbB levels have a higher validity than PbU levels as indicators of unacceptable exposure. PbB > 40 has maximum sensitivity (no false negatives : all individuals with PbA > 0.12 have PbB > 40); however, specificity is moderate (also PbB > 40 in subjects with PbA < 0.12 : 44% of false positives). PbB > 80 is highly specific (no individual with PbA < 0.12 has PbB >80, i.e., no false positives); but sensitivity is moderate (46% false negatives). On the basis of these results, Zielhuis and Verberk (1974) conclude : "If one wants to be certain that all subjects with PbA > 0.12 are selected out of a universe of exposed workers, PbB > 40 will serve this objective, however, at the cost of a number of false positives. If, on the other hand, one wants to select only individuals with PbA > 0.12, then PbB > 80 will serve this objectives, however, with many false negatives.".

The number of subjects in whom validity has been studied is limited (about 30 cases) but it is likely that even with a larger number of subjects the validity values will be similar since air sampling involves many limitations, i.e.:

- effect of particle size and solubility of particle;
- representation of only a small fraction of total volume of air inhaled;
- ingestion remains unmeasured;
- effect of contamination and position of sampling head;
- effect of the entry of particulates into sampling heads;
- failure to evaluate individual differences in pharmacokinetics according to age, type of respiration, congenital or acquired diseases, etc. (Lyman, 1975).

Table I - Mean values and 95% confidence limits of single determinations of lead in blood and urine which correspond to two lead-in-air concentrations

PbA (mg Pb/m^3)		PbB (µg/100 ml)	PbU (µg/l)
0.20	Mean	70	143
	95% C.L.	48-92	56-230
0 15	Mean	60	118
	95% C.L.	38-82	31-205

Table II - Validity of different PbB and PbU levels for predicting an unacceptable lead exposure (PbAir 0.12mg/m^3)

		Se	Sp	Validity
PbB µg/100 ml	> 40	1.00	0.66	1.66
	> 60	0.72	0.80	1.52
	> 80	0.56	1.00	1.56
PbU µg/l	> 60	0.88	0.53	1.41
	>120	0.56	0.95	1.51
	>160	0.12	0.95	1.07

Se = sensitivity, Sp = specificity, Sp + Se = validity

At the 2nd International Workshop on Permissible Limits for Occupational Exposure to Lead (Zielhuis, 1977) the conclusion was reached that a "standard" for lead in air based upon the relationship between PbB and PbA could not be established. Such a standard, it was felt, would best be based on PbB alone.

To conclude this section, it seems appropriate to make the following points:

a) Lead in the blood and lead in the urine are **indicators of exposure** since the levels of these parameters are closely influenced by the environmental concentration of lead.
b) Chelatable lead may be considered a "true" **indicator of dose,** the levels of which reflect the active lead deposit.
c) In currently exposed subjects the indicators of exposure permit prediction of the quantity of chelated lead.
d) In subjects no longer exposed the indicators of exposure do not permit a reliable evaluation to be made of chelatable lead.

Indicators of Effect in Adult Males

Biological tests which may be used as indicators of a biological lead effect are separated according to sex since in recent years it has been shown that some indicators of effect behave differently in males and females.

Erythrocyte delta-aminolevulinic acid dehydratase

The delta-aminolevulinic acid dehydratase (ALAD) of circulating erythrocytes is highly sensitive to inhibition by lead; inhibition of ALAD in red blood cells (RBC's) parallels inhibition in other tissues, e.g. liver (Secchi et al., 1974).

A very close negative correlation exists between erythrocyte ALAD and lead blood levels. The enzyme undergoes distinct inhibition in the range of PbB values below 40 µg/100 ml (Hernberg et al., 1970; Haeger-Aronson et al., 1971; Zielhuis, 1972; Lauwerys et al., 1974). There is suggestive evidence that the no-effect level is about 10 µg PbB/100 ml (Granick et al., 1973).

Up to 1974, studies on the relationship between ALAD and PbB have generally used the method of Bonsignore et al. (1965) or methods derived from this for the determination of ALAD. At present the European standardized method (Berlin and Schaller, 1974) is widely used. Determination of ALAD using the method of Bonsignore

is of little use in monitoring occupationally exposed subjects (see Fig. 5) (Alessio et al., 1976b). In fact, when PbB increases beyond 40 μg/100 ml, the enzymatic activity is reduced to a level too low to allow identification of different blood lead levels (de Bruin, 1968; Basecqz et al., 1971; Hernberg et al., 1972; Secchi and Alessio, 1974). However, ALAD can have a wider application in monitoring lead workers when it is measured with the CEC method, since a marked inhibition of the enzymatic levels occurs only when PbB values exceed 50-60 μg/100 ml (see Fig. 5).

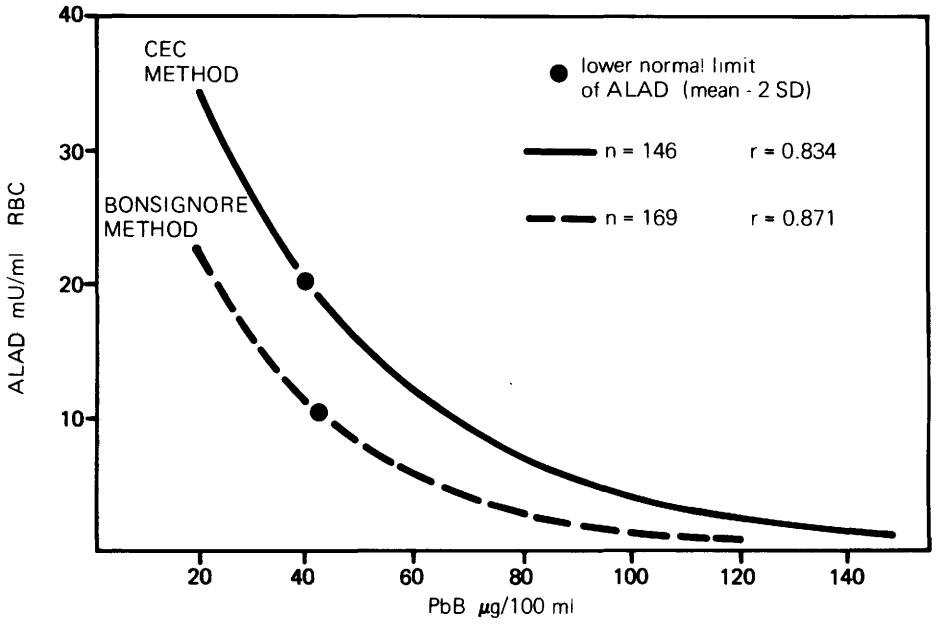

Figure 5 - Correlation between PbB and ALAD determined by two different methods in adult males currently exposed to lead

Validity of ALAD is rather moderate for PbB levels lower than 40 μg/100 ml which therefore implies a very high percentage of false classifications. Thus when subjects with only environmental lead exposure are studied according to Zielhuis (1974), "it is not possible to base a biological quality guide on individual ALAD levels". Validity of ALAD does, however, improve markedly for higher PbB levels. For example, the validity of ALAD (measured with the CEC method) is good at a PbB cut-off of 60 μg/100 ml. At this level, at cut-off of 15 m U/ml RBC the enzyme displays a sensitivity of 0.96 (i.e. 4% false negatives) and a specificity of 0.85 (i.e. 15% false positives). These data indicate that ALAD may be used as a screening test for occupationally exposed subjects (Table III).

After a worker's first exposure to lead, ALAD activity decreases rapidly without any appreciable time lag, parallel to the increase in blood lead concentration (Hernberg et al., 1972). According to Tola (1972) and Haeger-Aronson et al. (1974), when exposure to lead ceases, ALAD activity progressively returns to normal, parallel to PbB. Thus, according to these findings, ALAD does not indicate any former lead exposure that cannot be detected from an elevation of PbB.

Table III - Validity of ALAD for predicting different PbB levels. Analysis made on 108 adult males currently exposed to lead

PbB μg/100 ml	ALAD mU/ml RBC	Se	Sp	Validity
⩾40	⩽20	0.71	0.80	1.51
⩾60	⩽15	0.96	0.85	1.81
⩾70	⩽10	0.94	0.92	1.86

Se = sensitivity. Sp = specificity, Validity = Se + Sp
ALAD determined according to the European standardized method

However, other studies (Vergnano et al., 1969; Sakurai et al., 1974) indicate to the contrary, that in cases of severe past exposure, ALAD remains inhibited out of proportion to the current PbB. It has been conjectured that in this situation enzymatic inhibition is due to an inhibitor other than lead, probably of a thermolabile proteic nature (Vergnano et al., 1969).

The significance of erythrocyte ALAD inhibition due to lead in regard to health is still open to discussion (Zielhuis, 1975a). As regards ALAD inhibition for the range of PbB up to 40 μg/100 ml "its biological significance is dubious because it is unaccompanied by any detectable effects on the biochemical function of man" (NAS, 1972). According to Nordberg (1976), an inhibition of ALAD in the cells of the bone marrow is a subcritical effect which precedes an increased level of delta-aminolevulinic in blood and urine and the occurrence of anemia (critical effects). A decrease in ALAD activity in blood is an example of an indicator of subcritical effect of lead exposure.

Until recently, it was not clear whether the inhibition of peripheral erythrocyte ALAD by lead was a phenomenon which really occurred ***in vivo,*** or a phenomenon which only occurred ***in vitro,*** i.e. a result of membrane-bound lead getting access to the intracellular enzyme as a result of haemolysis in the test tube required for determining ALAD activity. Roels et al. (1974a) have shown that the decrease in the erythrocyte enzyme is a true reflection of the enzyme activity ***in vivo*** when PbB is below 120 μg/100 ml. ALAD inhibition is highly specific for increased lead absorption: e.g. no reduction of ALAD activity has been observed in workers occupationally exposed to cadmium and mercury (Lauwerys et al., 1974; Lauwerys and Buchet, 1973). A transitory inihibition of ALAD occurs after acute ingestion of high quantities of alcohol (Moore et al., 1971). In chronic alcoholism high PbB values may be found, but ALAD appears more depressed than might be expected from blood lead levels, and these levels remain low for a number of days after suspension of alcohol consumption (Krasner et al., 1974; Secchi and Alessio, 1974a).

In lead-exposed subjects, false negative results of ALAD may be obtained when hyper-regenerative erythropoietic disorders exist, e.g. bleeding anaemia, haemolytic anaemia (Bonsignore et al., 1970; Battistini et al., 1971).

The European standardized method for determination of ALAD proved to be accurate and with good reproducibility. The interlaboratory coefficient of variation in the intercomparison programme sponsored by the CEC in 1974 was 10% (Berlin et al., 1974).

In the view of Berlin and Schaller (1974), the routine use of ALAD is limited by technical problems, particularly the conservation of the blood sample at 0°C for a limited time interval. In our experience, when the sample is stored at 4°C, no loss of enzyme occurs after 24 hours (see Table IV).

Table IV - Erythrocyte ALAD activity (mU/ml) before and after storage at 4 °C

Samples	Before storage	After storage	
		24 hours	48 hours
1	38.0	37.6	33.0
2	21.4	20.8	17.2
3	19.8	19.9	16.2
4	16.0	16.0	13.0
5	9.8	9.7	9.0

Erythrocyte Protoporphyrin

Heme synthetase is extremely sensitive to the action of lead and the inhibition of this enzymatic activity causes an accumulation of EP in erythrocytes. This is related to the fact that the mitochondrial enzyme regulates the incorporation of iron in the porphyrin molecule. In occupationally exposed subjects, the concentration of this erythrocyte metabolite rises and can reach levels from 10 to 50 times higher than the values found in subjects not occupationally exposed to lead (Vigliani and Angeleri, 1935; Rubino et al., 1958; de Bruin, 1971).

The methods of erythrocyte protoporphyrin determination are numerous. Some permit selective measurement of different porphyrins (Schwartz and Wikoff, 1952; Sassa et al., 1973), others measure the total concentration of erythrocyte porphyrins (Piomelli et al., 1973). All these methods use extractive techniques.

The discovery that erythrocyte protoporphyrin that rises following an abnormal lead absorption (or following sideropenia) is not "free" but bound to zinc, revolutionized the determination methods. In fact, zinc protoporphyrin can be determined on capillary blood diluted with water or alcohol by direct fluorimetric reading (Lamola, 1974). Since 1976 instruments have been developed — hematofluorimeters — for the immediate determination of zinc protoporphyrin on undiluted blood (Blumberg et al., 1977).

Henceforth, protoporphyrin determined with extractive methods will be shown as EP and zinc protoporphyrin as ZPP.

For greater clarity, EP and ZPP are dealt with separately.

Erythrocyte protoporphyrin determined with extractive methods

EP measurement has made considerable advances in paediatrics as a result of studies carried out using microanalytical methods (Kammholz et al., 1972; Sassa et al., 1973; Piomelli et al., 1973; Chisolm et al., 1974). This test has not been used for monitoring occupationally exposed subjects until recently. A highly significant correlation was found between EP and PbB in adult males under stable lead exposure. In this situation EP is also closely correlated with urinary lead and chelatable lead (Roels et al., 1975; Tomokuni et al., 1975; Alessio et al., 1976a).

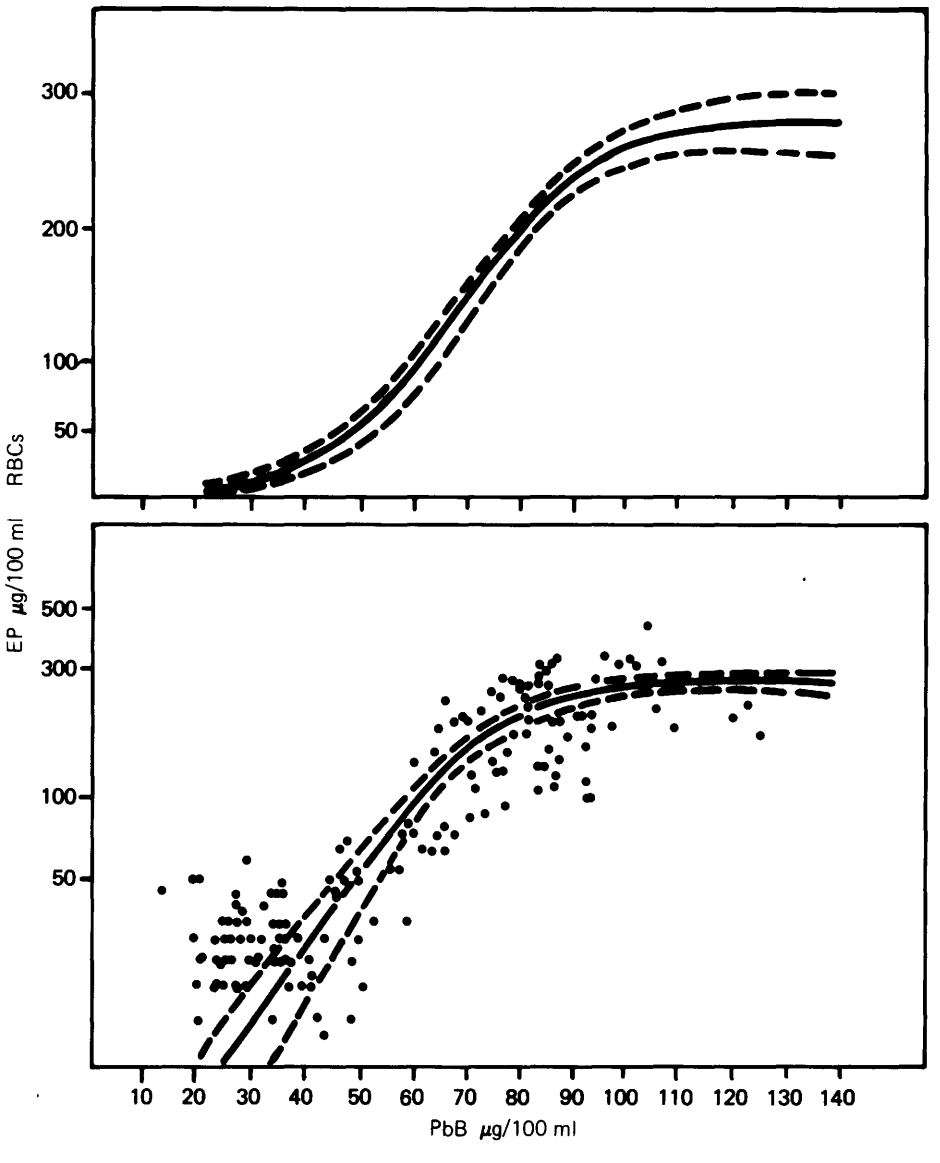

Figure 6 - Relationship between PbB and EP, in 201 adult males currently exposed to lead - r = 0.94.
Upper frame: linear scale on ordinate. Lower frame: logarithmic scale on ordinate.
EP determined according to the Schwartz and Wikoff Method

The behaviour of EP is uniform with respect to the three indicators of dose. EP first undergoes a modest increase with the elevation of the internal load within the normal values. Beyond such normal values a net increase occurs, which continues up to an asymptotic value which is not further altered by the increase in dose (Alessio et al., 1976a).

In adult males the increase in EP in the 40 to 80 μg/100 ml blood lead range appears very marked, so that the difference between "normal" subjects, subjects with "permissible" and subjects with "not permissible" absorption appears more distinct than that which can be revealed by blood lead (Fig. 6). It should be pointed out that at PbB levels which do not cause an elevation in EP, a reduction in ALAD levels is already in operation. On the other hand, the dose-response relationship calculation has shown that in adult males there is a no-response PbB level for an increase in EP of 25-35 μg/100 ml (Roels et al., 1975; Zielhuis, 1975a). The no-response PbB level for ALAD appeared to be 15-20 μg/100 ml (Zielhuis, 1975a).

Moreover, EP permits a fairly accurate prediction of the amount of chelatable lead (Alessio et al., 1976a). This seems particularly interesting since it is very likely that, as an indicator of biological effective internal dose, chelatable lead is more relevant than lead in blood.

EP can be reliably used as a screening test for monitoring occupationally exposed groups since in the 500-2000 μg/24 h range for PbB EDTA (see Table V; Alessio et al., 1976a).

Table V

A) Validity of EP for predicting different PbB levels. Analysis made on 201 adult males currently exposed to lead

PbB (μg/100 ml)	EP (μg/100 ml RBC)	Se	Sp	Validity
⩾40	⩾ 50	0.83	0.98	1.81
⩾60	⩾ 75	0.97	0.99	1.96
⩾70	⩾100	0.98	0.90	1.88

B) Validity of EP for predicting different PbU EDTA levels. Analysis made on 92 adult males currently exposed to lead

PbU-EDTA (μg/24 ore)	EP (μg/100 ml RBC)	Se	Sp	Validity
⩾ 500	⩾ 50	0.84	1.00	1.84
⩾1000	⩾ 75	0.92	1.00	1.92
⩾1500	⩾100	0.96	0.97	1.93
⩾2000	⩾150	0.93	0.83	1.78

Se = sensitivity, Sp = specificity, Validity = Se + Sp
EP determined according to the Schwartz and Wikoff Method

For example, at a blood lead level of 60 μg/100 ml, EP at a cut-off of 75 μg/100 ml correctly classified 97% of positive subjects and 99% of negative subjects. Thus only 3% false negatives and 1% false positives were obtained.

When examining recently exposed subjects, account must be taken of the fact that between the beginning of lead absorption and the increase in EP there is a time lag evaluated by Sassa et al. (1973) as 2 months, and by Stuik (1974) as 2 to 3 weeks.

Normalization of EP after cessation of exposure is slower than that of PbB, ALAU and CP. In fact, in erythrocytes of subjects who have been exposed to lead there is a surplus of EP which persists until the red blood cells are destroyed (Albahary, 1972). However, in severely exposed subjects, EP stays at high levels even for many years after cessation of exposure (Saita et al., 1954; Gajdos, 1957; Rubino et al., 1958). Alessio et al. (1976c) recently demonstrated that the correlation existing between EP and PbB is decidedly lower in male subjects no longer exposed to lead than in currently exposed subjects, and that for the same PbB values, the EP levels are markedly higher in subjects who are no longer exposed.

EP and chelatable lead are closely correlated both in currently exposed subjects and in subjects with past exposure, and the regression curve in both groups takes on an

almost identical profile (see Fig. 7; Alessio et al., 1976c). These data seem to indicate that EP remains at high levels for a long period of time due to a direct inhibition of heme synthetase by the lead released from the deposits.

The erythrocyte metabolite can therefore be used to detect the existence of past exposure and to determine whether a patient who has had past exposure should resume work with lead.

EP levels as high as those occurring in severe lead poisoning might be found in erythropoietic protoporphyria, a rare congenital disorder, and in thalassemia major. Moderate increases have been found in cases of iron deficiency, serious liver diseases, and tumours (Baloh, 1974; Saita et al., 1966).

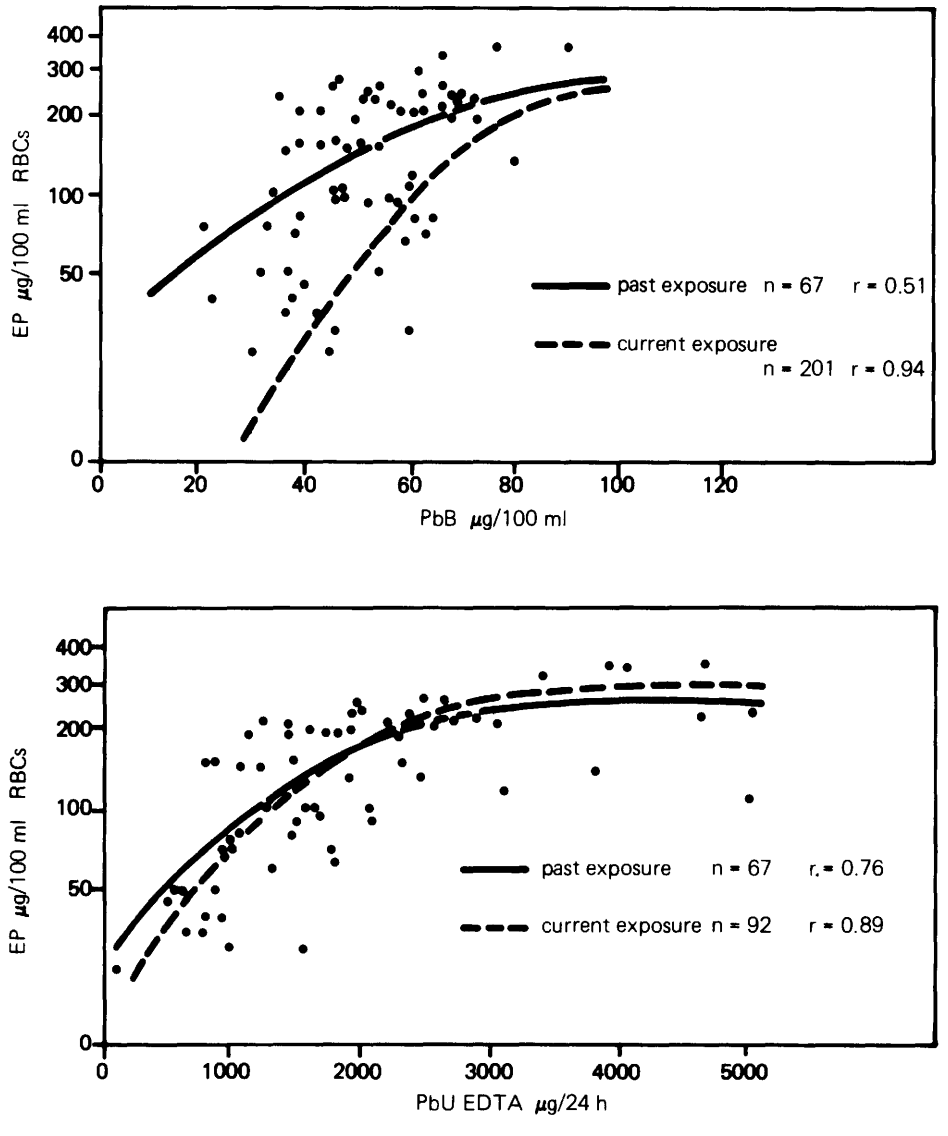

Figure 7 - Relationships between EP and PbB (upper frame) and EP and PbU-EDTA (lower frame) in adult males with past lead exposure.
Scatter diagram: individual date of past-exposed subjects.
Logarithmic scale on ordinate

Zinc protoporphyrin

Determination of zinc protoporphyrin with portable hematofluorimeters is a very practical test which is easier to perform and lower in cost than the extractive methods.

There is a very close correlation between ZPP and PbB in adult males: The regression curve between the indicator of exposure and effect takes on the same profile as already observed for EP (Fig. 7); in fact, at PbB levels below 35-40 µg/100 ml, ZPP undergoes only a moderate increase, but subsequently the increase is very marked (Schaller and Schiele, 1977; Alessio et al., 1978; Fig. 8).

Without cases having PbB levels above 90 µg/ml it is not possible to check whether the regression curve takes on the asymptotic slope observed for EP determined with the Schwartz and Wikoff method.

Research in progress in our laboratory has shown a high predictive validity of ZPP and PbB levels ≥ 60 µg/100 ml : using a cutt-off of ZPP ≥ 80 µg/100 ml validity was 1.77, with very high sensitivity (0.98), signifying 2% false negatives. The test may therefore be used to advantage in screening studies of occupational exposed subjects. Such studies are facilitated by the fact that the instrument is portable, gives immediate results and allows a large number of subjects to be examined in a short time.

As the hematofluorimeter takes account of the absorption spectrum of oxyhemoglobin, the ZPP levels determined on capillary blood are decidely higher than those determined on venous blood; whereas they are identical to those determined on venous blood after oxygenation (Alessio et al., 1978).

A close correlation exists between ZPP and EP; but it should be noted that while Alessio et al. (1978) found that EP levels, determined according the Piomelli method, were higher than ZPP levels, Blumberg et al. (1977) found that ZPP levels were higher than EP levels, and Schaller and Schiele (1977) found that ZPP levels were practically indetical to erythrocyte protoportphyrin levels. A tentative explanation of the discrepancy in results might be the use of a different standard.

It should moreover be noted that the research in progress in our laboratory has shown that by using three hematofluorimeters of different make, significantly different values are obtained.

It is hoped that hematofluorimeter manufacturers carry out a joint study as soon as possible to standardize the calibration of the instrument, so that ZPP values may be readily compared in all laboratories.

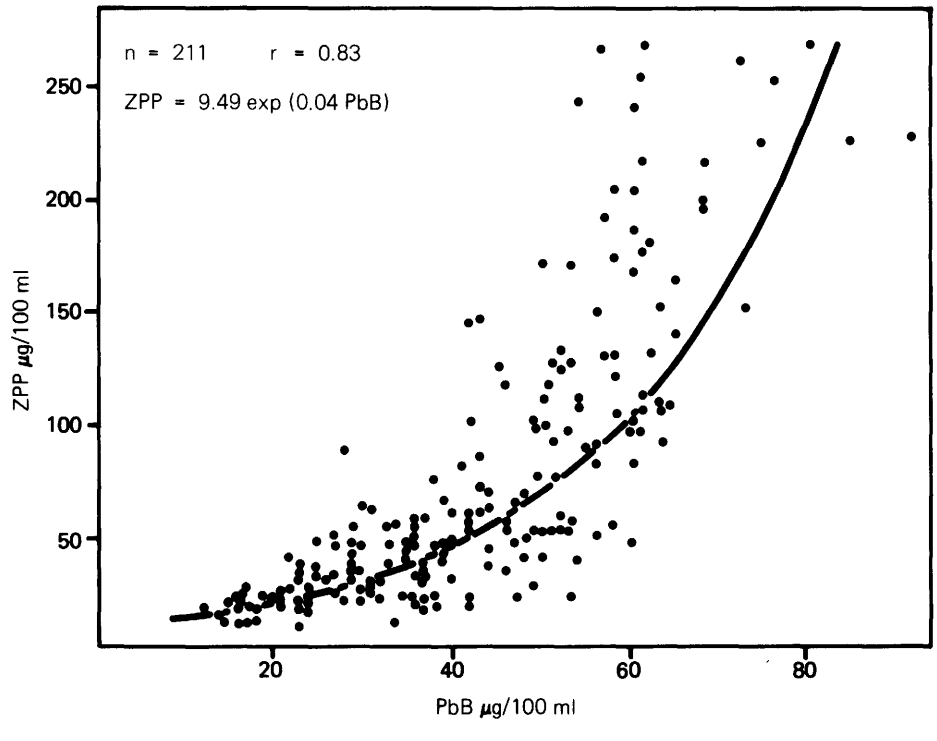

Figure 8 - Relationship between PbB and ZPP in 211 adult males currently exposed to lead. ZPP determined with an ESA 4000 apparatus

Delta-aminolevulinic acid in urine

Due to the inhibition of the ALAD of the maturing RBC's by lead, the transformation of ALA into prophobilinogen is obstructed, resulting in an increase in ALA in the serum and in the urine. On the subject of behaviour of ALA in the serum, a few studies on children with acute encephalopathy are available. However, at present it does not appear that the test can be used routinely, since detection of only moderately increased levels of ALA requires more than 10 ml of plasma (Chisolm, 1975).

Many studies are, however, available on ALAU. Researchers have found a good correlation between the urinary metabolite, PbB and PbU (Williams et al., 1969; Selander and Cramer, 1970; Haeger-Aronsen, 1971; Soliman et al., 1972; Lauwerys et al., 1974). The coefficient of correlation between PbB and ALAU is usually between 0.5 and 0.7, and therefore is not as close as the correlation which generally exists between PbB and the blood tests (ALAD and erythrocyte protophorphyrin). A significant increase in ALAU can be seen at PbB levels slightly higher than those at which there is an increase in erythrocyte protoporphyrin (see Fig. 10; Alessio et al., 1976b). This phenomenon is clearly seen from examination of the dose-response relationship. In fact, the approximate no-response PbB level for ALAU is 35-45μg/100 ml (Roels et al., 1975; Zielhuis, 1975b).

Erythrocyte protoporphoryn permits better discrimination between exposed workers with "permissible" absorption and those with "potentially dangerous" absorption than ALAU. In fact, at a PbB concentration below the currently accepted TLVs, the progressive elevation of erythrocyte protoporphyrin is more marked than that of ALAU (see Fig. 9). It should be noted, however, that the levels of ALAU, like the levels of CPU, also undergo increasing elevation when PbB values exceed 80-90μg/100 ml, while erythrocyte protoporphyrin values do not undergo any further increase. (This phenomenon has not yet been verified for ZPP.) Therefore, urinary tests may have an important application when metabolic damage such as that which can occur in lead intoxication must be evaluated (Alessio et al., 1976b).

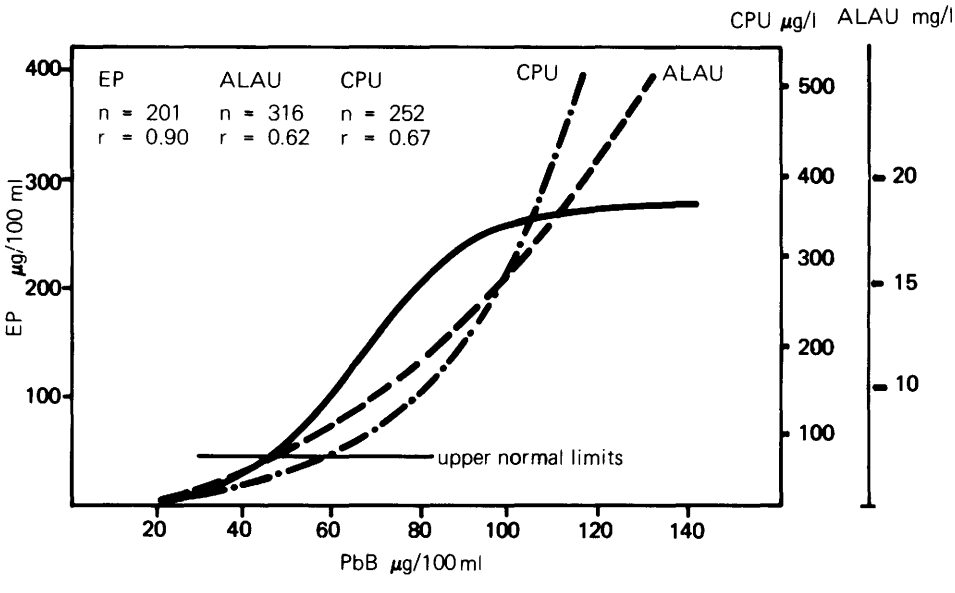

Figure 9 - Relationship between PbB and indicators of effect in adult males currently exposed to lead

The validity of ALAU for predicting PbB appears to be distinctly lower than that of erythrocyte protoporphyrin.

To predict a PbB level $\geq$ 60 μg/100 ml using a cut-off of ALAU > 10 mg/l, validity was 1.67, with sensitivity = 0.75, and specificity = 0.52; the number of false negatives is therefore very high (25%) (Alessio et al., 1976b).

In recently exposed subjects, there is a latency period of about two weeks before the urinary metabolite increases (Tola et al., 1973; Benson et al., 1976).

After cessation of lead exposure, the excretion of ALA in the urine becomes "normal" relatively quickly. This parameter is therefore not suitable for detecting past lead exposure (Haeger-Aronsen et al., 1974).

For the determination of ALAU, as for all the other urinary tests, it is difficult to obtain 24-hour urine samples or urine samples for precise periods of time, e.g. 4-8 hours.

Generally the determination is therefore performed on spot samples. Owing to the different density of daily samples, widely varying levels of the metabolite, e.g. from "normal" to "pathologic" can be obtained from the same subject. For an example, see Fig. 10. To overcome this difficulty, the sample is currently corrected according to its specific gravity or creatinine. This correction will probably be useful in studies on groups of subjects, but in single subjects it does not permit approximation of the value expressed in mg/l or mg/24 h.

The ALAU reported in the literature for the subjects not occupationally exposed to lead are below 6 mg/l or 4.5 mg/g of creatinine. High values of ALAU can also be found in subjects with acute intermittent prophyria.

Various chromatographic and non-chromatographic methods are available for the determination of ALAU. A critical evaluation of some of these techniques has been made by Roels et al. (1974b).

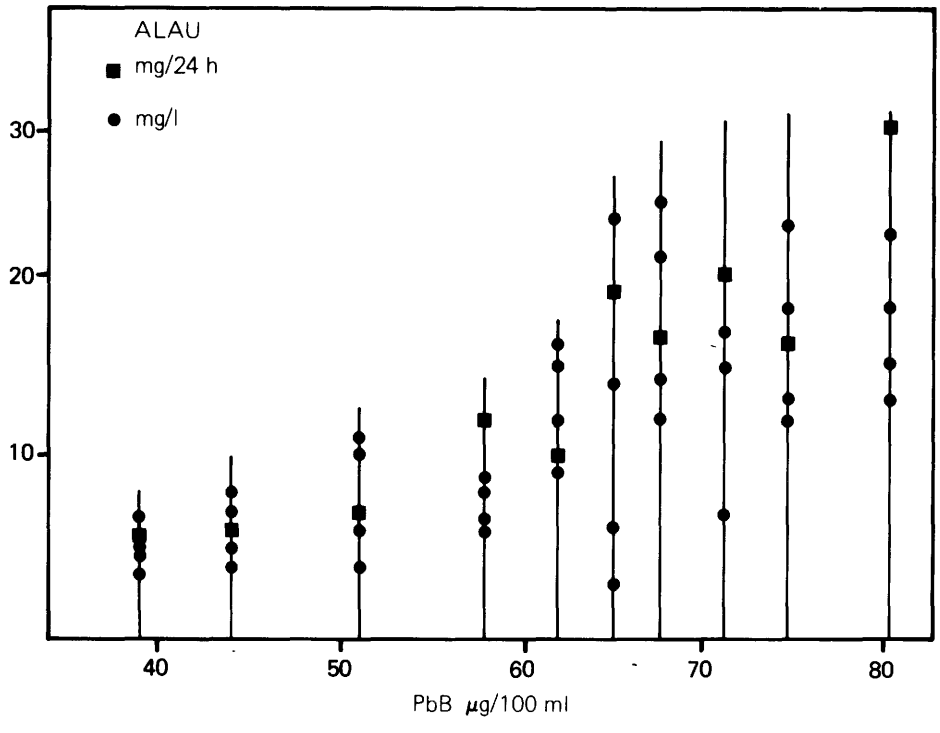

Figure 10 - Dayly fluctiations of ALAU in lead workers with different degrees of exposure: PbB renging between 37 and 84 µg/100ml. Each vertical bar represents data from a single worker

Coproporphyrin in urine

In subjects under continuous exposure, there is a good correlation between PbB and CPU (Williams et al., 1969; Soliman, 1972; Alessio et al., 1976b).

An excretion of coproporphyrins in the urine (mainly isomer III) beyond the upper normal limits occurs when the PbB levels are slightly higher then those at which an increase in ALAU values occurs (see Fig. 10). This phenomenon is also evident in the examination of the dose-response relationship between the two urinary metabolites and PbB (Wada, 1976).

From commencement of exposure to increase in CPU there is a time lag of about 2 weeks in recently exposed subjects (Tola et al., 1973a; Benson et al., 1976). With cessation of exposure, the urinary coproporphyrins return to normal within a few weeks and sometimes within a few days (Saita, 1962).

Urinary coproporphyrin is not a specific test of lead exposure. Increases in the urinary metabolite may occur also in porphyria cutanea tarda, liver diseases, haemolytic anaemias, malignant blood diseases, infectious diseases, and also after consumption of alcohol. However, subjects with severe lead exposure may in some rare cases show normal levels of coproporphyrin in the urine (Saita et al., 1966; Lauwerys, 1975).

The same limitations given for ALAU apply for this test as well.

The validity of CPU (determined on spot samples) to predict different PbB levels is rather modest, so its use as a screening test is limited (Alessio et al., 1976b).

Other porphyrins are not as common in urine, although increased uroporphyrin levels may occasionally be detected, especially in severe cases of lead poisoning (Stankovic et al., 1973).

Haemoglobin and stippled cells

These two tests are only marginally important for the routine monitoring of lead exposure. Haemoglobin and PbB are generally poorly correlated; a reduction in Hb occurs when the PbB level exceeds 100-110 μg/100 ml (Williams, 1966; Cooper et al., 1973).

In the past, stippled cell count was "an early indicator of abnormal lead absorption", since the appearance of stippled cells precedes the onset of anaemia (Saita, 1962). This test is not used today because it does not accurately reflect the amount of lead absorbed and because the number of stippled cells increases with a much greater time lag than the other biological changes discussed above (Lauwerys, 1975). Furthermore, the test is not specific for lead intoxication since stippled cells may be present in thalassemia, pernicious anaemia and anaemia due to renal insufficiency (Saita, 1962).

Indicators of effects in adult females

Because of its relatively recent interest, the number of studies of female exposures is rather limited. They generally involve a small sample of subjects with a moderate degree of exposure.

Erythrocyte delta-aminolevulinic acid dehydrase

Studies made on groups of subjects not occupationally exposed have shown that adult females living in the same place and of the same age as a group of male controls had a higher mean value of erythrocyte ALAD activity and a lower mean value of lead in blood (Haeger-Aronsen et al., 1971; Secchi et al., 1973).

In the women it was also observed that the reduction in ALAD activity with age is less marked than in men (Secchi and Alessio, 1974b). The difference found between the two sexes was attributed to a different lead intake with food, wine, and smoking. Tola (1973), who examined 171 women and 1199 men with PbB levels between 9 and 90 μg/100 ml, found no consistent differences between the ALAD values of men and women at the same blood levels. Similar results have been obtained in a study of 93 women and 95 men with PbB levels ranging from 8 to 80 μg/100 ml (Alessio et al., 1977). From these data it therefore appears that there are no differences in ALAD level between males and females with the same level of internal lead load.

Erythrocyte protoporhyrin

Stuik (1974) has shown that increase in EP occurs in adult females at a lower concentration of PbB than in adult males (for females at a PbB level of 25 - 35 μg/100 ml; for males at 35 - 45 μg/100 ml), and that the increase in EP was steeper in females with the increase in PbB values.

EP was observed to behave similarly by Roels et al. (1975) in 40 male and 24 female adults with moderate occupational exposure (Pb 50 μg/100 ml). EP and PbB were closely correlated in the the two groups; EP was markedly higher in the women at the same internal lead dose, i.e. PbB. This phenomenon can also be clearly observed in the groups considered in a study by Alessio et al. (1977), see Fig. 12, which consisted of subjects with more severe exposure. Similar results are obtained when erythrocyte protoporphyrin is determined with hematofluorimeters, like ZPP.

Study of the dose-response relationship does however show that the no-response PbB levels for an increase in EP are 25 - 35 μg/100 ml for females (Roels et al., 1975).

In non-occupationally exposed women, the EP levels are higher than in males (Roels et al., 1975; Wibowo et al., 1977).

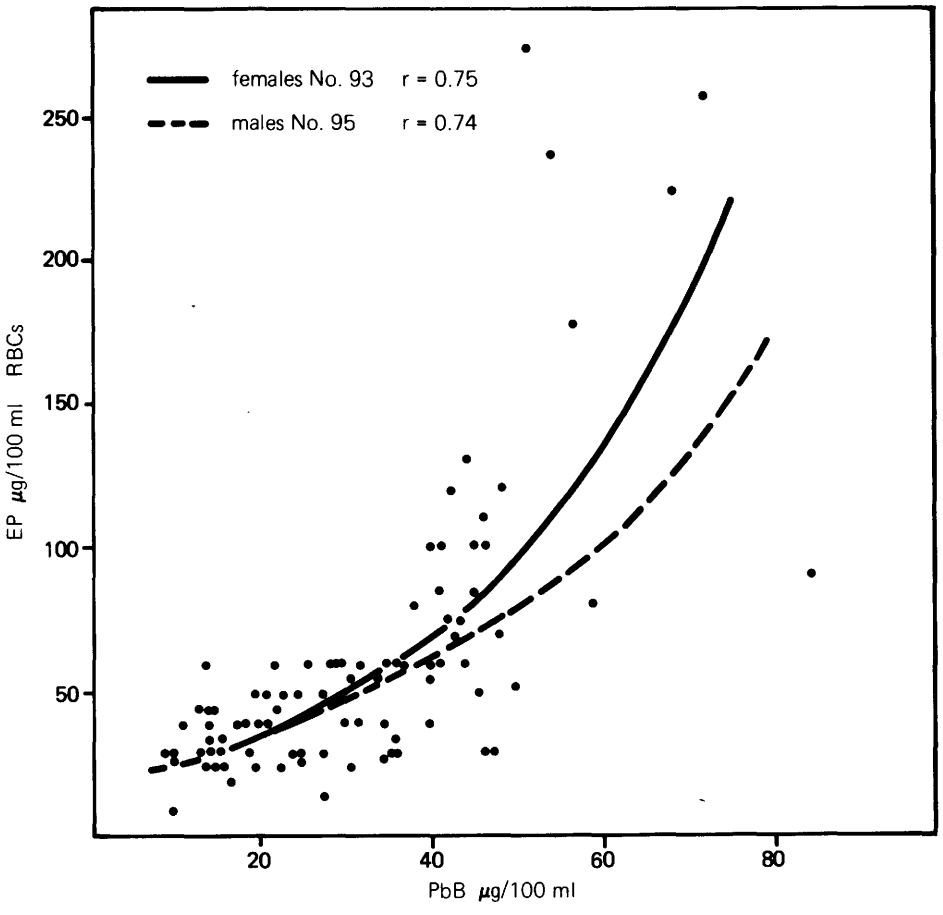

Figure 11 - Relationship between PbB and EP in adult males and females

Delta-aminolevulinic acid in urine

In occupationally exposed women, ALAU and PbB are well correlated. Levels of the urinary metabolite in the women seem slightly higher than in men, at the same PbB level (Roels et al., 1975).

Study of the dose-response relationship shows that the no-response levels for an increase in ALAU are 35 - 45 µg/100 ml for males and 30 - 40 µg/100 ml for females (Roels et al., 1975). It does not, however, appear that there is a significant difference for ALAU values in non-occupationally exposed subjects in the two sexes.

Urinary coproporphyrin

Results by Alessio et al. (1977) show that CPU and PbB are significantly correlated. The relationship between the two parameters does not seem to indicate the existence of a difference in behaviour of the urinary metabolite in the two sexes.

From the available data on adult women, the following conclusions can be drawn:

- in adult women a significant correlation exists between the indicators of internal lead dose and indicators of effect, as had already been confirmed in adult men;
- the relationship between indicators of dose and indicators of effect, evaluated with the regression curve and/or the dose-response curve, shows that in the female, the "qualitative" behaviour of the indicators of effect is identical to that observed in males. In males the erythrocyte ALAD undergoes a distinct inhibition in the range of PbB values below 40 µg/100 ml. The erythorcyte protoporphyrin initially increases rather moderately; then, beyond a PbB level of 40 µg/100 ml, the increase is very marked. ALAU and CPU increase above normal for PbB values higher than those at which an increase in protoporphyrin occurs. The increase in the two urinary metabolites in relation to the increase in internal lead load is not as steep as the increase in the erythrocyte metabolite.

- There is a clear difference in the "qualitative" behaviour of protoporphyrin (and perhaps of ALAU) in the two sexes at identical levels of internal dose. This phenomenon appears to be due to a greater susceptibility of haemopoiesis to lead in women. The cause of such hypersensitivity might be a relative iron deficiency in women, causing increased alterations in haemopoiesis induced by lead (Stuik, 1974; Zielhuis, 1975a). Synergic action between sex hormones and lead on the enzymatic activity of heme synthesis has also been suggested (Roels et al., 1975).

Conclusions

A vast number of tests which permit a sufficiently accurate evaluation of the degree of exposure, body burden and toxic affect are available for monitoring lead workers. Given the advantages and limitations of each test, the choice of indicator or indicators will depend on the type of investigation.

Two tests should be used simultaneously for the ***periodic surveillance*** of workers exposed to lead concentrations sufficient to cause alterations in biological indicators close to the "permissible" limits. One test should be designed to indicate internal dose and another to indicate effect. In monitoring individuals, blood tests are preferable to urinary tests, the latter being subject to considerable variation due to differences in urine density. Furthermore, elevation beyond the "normal" limit values of the urinary indicators of effect (i.e. delta-aminolevulinic acid and coproporphyrin), occurs at internal dose levels higher than those at which an alteration occurs in the blood indicators of effect (i.e. delta-aminolevulinic acid dehydrase activity of erythrocytes and erythocyte protoporphyrin).

In general, it is advisable to use blood lead levels and erythrocyte protoporphyrin for periodic monitoring as these two tests integrate well. This is not only because one evaluates internal dose and the other the effect but also because blood lead evaluates a momentary situation (present exposure) while the erythrocyte metabolite permits evaluation of body burden and past exposure. These features are important in relation to the fact that industrial levels of exposure are rarely stable, so that PbB alone might give only partial information in cases of non-steady-state exposure. On the other hand, protoporphyrin does not permit assessment of current absorption.

A ***screening test*** wich is inexpensive, easy to perform, sensitive, specific, precise and accurate should be used to identify subjects with the highest exposure from a group. The percentage of false negatives should be minimal, but too many false positives may give rise to excessive referrals for diagnostic evaluation, cause alarm and overcrowd busy outpatients facilities (Chisolm et al., 1974). Both erythrocyte protoporphyrin and delta-aminolevulinic acid dehydratase comply on the whole with these requirements: both tests have been shown to possess high predictive validity of the "true situation", i.e. internal lead load measured with PbB.

Protoporphyrin offers the following advantages over ALAD: a) it also permits quantification of situations in which an internal lead load has already caused a marked inhibition of ALAD; b) it can be measured using capillary blood with micromethods which are rapid to perform. The fluorimetric zinc protoporphyrin technique appears to offer a simple, instant and repeatable measurement; c) a higher number of analyses can be performed in the course of the day; d) the sample for analysis can be stored longer periods of time.

The urinary test may be used for assessment of the ***environmental conditions of a place of work*** on a group basis, although blood tests provide more accurate information. If urinary tests are used, it will be appropriate to take the density of the samples into account, rejecting those with density lower than 1010, or with creatinine concentration below 0.5 g/l.

For a correct evaluation of a group investigation, it will not be sufficient to express the data solely as a mean and standard deviation or range. This procedure can be applied only if the parameter follows a Gaussian distribution, and it will be appropriate to consider the percentage distribution of the data as well (Zielhuis, 1974).

The choice of biological tests must also be made on the basis of the availability of suitable equipment and trained technical staff, the possibility of easy and rapid performance, transport and cost.

The problem of ***biological limit values*** for workers exposed to inorganic lead has been considered by many authors and has been discussed at numerous meetings of experts. Limit values have been proposed or established by national and international bodies responsible for the protection of workers health.

In September 1976 a workshop was organized in Amsterdam under the auspices of the Permanent Commission and International Association on Occupational Health and the World Health Organization, which re-examined the problem of permissible limits for occupational exposure to inorganic lead (Zielhuis, 1977).

At the workshop the following recommended guidelines for PbB based on health criteria were drawn up: "for **male** workers **Individual** PbBs should not exceed 60 μg/100 ml in the light of present knowledge available to this group". It is however desirable to reduce individual exposure below this level, taking into account the effects on the haematopoietic system at concentrations above 45 - 50 μg/100 ml and on nerve conduction velocity at concentrations between 50 - 60 μg/100 ml. The group could not agree on what level should b regarded as a health based permissible level for occupational exposure. So far as **female** workers of child-bearing age are concerned the risk of harm to the foetus at above mentioned PbB levels is not supported by factual evidence but is based on theoretical possibility. Nevertheless, because of potential effects on the foetus, a safe practice would be to avoid employment of women of child-bearing age on lead work where blood levels might regularly exceed 40 μg/100 ml.

In 1978, recommendations were also made in the U.S.A. by the National Institute for Occupational Safety and Health (NIOSH, 1978), and by the Italian Society of Occupational Medicine and Industrial Hygiene (Foa et al., 1978), while in 1980 a report of a group of experts was issued by (WHO (WHO-1980).

In 1982, the Council of Ministers of the European Communities (**CEC 1982**) adopted a directive on the protection of workers exposed to inorganic lead. This directive sets action levels and limits both for lead in air and for biological indicators (Table VI). The exceeding of the limit values may require removal of workers from exposure.

The above limit values are to be considered as maxima; Member states are encouraged to set lower limits. Such is the situation already in some of the Member states.

Table VI. CEC biological action levels and limit values for lead exposure at work.

Action Levels	
a)	PbB > 40ug/ 100 ml information of workers
b)	PbB > 50ug/ 100 ml full application of Directive
Limit Values	
a)	PbB 70 ug/ 100 ml
b)	PbB between 70 - 80 ug / 100 ml - additional biological indicators must be used - limit value exceeded if ALA > 20 ug / g creatinine or ZPP > 20 ug / g haemoglobin or ALAD < 6 E.U.

Research Needs

In spite of the fact that lead is the most extensively studied metal from the point of view of industrial toxicology, further research is still necessary to establish safe permissible limits for exposure. The following are recommendations for further research:

- Standardization of tests. Standardization of analytical methodology would allow a comparison of the studies being carried out by the different laboratories and research workers and would facilitate discussion and application of normal values and permissible limits internationally.

  As a result of the inter-laboratory variability of lead blood levels, the relationship between these levels and other biological indicators cannot be precisely determined.

The opinions of the various research workers on the usefulness of correcting the results of urinary tests made in spot samples are conflicting. This question should be dealt with to verify whether the corrections made for the individual subject allow a value to be obtained which is sufficiently similar to the value obtained on the same day on 24 h urine (considering the value expressed as quantity of substance per litre and/or quantity of substance per 24 hours).

Daily variations in the results obtained from the various biological tests should be investigates as there are very few references to this in the literature.

- Determination of the relationship between external and internal exposure. More extensive studies involving larger groups of subjects and taking particle size and solubility into consideration are necessary for this determination.
- Establishment of better indicators of internal lead dose. Very few studies are available on lead in plasma although diffusable plasma lead may offer the best approximation of the biologically effective body burden. It should, however, be taken into consideration that the plasma fraction is not a constant fraction of the total blood concentration.

  Chelatable lead may provide a more direct measurement of the rapid exchange pool and it may be used as a rough measure of plasma lead concentrations, since it is normally found in plasma and not in cells. It therefore appears necessary to determine the dose-effect relationship between chelatable lead and other indicators.

  The dose and rate of administrations of chelating drugs for estimating the mobile portion of the body burden should also be standardized.
- Research on hypersensitivity to lead. The few data available in the literature (Saita and Moreo, 1959; Girard et al., 1967; Albahary, 1972; Saita and Lussana, 1971), indicate that subjects with genetic alterations (thalassemia, haemoglobinapathy, G6PD deficiency) may be hypersensitive to the action of lead. The high incidence of these alterations in some countries of the European Community and the increasing transient population, e.g. immigrants from Mediterranean area, point to the necessity of a re-examination f the problem.

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Human biological monitoring of industrial chemicals series

Manganese

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Summary

This document reviews the metal manganese (Mn) and manganese(IV)oxide (brownstone, MnO_2) as related to occupational exposure and the possibilities of the biological monitoring of exposure.

Chronic manganism has been observed in workers exposed through inhalation to manganese for periods of more than two years, but studies attempting to correlate degree and length of exposure with manganese levels in blood, urine, faeces and hair show contradictory results and dose/response relationships have not been established. Most authors assume that there is not direct connection between manganese concentrations in biological materials and the severity of chronic manganese poisoning. Individual susceptibility to the disease is more likely the decisive factor.

Until further information is available, the principle of biological monitoring can therefore only be recommended with reservation for manganese. An improvement in industrial hygiene measures at workplaces and a regular neurological examination of workers exposed to manganese would seem to be the best currently available methods of preventing chronic manganism.

Manganese

Chemical and Physical Properties

Manganese is a very hard, brittle metal, greyish-white in colour.

Table I - Physical properties of manganese

Symbol	Mn
Atomic weight	54.94
Melting point	1247 °C
Boiling point	2030 °C
Specific gravity	7.2 g/cm^3
Solubility (Mn and MnO_2)	Soluble in diluted acids
Valency states	From III to VII, mostly II, IV and VII

Pure manganese is seldom used in industry. It is a typical alloying metal. Manganese(IV)oxide (brownstone, MnO_2) is the most important manganese compound from the viewpoint of occupational medicine.

The most significant organic manganese compounds are the antiknock compound, methyl cyclopentadienyl manganese tricarbonyl (MMT), the fungicide, manganese ethylene bis-dithiocarbamate and the catalyst and drying agent, manganese stearate. The toxicity of these compounds for humans is unknown, and thus organic manganese compounds will not be taken into account.

Although manganese(IV)oxide is the most important manganese compound as far as occupational medicine is concerned, in many cases the exact identity of the manganese compound which causes a particular effect is not reported in the literature. It has therefore often been necessary to refer to manganese generically in this document.

Effects on Humans

The significance of manganese as an essential trace element for humans has not been proven. Manganese deficiencies in humans are not known. The daily manganese requirement for man is estimated at about 3 mg. In general, this is probably covered by food intake.

There is a possible correlation between exposure to manganese and collagenosis and allied diseases (Schroeder et al., 1966). The pathogenic significance of mangenese is not known, however. Saric and Hrustic (1975) recently confirmed the long-established significant drop in systolic blood pressure in persons occupationally exposed to manganese.

In view of the relatively small quantity of manganese in food, oral manganese intake is only very rarely the case of chronic manganese poisoning. Acute intoxication caused by potassium permanganate is due less to the manganese content than to the strong oxidation potential and the potassium content in the compound.

Manganese poisoning is nearly always due to occupational contact where manganese is mainly absorbed through the lungs. Inhalation of manganese fumes can lead to acute metal-fume fever.

Pneumonia has also frequently been observed as a result of the effects of fine manganese dust. This is marked by poor response to antibiotics. In other respects manganese pneumonia cannot be differentiated from bronchopneumonias from other sources. Permanent damage to the lungs, especially pulmonary fibrosis or straight pneumoconiosis, does not seem to occur.

Features of chronic manganese poisoning are disorders of the central nervous system, the main factors being changes in state of mind and in the extrapyramidal motor and vegetative nervous system. The clinical picture of 'manganism' is similar in many ways to Parkinson's disease with rigor, tremor and akinesia. In contrast to Parkinson's syndromes of other genesis, the disease often takes shape suddenly and also affects younger persons after generally more than two years of occupational exposure.

In pathological anatomy, degenerative changes of the ganglion cells in the putamen, pallidum, caudate nucleus and thalamus are found.

Enzyme inhibition in the metabolism of the biogenic amines is considered to be the cause of the damage to the central nervous system. Manganese is thought to disturb the synthesis of the central transmitter substances, dopamine and serotonin. It is still not known, however, why the effect is irreversible.

When the manganese source is removed the disease generally shows no progression, but also no improvement. In terms of differential diagnosis, manganese-induced Parkinson's disease must be considered along with idiopathic, postencephalitic and arteriosclerotic types of Parkinson's syndrome. As with other types of parkinsonism, improvements were obtained after treatment with L-dopa (Rosenstock et al., 1971).

Mutagenic, teratogenic and carcinogenic effects of manganese in humans have so far not been reported.

Metabolism

The main route of occupational exposure is absorption of dust and fumes containing manganese via the respiratory tract. Cutaneous absorption seems to be of no significance. Mangenese intake in the case of persons not occupationally exposed is mainly oral with food in the form of the water-soluble compounds of manganese.

Long-term balance studies show the gastrointestinal absorption of manganese compounds to be slow and slight. Values of between 3 and 12% have been calculated. The usual daily intake is approximately 2-5 mg Mn/day (Schroeder et al., 1966). Fish and other forms of marine life are relatively rich in manganese. The highest concentrations were found in tea leaves.

Drinking water and air intake, approximately 2 μg and 5-6 μg respectively, accounts for only a small proportion of the average daily manganese intake.

There are no reliable data available on the extent of retention and resorption of respirable dust and fumes containing manganese. Schroeder et al. (1966) maintain that manganese can accumulate in the lungs. It is thought that manganese is transported slowly and continuously from the lungs to the blood. The content of manganese in the lungs of normal persons, however, does not appear to be age-dependent.

Manganese is transported in blood partially in trivalent form, bound to a betaglobulin, transmanganin. The linkage probably takes place in the liver (Rosenstock et al., 1971). The manganese content in red blood cells is about five times the plasma or serum manganese level. The individual organs are reported to have a relatively constant manganese level. No correlation between organ concentrations and age has been determined (Schroeder et al., 1966). The highest organ concentrations found are in the liver, pituitary gland, small intestine and pancreas.

About 43% of the total body burden is contained in the bones. Manganese is particularly associated with the mitochondrial fraction.

Manganese is excreted primarily with the faeces. 92% of the total excretion is thought to be via the feaces. It is also thought that manganese elimination via the bile contributes to elimination with the feaces. Some of the manganese probably undergoes enterohepatic circulation.

Urinary manganese excretion is low, amounting to only about 6% of total excretion (Schroeder and Nason, 1971). Calcium EDTA, on the other hand, appreciably increases urinary excretion and this test might be used to establish elevated exposure (Rosenstock et al., 1971).

Based on the figures supplied by Schroeder and Nason (1971) a proportion of the daily excretion of about 2% can be attributed to perspiration. The deposition of manganese in the hair is negligible, about 1 μg/g.

Mohany and Small (1968) established by intravenous administration of $^{54}MnCl_2$ that manganese excretion is two phased. They calculated a biological half-life of 4 days for the rapid phase and a half-life of 39 days for the slow phase.

In view of the constant level of manganese in the body tissues it is assumed that manganese does not accumulate in the body. Long-term balance studies performed by Tipton et a. (1969) and by McLeod and Robinson (1972), however, do suggest that a certain amount of the daily intake of manganese is retained. These studies did not take into account all possible pathways of excretion, however, and errors in method cannot be ruled out.

Human manganese metabolism seems to be closely connected with iron metabolism. Thomson et al. (1971) found increased manganese absorption in patients with iron deficiency but no increased retention. Haemochromatosis due to excessive iron absorption is also connected with increased levels of manganese in the liver (Alstatt et al., 1967). Another factor, which may influence manganese metabolism, is lead exposure. Zielhuis et al. (1978) observed a tendency for manganese in blood to increase with increasing blood-lead levels, but the reason for it is still unclear.

In general there is probably good autoregulation of the manganese levels in the organs and body (Schroeder et al., 1966). The same authors found considerably increased manganese levels in certain persons as compared with controls. They therefore suggest a genetically determined disorder of manganese metabolism. The studies performed by Mena et al. (1969) also point to individual differences in manganese metabolism and its adaptation. Healthy manganese workers show a higher metabolism rate of radioactive 54-manganese than control persons and persons suffering from manganism. The reason for a predisposition to the disease in the latter may therefore be a poor adaptation to the increased intake.

Factors, which possibly influence the susceptibility to manganism, are alcoholism, chronic infections, nutritional deficiencies, especially iron deficiency, a high nutritional intake of manganese, and dysfunction of liver and kidneys. The mechanisms which may cause an increased susceptibility are still unknown.

Biological Indicators

Methods for Assessing Concentrations in Biological Material

Neutron activation analysis, atomic absorption with and without flame, colorimetric and catalytic processes, X-ray fluorescence and polarography are used to determine manganese levels in biological material. Neutron activation is a very sensitive and specific technique, but it is impractical due to the time involved and to the requirement of a neutron source (d'Amico and Klawans, 1976).

As a reference method, however, neutron activation analysis is indispensable. Colorimetric methods require careful preparation of samples and are neither very sensitive nor sufficiently specific. X-ray fluorescence is not suitable for biological material due to its poor detection limit. Not enough work has been done with polarography to determine its potential.

Atomic absorption, particularly flameless, shows good sensitivity and, given suitable sample operation, also high specificity. The material to be analysed can sometimes be used directly and sample requirements are minimal (d'Amico and Klawans, 1976). Studies conducted by Smeyers-Verbeke et al. (1976) with the use of flameless atomic absorption show that calcium and magnesium, in particular, interfere with manganese determination but only in unphysiologically high concentrations. Suitable temperature programmes, the use of an addition process and compensation by deuterium background correction by and large enable matrix effects to be avoided: Conventional flame atomic absorption lacks sensitivity in manganese determination. The mineralization and extraction procedures required are time-consuming and samples may easily become contaminated with manganese.

A comparison of normal blood and blood component values measures by different authors using different methods (Tables 2 and 3), shows a wide range of analytical results. This is primarily due to the methods employed. There is a marked trend towards lower values which suggests a lack of specificity in earlier methods. Similar drops in the normal values of trace elements in biological material, due purely to method, have been recorded in recent years for a number of other elements (Mertz, 1975).

Concentrations in Blood and Blood Components

Generally only manganese-free materials may be used for collecting and storing biological samples as indicators for exposure to manganese. In the case of blood-samples especially, the venipuncture by steel needles can result in elevations of the manganese levels up to 10%. Therefore plastic or nickel needles should be preferred.

A number of blood and blood-component manganese levels recorded in the literature for normal subjects are summarized in Table 2. Manganese levels in whole blood measured by spectrometry are about ten times higher than the results obtained with neutron activation and atomic absorption.

Table II - Normal manganese levels in blood and blood components

Country	No.	Test material	Average (µg/100 ml)	Range (µg/100 ml)	Method	Author	Year
U.K	-	Whole blood	0.24	S.D. ± 0.08	Neutron activation	Bowen	1956
U.S.A	16	Serum	0 25	0.205 - 0.297	Neutron activation	Papavasiliou and Cotzias	1961
	7	Plasma	0 269	0.21 - 0.302	Neutron activation	Papavasiliou and Cotzias	1961
	7	Whole blood	1.160	0.901 - 1.45	Neutron activation	Papavasiliou and Cotzias	1961
U.S.A.	48	Serum	1 3		Spectrometry	Butt et al.	1964
	47	Whole blood	4.0	S.E. 0.1	Spectrometry	Butt et al.	1964
U S A	12	Plasma	0.43	± 0 05	Neutron activation	Olehy et al.	1966
	15	Erythrocytes	1.6	± 0 1	Neutron activation	Olehy et al.	1966
F R.G.	62	Serum	1.68	0.05 - 2.1	Spectrometry	Mertz et al.	1968
U.S A.	40	Serum	2.4	1 2 - 3.8	AAS (with flame)	Mahoney et al.	1969
JAPAN	-	Whole blood	3.47	To 8.85	Spectrometry	Horiuchi et al.	1970
U.S.A.	-	Plasma	0.83	From 0.25	Colorimetry, Spectrometry	Schroeder and Nasan.	1971
U S.A	-	Whole blood	3	-	AAS (with flame)	Smyth et al.	1973
U.S.A.	19	Serum	1.02	0.74 - 1 25	AAS (flameless)	d'Amico and Klawans	1976
BELGIUM	20	Whole blood	1.22	S.D. ± 0.39	AAS (flameless)	Buchet et al.	1976

The red blood cells contain abot five times more manganese than plasma or serum. The total blood mangenese content is approximately 0.14 mg, i.e. one hundreth of the entire body burden of 12 to 20 mg (Schroeder and Nason, 1971).

The analysis results of the various authors differ appreciably. The blood manganese levels recorded for control subjects and persons exposed to manganese can therefore only be taken as relative values. A certain value is attached to the establishment of the blood manganese level in the determination of high exposure levels. Jonderko et al. (1971) consider the blood mangenese level as a parameter of some value in the identification of chronic manganese poisoning.

Smyth et al. (1973) found no significant correlation between manganese exposure and the blood manganese level. Nor did the blood manganese level differ appreciably between persons exposed to manganese, with an average of 4 µg/100 ml, and a non-exposed comparative group, which averaged 3 µg/100 ml.

Mahoney et al. (1969) also found no change in the serum manganese level after daily oral intake of 800 mg manganese chloride for three months and 200 mg for four weeks.

Jonderko et al. (1971) found an increase in the serum manganese level only in persons who had been exposed for more than four years to manganese. They attribute this to

non-occupational influences which could favour manganese retention, e.g. consumption of alcohol. After the exposure source was removed the authors found that the serum manganese reverted to normal within 10 months.

Horiuchi et al. (1970) determined by spectrometry the blood manganese level of three groups of persons exposed to different degrees of manganese (see also Table 4). The most highly exposed group, consisting of 43 workers in a mangenese mill, showed a median of 9.5 μg/100 ml (range: 4-5 μg/100 ml). The blood manganese levels of the occupationally exposed groups showed a statistically significant difference ($p = 0.0113$) in comparison with the control group with a mean blood manganese level of 3.47 μg/100 ml. Horiuchi et al. (1970) also established a positive correlation ($p = 0.097$) between blood manganese and neurological findings (see also Table 5). This significant relationship between blood manganese and neurological findings is not confirmed by the studies of Smyth et al. (1973), Jonderko et al. (1971) and other authors.

The studies, although partially contradictory, do not suggest any dose/response relationship between blood manganese lvels and health disorders. On the other hand, the results, often obtained by unreliable methods, do not prove that such dose response relationships do not in fact exist. The relatively short biological half-life of manganese in the human body means that dose/response relationships can be expected at best for the period in which the disease occurs, but not after exposure has ended.

In view of the substantial individual difference, assessment of the degree of exposure by determination of blood manganese level would seem to be possible on a group but not on an individual basis.

As things stand at present, the proposed exposure limit of 10 μg/100 ml blood (Department of Employment U.K., 1974) presents problems both from toxicological and analytical viewpoints. Considerably higher as well as considerably lower blood levels are considered to be normal by certain authors.

Concentrations in Urine

As the results summarized in Table 3 show, the normal excretion levels of manganese in the urine, established by the different authors, show considerable discrepancies. According to the biological criteria of the Department of Employment (U.K.) (1974), the normal manganese excretion in the urine is less than 10 μg/l. 50 μg/l has been proposed as a value used for establishing a provisional maximum exposure level. Urine excretion can be increased considerably by administering calcium EDTA. Rosenstock et al. (1971) see this as a potentially valuable test for determining manganese exposure which has occurred a considerable time back. Increased manganese excretion in the urine several years after exposure to manganese has terminated is considered by Browning (1961) to be due to the existence of lung deposits from which manganese is transported to the body.

The persons examined by Smyth et al. (1973) showed an average urinary manganese excretion rate three times higher than non-exposed control persons. Manganese excretion by the exposed group averaged 19 μg/l urine. Nevertheless, individual results varied greatly. There was only a slight correlation between the degree of exposure measured by the air concentration and the manganese excretion in the urine. Smyth et al. (1973) do not consider urinary manganese excretion to be a suitable parameter for manganese exposure due to its low proportion in the total elimination of manganese.

Tanaka et al. (1969) found a positive correlation between manganese air level and manganese excretion in the urine, but not between manganese excretion and neutrological symptoms. The authors assume that the manganese level in urine is basically a measure of current exposure.

Horiuchi et al. (1970), on the other hand, found differences in urinary manganese excretion in the groups they examined, depending on the degree of exposure. The differences were significant ($p = 0.00049$) on reference to the authors' normal value for manganese in urine with a mean of 6.3 μg/l. In addition, the authors established statistically significant correlations between blood level and urinary excretion ($r = 0.283$) and neurological symptoms ($p = 0.097$) for blood, and $p = 0.001$ for urine) for all three groups together. The results of the study of Horiuchi et al. (1970) are shown in Table 4. The results of the statistical evaluation are presented in Table 5.

Confirmation or contradiction of the results provided by Horiuchi et al. (1970) would require further studies. Studies to date do not show any dose/response relationships for urinary manganese excretion and health disorders. The establishment of an

exposure limit of 50 µg/l currently presents problems from the toxicological and analytical viewpoints. Nevertheless, Smyth et al. (1973) determined manganese excretion levels of more than 45 µg/l in four out of five persons exposed to manganese who showed neutrological symptoms. Therefore, the proposed limit of 50 µg/l (Department of Employment, U.K., 1974) would not appear to be too low.

Table III - Normal manganese excretion through urine

Country	No.	Average (µg/l)	Range (µg/l)	Method	Author	Year
U S.A.	-	-	1 - 10 µg/l	AAS (with flame)	Ajemian and Withman	1969
U.S.A.	2	43 µg/d 53 µg/d	± 0.8 µg/d ± 1.6 µg/d	Spectrometry	Tipton et al.	1969
JAPAN	-	6.31	to 30 2	Spectrometry	Horiuchi et al.	1970
U.S.A	-	300 33	-	Colorimetry Spectrometry	Schroeder and Nason	1971
U.S.A.	3	-	4 - 19 µg/d	AAS (with flame)	McLeod and Robinson	1971
U.S.A.	-	7	-	AAS (with flame)	Smyth et al.	1973
BELGIUM	20	0.65	S.D. ± 0.53	AAS (flameless)	Buchet et al.	1976

Table IV - Manganese in air, in the whole blood and urine (ranges and medians) for different types of work (Horiuchi et al. 1970)

Type of work number of workers	Mn in air (mg/m^3)	Mn in blood (µg/100 g)	Mn in urine (µg/l)
Manganese mill n = 43	2.3 - 17.1 8.4	4 - 54 9.5	8 - 165 68.5
Battery factory n = 35	1 5 - 21.1 4.3	4 - 20 8	1 - 42 6
Electrode factory n = 31	3.1 - 8.1 4.9	4 - 17 6	3 - 19 5

Table V Results of the statistical evaluation from the study of Horiuchi et al. (1970) (p-values by Fisher's direct probability method)

Groups under examination	Positive neurological findings and Mn in whole blood	Positive neurological findings and Mn in urine
Manganese mill	0.048 +	0.013 +
Battery factory	0.520 (n.s.)	0 180 (n.s)
Electrode factory	0.072 (n.s.)	0.206 (n.s.)
Total	0.097 (n.s.)	0.001 +

+ = statistically significant, n.s. = not significant

Concentrations in Faeces and Hair

The manganese content in the faeces represents both the manganese not resorbed into the gastrointestinal tract and the manganese actively eliminated by the body. The normal content in the faeces of persons not occupationally exposed to manganese was established as between 1.6 mg/100 g faeces (Jindrichova, 1969) and 4.1 mg/day (Horiuchi et al., 1970). Jindrichova (1969) examined 390 persons, some of whom were occupationally exposed to considerable quantities of manganese, and found average faecal manganese levels of 6.23 mg/100 faeces. There was no significant correlation between exposure and faecal concentration. The author is of the opinion that values over 6 mg manganese/100 g faeces are a sign of occupational exposure to manganese. However, the manganese content in the faeces differs appreciably from individual to individual. For this reason connections between faecal concentration and exposure levels can be applied to groups, but not to individuals. This study does not indicate whether the faecal manganese concentration provides a better parameter for

assessing the hazard than the manganese levels in the blood and urine. There is also a lack of data on dose/response relationships which could justify the establishment of a toxicologically substantiated limit for manganese excretion through the faeces.

Studies on the manganese content of hair are contradictory. Schroeder and Nason (1971) quote a value of approximately 1 µg/g whereas Cotzias et al. (1964) maintain that the manganese content of heir depends on its pigment and melanine content.

Creason et al. (1975) found in New York City iinhabitants an average manganese level of 0.56 µg/g hair in children and 0.95 µg/g hair in adults. The manganese content in the hair ranged from 0.05 to 12 µg/g. In persons with both suspected and established chronic manganese poisoning, Teisinger et al. (1956) found lower manganese levels in the hair of the experimental group than in the control group.

Rosenstock et al. (1971) detected no manganese in the hair of normal adults by atomic absorption spectrometry, while patients with chronic manganese poisoning showed 29 µg Mn/g head hair and 107 µg Mn/g chest hair. The authors thought this discrepancy was due to different growth rates in the two types of hair.

The studies on manganese content in hair do not permit any final judgement as to the value of this test material as a measurement of increased manganese exposure. It would appear feasible, however, that the manganese content of hair might allow assessment of previous manganese exposure which occurred over a longer period than could manganese levels in the body fluids and excreta (Rosenstock et al. 1971).

Biological Parameters for Assessing Exposure and Early Reversible Effects

Specific early biochemical symptoms of manganese poisoning in humans are not known. The effects of manganese of various biochemical and clinico-chemical parameters have been examined by numerous authors. The studies by Rodier (1955), in particular, are of importance. Rodier found a reduction in urinary excretion of 17-ketosteroids in 81% of patients with chronic manganese poisoning. The basal metabolic rate was higher in 53% of the 84 persons examined. It is not known whether these are the symptoms of overexposure or early signs of intoxication (Tanaka et al., 1969). Jonderko et al. (1971) ran a widescale clinico-chemical programme and found changes indicating liver function disorders in persons exposed to manganese. After exposure ended the parameters tended to revert rapidly to normal.

In addition to an increased blood manganese level, the authors considered the following as early symptoms of chronic manganese poisoning :

- dysproteinaemia with hypalbuminaemia and hyper-beta globulinaemia
- increased bilirubin level
- increased transaminase and asparate aminotransferase activities
- reduced LDH activities
- reduced magnesium and increased calcium levels in the serum
- reduced haemoglobulin and glutathione levels in the erythrocytes.

The changes most likely do not represent a specific effect of manganese as only slight diagnostic value can be attached to the individual case. The same probably also applies to coproporphyrinuria, which is frequently observed in manganese workers (Baader, 1960).

The findings of Rodier (1950) and Jonderko et al. (1971) have so far not been confirmed by other authors. Dose/response relationships cannot be established on the basis of the available test material.

Conclusions

Most authors assume that there is no direct connection between the manganese level in the body fluids and faeces and the severity of chronic manganese poisoning. In fact, individual susceptibility to the disease seems to be the decisive factor. As far as diagnosis is concerned, detection of the pollutant in biological material shortly after the illness has set in gives little more than an indication of the actual exposure.

The main points of interest for occupational medicine are the manganese levels in the blood, faeces and urine.

On the basis of our current knowledge, biological monitoring can only be recommended with reservation for manganese.

Further studies should deal with the establishment of dose/response relationships and the limit values based on them, as well as with the determination of effects which are reversible at an early stage. In particular, the causes of the increased sensitivity of some persons must be recognized and suitable examination methods devised to trace this group of people.

Until then an improvement in industrial hygiene measures at worksplaces and a regular neurological examination of the workers exposed to manganese would seem to be the best methods of preventing chronic manganism.

Research Needs

The following are recommendations for further research:

- pure research and development of suitable methods to determine the particular predisposition of certain persons to manganism;
- substantiation of other adverse effects and examination of the applicability of the principle of biological monitoring;
- establishment of toxicologically justified exposure limits for manganese in biological material if dose/response relationships are found to exist.

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Human biological monitoring of industrial chemicals series

Titanium

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Summary

This document reviews the metal titanium (Ti) and its compounds of industrial interest (chlorides and oxides) as related to occupational exposure and the possibilities of the biological monitoring of exposure.

The detailed metabolism of titanium is not known; the lungs, however, accumulate the largest quantities as a result of inhalation and are the primary target organ. Titanium appears to be a substance with a low resorption rate and one which finds a wide range of tolerance in the human body.

Titanium has been measured in blood and urine using mainly spark-source mass spectrometry and X-ray fluorescence analysis. The reported concentrations vary widely and do not appear to be related to exposure.

It is not recommended that titanium be brought within the scope of human monitoring. This is particularly true of titanium dioxide, the titanium compound most widely used in industry. It has not yet been determined, however, to what extent human monitoring is applicable to other titanium compounds such as organic titanium derivatives.

Titanium

Chemical and Physical Properties

Titanium is a grey metal which in the form of powder or dust is extremely inflammable and explosive. Its atomic weight is 47.9 and its atomic number is 22.

Titanium is found in various valencies and has metallic and non-metallic properties. The principal valence state is 4+ (titanic), but 3+ (titanous) and 2+ states are also known. There are also oxidized compounds such as titanyl chloride ($TiOCl_2$). Titanium is found in its metallic state in compounds such as titanates, e.g. calcium, iron and potassium titanate. Titanium IV compounds are easily hydrolysed into titanium dioxide (Table 1).

In addition to metallic titanium, titanium dioxide and titanium tetrachloride are the main compounds used in industry. See Table 2. In many cases, however, the exact identity of the titanium compound which causes a particular effect is not reported in the literature. Titanium has therefore often been referred to generically in this document.

Table I - Melting points and solubility of titanium and its principal compounds

Components	Melting point	Solubility
Ti	1800	Insoluble in cold water, soluble in dilute acids
TiO_2	1640 (decomposition)	Insoluble in hot and cold water, soluble in sulphuric acid and alkalis
$TiCl_4$	30	Soluble in cold water (with) hydrochloric acid, alcohol, and hot water

Table II - Some industrial uses of titanium and its compounds

Substance	Use
Titanium Ti	Alloys, Aerospace, Chemical Processing Industries
Titanium Dioxide TiO_2	Pigments, Paints, Lacquers, Printing Ceramics, Food Additives, Drug and Cosmetics Applications
Titanium Tetrachloride $TiCl_4$	Polymerization (Ziegler Type) Catalyst Starting Material for Most Organic Compounds
Titanous Chloride $TiCl_3$	Polymerization Catalyst
Organic Titanium Compounds	Cross Linking Agents, Catalysts

Titanium has a number of organic derivatives. The principal ones are alkyl and aryl titanates of the general compound type $Ti(OR)_4$.

Ambient Measurements

Titanium uptake at the workplace is due exclusively to inhalation. The principal methods used in analysing air samples are atomic absorption spectrometry, X-ray fluorescence analysis, neutron activation analysis and, occasionally, photometric techniques.

Dittrich and Cothern (1971), using X-ray fluorescence analysis, examined dust samples from filter papers used a collectors for 25 hours in a high-volume air sampler. Similar analyses were carried out by Rhodes et al. (1972) in Texas. The lower detection limit for titanium using X-ray fluorescence was 0.011 $\mu g/m^3$. Dams et al. (1970) determined the level of titanium in air samples by neutron activation analysis.

The lower detection limits were 0.2 ug titanium per filter. In a more recent work this group (Dams et al., 1972) describes how the titanium concentration on very clean filters or impactor surfaces was determined by neutron activation. Analysis of a number of filters showed very high levels of trace elements, a factor which considerably reduces the analytical sensitivity of the method.

Atomic absorption spectroscopy was used to analyse a large number of metals in air samples after enrichment by means of suitable filters (Beyer, 1969; Burnham et al., 1970; Hwang 1972). Ranweiler and Moyers (1974) described an atomic absorption spectrometry method for the analysis of dust samples collected on polystyrene filters using high-volume samplers. One of the 22 metals found was titanium. The lower detection limit for titanium in practice was 0.07 $\mu g/m^3$.

Yound and White (1959) developed a colorimetric method for the determination of airborne titanium. Titanium was found as titanium diocyanate with tri-n-octylphosphine in the organic milieu.

Effects on Humans

There is no epidemiological evidence that titanium dust causes titanium-induced pulmonary fibrosis (Moschinski et al., 1959). No pathological changes were detected in clinical and X-ray tests carried out by Vernetti-Blina (1928) on subjects who had been exposed for a long period of time to titanium dioxide dust, nor were any such changes evident in the blood count. In addition, an autopsy carried out by Schmitz-Moormann et al. (1964) on a person who had been employed for 15 years on the manufacture of titanium dioxide pigments revealed no signs of inflammation or fibrosis in the lungs. These authors considered titanium dioxide to be an absolutely inert substance. Uragoda and Pinto (1972) examined 136 workers in a factory in Ceylon in which ilmenite was processed. The workers were exposed to a large number of minerals, principally ilmenite, rutile and zircon ores. These workers showed no greater incidence of pulmonary disease than that found in a control group of normal subjects.

Elo et al. (1972) carried out lung tests on three workers who had been engaged for nine years in the manufacture of titanium dioxide pigments. Biopsies showed a significantly higher level of titanium than that found in autopsies on normal subjects. Deposits in the pulmonary interstices were found in association with cell destruction and slight fibrosis. Titanium dioxide was also found in the lymphatic system. The authors concluded from this that the lymphatic system was responsible for elimination of titanium dioxide from the lungs. The electron microscope revealed the presence of titanium dioxide particles in the lysosomes of the alveolar macrophages. On the basis of these findings, Elo et al. (1972) classified industrially manufactured titanium oxide, either alone or in conjunction with silicates, as a substance with a slight irritant effect on the pulmonary interstices. In a more recent work, the "adverse effects" noted are attributed to substances such as quartz or silicates rather than to titanium dioxide. These substances are present in the manufacture of titanium dioxide pigments (Määttä and Arstila, 1975).

Heimendinger and Klotz (1956) report on a case of accidental exposure to titanium tetrachloride fumes. Contact with jets of $TiCl_4$ at a temperature of 100°C and the inhalation of fumes of titanic acid and titanium oxychloride resulted in superficial burns and the formation of scar tissue. There was considerable inflammation of the mucous membrane of the pharynx, the vocal cords and the airways, with the formation of scar tissue and the later development of laryngostenosis. Histological examination

revealed phagocytized TiO_2 in the lungs. Substantial deposits of dust were found in association with localized small areas of emphysema, but no specific lesions were observed.

No cases are known of damage caused to skin or tissue by titanium (Hygenic Guide Series, 1973). Titanium compounds such as salicylates, oxides, and titanates have been used in the treatment of skin lesions and as aids in surgery (Ereaux, 1955; Browning, 1969).

There is no evidence that titanium or its compounds have any carcinogenic, mutagenic or teratogenic effects on man.

Absorption, Distrubution and Excretion

Little is known about the absorption of TiO_2 in man. Schroeder et al. (1963) report an uptake of 300 μg Ti per day in a normal subject. The daily excretion rate is also about 300 μg Ti per day. While approximately 10 μg are excreted in urine and 290 μg in faeces, approximately 0.4 μg of titanium are thought to be retained in the lungs.

Perry and Perry (1959) found a mean level of 10.2 μg Ti/l in a pooled sample of normal urine. This would mean that approximately 3% of the titanium taken into the body is absorbed and excreted via the urine. On the other hand, West and Wyzan (1963) found no change in the renal excretion of titanium in five volunteers to whom 5 g of titanium dioxide were administered on three consecutive days. The urine was analysed for five days after the beginning of the uptake.

Wide variations were found in the titanium level in different human organs. Generally the highest concentration of titanium was found in the lungs. Hamilton et al. (1972) found a titanium concentration of 3.7 μg/g wet weight in the lungs of subjects from the United Kingdom. The lowest concentration was 0.8 μg/g and was found in the brain. Similar results were found by Anspaugh et al. (1971).

Crable et al. (1967, 1968) analysed the metal and dust levels in the lungs of chromium workers in West Virginia. The average titanium level in the lungs of 26 miners was 119 μg/g dry weight. The corresponding figure for titanium levels in normal subjects given by the same authors is 19 μg/g dry weight. Roething and Wehran (1972) found that titanium also accumulates in the lungs as silicosis progresses. The titanium level in the lungs was 4.0 - 24.3 mg/kg. Concentrations in the lymph nodes ranged from 12.2 - 120 mg/kg.

The mean level of titanium increased with the severity of the silicosis. The lymph nodes contained an appreciably higher level of titanium than the lungs. On the other hand, Einbrodt and Liffers (1968) reported that the concentration of titanium dioxide in the lymph glands is less than half of that in the lungs. Only approximately 5% of all retained titanium dioxide is transported to the lymph glands.

Biological Indicators

Methods for Assessing Concentrations in Biological Material

Information on titanium analyses in biological material is limited and the results of various analyses fluctuate widely; special attention should be paid to these two factors when biological material is being analysed for titanium.

Hamilton et al. (1972) examined a large number of elements in human tissue. The methods of analysis used were spark-source mass spectrometry, X-ray fluorescence analysis and various methods of neutron activation analysis. The lower detection limits for titanium in human tissue, using spark-source mass spectrometry, were 0.007 μg/g wet weight and with X-ray fluorescence analysis they were 0.3 μg/g wet weight. Tipton et al. (1969) used ARC emission spectroscopy in their examination of faeces and urine. Lower detection limits were 9 mg/kg for faeces and 30 μg/kg for urine, each related to ash weight. The comprehensive studies by Schroeder et al. (1963) employed the technique of Sandell (1959).

Chromotopic acid used as a reagent gives a sentitivity of approximately 0.25 μg/g of test material. McCue (1973) compared various methods for determining titanium in human blood and concluded that the most suitable was a method of Clarks (1970) for the determination of titanium in ceramic material. This is a photometric method in which tiron (4.5-dihydroxy-m-benzenedisulphonic acid disodium salt) was used as a

reagent. The limit detection was 50 μg/kg. This method appears to be preferable to emission spectrometry, atomic absorption spectrometry and X-ray fluorescence analysis.

Concentrations in Blood and Blood Components

The mean blood titanium level in test material from the United Kingdom was 0.07μg/g blood. These tests were carried out by Hamilton et al. (1972) using spark-source mass spectrometry. Timakin et al. (1967), on the basis of an examination of 100 healthy men and women in the USSR, obtained similar results with an average titanium level of 54.1 μg/kg of blood. The blood titanium concentration of 0.03 μg/g analysed by Maillard and Ettori (1936) was within the same range. Mozhaitseva (1970) analysed the blood titanium level of 20 subjects between 20 and 43 years of age. The titanium concentration varied from 75-159 μg/l of blood, the mean reading being 123 ± 5 μg/l of whole blood.

According to the investigations of Smyshlyaeva et al. (1971), the titanium level in erythrocytes and in plasma was in the ratio of 2 : 3. The ratio lowered slightly as the subject's age increased.

There is some evidence that the level of blood titanium is influenced by a number of diseases. Carroll and Tullis (1968), and McCue (1973) found a higher level of titanium in the leukocytes of persons suffering leukaemia or Hodgkin's disease than in normal subjects. Differences have also been noted between the blood titanium levels in normal subjects and those found in persons with various forms of cancer or cardiac disease.

No reference to measurements of the titanium level in persons occupationally exposed to titanium was found in the literature.

Concentrations in Urine

The literature provided little information on renal elimination of titanium. Perry and Perry (1959) found a titanium concentration of 10.2 μg/l in normal urine. The high figures obtained by Tipton and Cook (1963) are probably affected by the method used. Kvirikadze (1967) reported increased renal excretion of titanium in the urine of patients with bladder tumors.

No references to the titanium level in the urine of persons exposed to titanium have been found.

Concentrations in the Lungs

As stated in the section "Absorption, Distribution and Excretion", the highest titanium levels in adults are found in the lungs (Tipton and Cook, 1963; Hamilton et al., 1972). Schroeder at al. (1963) examined the relationship between the titanium level in the lungs and the age of the subject. In two cases out of five they found no evidence of titanium in the lungs of new-born infants and children. American studies have shown that titanium accumulates in the lungs throughout life. This phenomenon was not observed in test materials such as kidneys, skin or the aorta. Schroeder et al. (1963) also noted that regional differences in lung titanium levels should be taken into account.

Persons occupationally exposed to titanium show higher titanium levels than normal subjects. Crable et al. (1967, 1968) found a mean titanium level of 119 μg/g dry weight in the lungs of 26 mine workers. Titanium levels in the lungs of subjects not occupationally exposed were 19 μg/g dry weight. Roething and Wehran (1972) were able to show that the level of titanium in the lungs of silicosis sufferers increased with the severity of the disease. Finnish working parties have also recently noted deposits of titanium dioxide in the lungs of occupationally exposed persons (Elo et al. 1972; Määttä and Arstila, 1975).

Biological Parameters for Assessing Exposure and/or Early Reversible Effects

There are many references in the literature concerning human occupational exposure to titanium where titanium-bearing ores are smelted and where titanium metals and titanium dioxide and carbide are produced. The specific conditions of such exposure are described by Mogilavskaya (1972, 1973) and Stokinger (1962).

On the other hand, there is no information in the literature on dose-response relationships at the workplace. In the absence of such information it is not possible at present to state occupational tolerance limits for levels in blood, urine or lung tissue.

Blood and urine analyses have been carried out to determine the presence of titanium in biological material. According to Schroeder and Nason (1971), titanium accumulates in the lungs. The literature also contains details of titanium analyses of lung tissue obtained by biopsy or autopsy. Determination of the titanium content of these organs is, however, of secondary importance. The literature offers no suggestion for the definition of biochemical parameters.

Conclusions

The highest titanium levels in adults are found in the lungs. Uptake at the workplace is due exclusively to inhalation. Persons occupationally exposed to titanium show higher titanium levels than normal subjects.

Schaller and Valentin (1976) recommended that titanium not be brought within the scope of human monitoring as it is a substance with a low resorption rate which finds a wide range of tolerance in the human body. This is particularly true of titanium dioxide which is the titanium compound most widely used in industry.

It has yet to be determined to what extent human monitoring is applicable to certain titanium compounds such as organic titanium derivatives.

Research Needs

The following are recommendations for further research :

- improve the methods of analysing titanium in biological material and test their reliability criteria;
- calculate the dose-response effect relationships at certain workplaces;
- test the validity of dose-response relationships for the various compounds.

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Human biological monitoring of industrial chemicals series

Toluene

R. Lauwerys

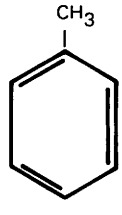
Summary

Absorption of toluene occurs mainly through inhalation of vapours and by skin contact with the liquid form.
Toluene exerts its main toxic action in the central nervous system.
Toluene gives rises to hippuric acid ***in vivo*** and therefore the measurement of hippuric acid has some practical application for evaluating current exposure to toluene. In a group of workers exposed to 100 ppm toluene for 8 hours, the mean hippuric acid concentration in urine at the end of the workshift amounts to about 2.5 g/g creatinine. On an individual basis, this threshold has a good specificity, but a low sensitivity. Currently, the determination of O-cresol in urine does not seem to offer additional advantages over the analysis of hippuric acid, but further studies are required to test the validity of this test. During exposure to 100 ppm toluene at rest, the average concentration of toluene in venous blood and in air amounts approximately to 40 μg/100 ml and 70 mg/m^3 respectively. Not enough investigation has been performed to evaluate whether analysis of expired air (or blood) collected 16 hours after the end of exposure can provide an estimation of the magnitude of previous day intake of toluene.

Toluene

Chemical and Physical Properties

- colourless liquid at normal temperature
- boiling point (760 mm Hg) 110.6°C
- vapour pressure (25°C) 28 mm Hg
- vapour density : 3.14
- molecular formula : $C_6H_5CH_3$
- structural formula



1 ppm = 3.75 mg/m^3

1 mg/m^3 = 0.267 ppm

It is important to keep in mind that if purified toluene contains usually less than 0.01% benzene industrial grade may contain up to 25% benzene (NIOSH 1973).

Chronic effects on humans

It is now recognized that on the contrary to benzene, toluene is not myelotoxic and does not modify the blood picture (Browning, 1965; von Oettingen et al., 1942). Previous reports suggesting that toluene has myelotoxic properties resulted from investigations on workers handling toluene contaminated with benzene.
No specific biological changes have been attributed to toluene which exerts its toxic action mainly on the central nervous system and secondarily on the liver and kidney (Lauwerys, 1982).

Metabolism

Metabolic Pathway

Absorption of toluene occurs mainly through inhalation of vapours and by skin contact with the liquid form. Skin absorption of toluene vapour is negligible (Piotrowski 1967).

Although Gerarde (1960) has stated that liquid toluene is poorly absorbed through the intact skin, Dutkiewics and Tyras (1968) have calculated that the rate of absorption of liquid toluene through the skin of the hand and forearm of human volunteers ranges

from 14 to 23 mg/cm²/hour. These data have been confirmed by Guillemin et al (1974). Comparing these results with those obtained by Hanke et al. (1961) with benzene it can be concluded that the rate of toluene absorption through the skin is 30 to 50 times that of liquid benzene and hence skin absorption from contact with liquid toluene should be taken into account in the evaluation of toluene exposure.

Teisinger and Srbova (1955) have estimated at about 40% the absorption rate of inhaled toluene. A fraction (± 20%) of the absorbed toluene is excreted unchanged in the expired air (Srbova and Teisinger 1952). The fraction (± 80%) of toluene which is not eliminated unchanged in the expired air is mainly oxidized by transformation of the methyl radical into a carboxyl radical (by the microsomal monooxygenase system), which is then conjugated with glycine to produce hippuric acid (Fig. 1).

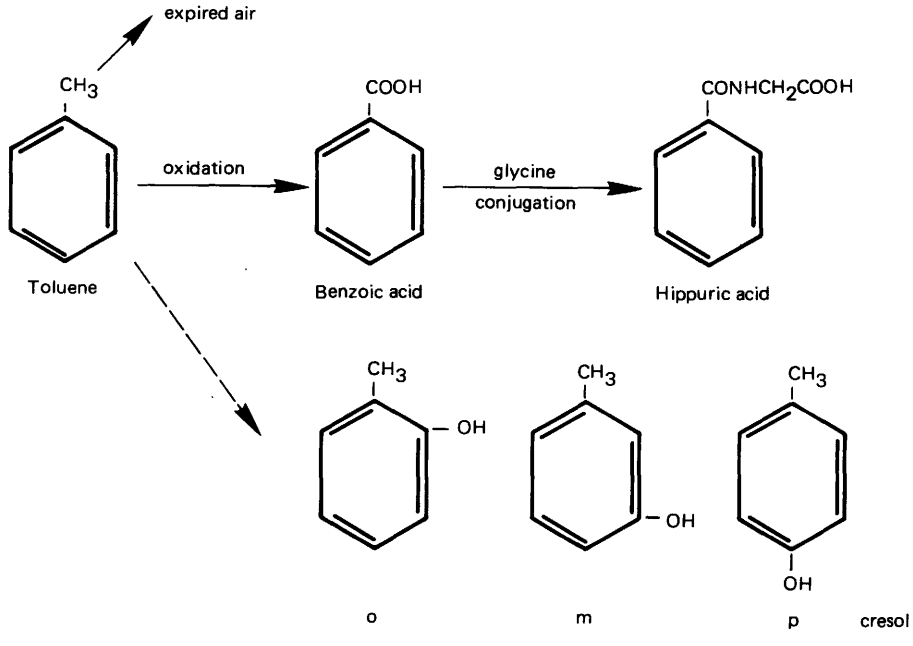


Figure 1

According to Teisinger and Srbova (1955), about 20% of the benzoic acid intermediate is also conjugated with glucuronic acid with formation of benzoylglucuronic acid. It seems, however, that this alternative pathway is of much less importance than suggested by Teisinger and occurs only when heavy toluene absorption takes place (Pagnotto and Lieberman, 1967).

Hippuric acid is excreted in urine. Ogata et al. (1970 and 1971) have found during experiments on volunteers that 63 to 68% of the toluene ***absorbed*** are excreted as hippuric acid and Tokunaga et al (1974) have estimated that around 31% of the toluene ***inhaled*** are excreted as hippuric acid. Its biological half-life is about 3 hours and therefore elimination is practically complete in 18 hours (Ogata et al., 1970; von Oettingen et al., 1942).

On the contrary to benzene, only a very small fraction of toluene (less than 1% of the dose) is oxidized on the aromatic ring with production of ortho-meta- and para-cresol (Bakke and Scheline, 1970; Woidowe et al., 1979). This difference in the oxidative metabolism of benzene and toluene probably explains the different myelotoxic properties of both solvents.

Factors Influencing Metabolism

Enzyme inducers (like phenobarbital) can stimulate the rate of biotransformation of toluene (Ikeda and Ohtsuji, 1971). Toluene and trichlorethylene can reciprocally inhibit their oxidative degradation (Ikeda 1974).

Biological Indicators

The biological tests which have been proposed for evaluating toluene exposure are :

- hippuric acid in urine
- benzoic acid in urine
- O-cresol in urine
- hippuric acid in blood
- toluene in blood
- toluene in expired air.

It should be noted that like benzene the metabolism of toluene is rapid and therefore the time of biological material sampling is critical.
The reader should refer to the report on benzene for a critical appraisal of the various methods of expressing the results when the analysis is performed on urine (Lauwerys, 1979).

Hippuric Acid in Urine

All the methods described in the literature for hippuric acid determination in urine have not the same specificity. The spectrophotometric and fluorometric techiques which are not preceded by chromatography lack of specificity because other urinary metabolites (methylhippuric acid, uric acid, etc.) interfere (Pagnotto and Lieberman, 1967; Tomokuni and Ogata, 1972; Ellman et al., 1961; Kaneko et al., 1975). Spectrophotometric measurement of hippuric acid after its separation from other urine metabolites by paper (Gaffney et al., 1945) or thin layer chromatography (von Kufner et al., 1973; Ogata et al., 1969) is more specific but is too elaborate and time-consuming for the routine monitoring of workers exposed to toluene.

Table I - Concentration of hippuric acid in urine of subjects not occupationally exposed to toluene

Subjects	Hippuric acid in urine		Method	Reference
Males 31	$\bar{X}$ = 1.1 g/l or 0.8 g/g creatinine SE = 0.84 g/l or 0.45 g/g creatinine 97th percentile 2.8 g/l or 1.7 g/g creatinine		3	Buchet and Lauwerys 1973
Males 31	$\bar{X}$ = 0.92 g/l or 0.41 g/g creatinine SD = 0.64 or 0.29 g/g creatinine		2	Von Kufner et al., 1973
Not indicated	$\bar{X}$ = 0.44 g/l SD = 0.20 g/l		1	Tomokuni and Ogata 1972
Males 21	$\bar{X}$ = 0.53 g/l (adjusted SG 1016) range 0.21 - 1.09 g/l or $\bar{X}$ = 0.47 g/g creatinine range = 0.18 - 0.89 g/g creatinine		1	Mikulski and Wiglusz 1970
Females 21	$\bar{X}$ = 0.52 (adjusted SG 1016) range 0.2 - 1.00 g/l or $\bar{X}$ = 0.49 g/g creatinine range = 0.21 - 0.99 g/g creatinine		1	Mikulski and Wiglusz 1970
Not indicated	$\bar{X}$ = 0.8 g/l range = 0.4 to 1.4 g/l		1	Pagnotto and Lieberman 1967
Male students	geometric mean	range	2	Ikeda and Ohtsuji 1969 (a)
36	0.3 g/l (uncorrected)	(0.07 - 1.27)		
	0.29 g/l (SG 1016)	(0.06 - 1.39)		
	0.23 g/g creatinine	(0.04 - 1.17)		
Male workers	0.35 g/l (uncorrected)	(0.11 - 1.08)	2	
36	0.29 g/l (SG 1016)	(0.09 - 0.91)		
	0.24 g/g creatinine	(0.08 - 0.71)		
Female students	0.38 g/l (uncorrected)	(0.11 - 1.43)	2	
30	0.57 g/l (SG 1016)	(0.15 - 2.13)		
	0.45 g/g creatinine	(0.16 - 1.24)		
39 traimen	$\bar{X}$ = 0.92 g/l (SG 1016) $\bar{X}$ = 0.74 g/g creatinine	(0.11 - 4.3) (0.86 - 2.3)	3	Engström et al., 1976
30	$\bar{X}$ = 0.79 g/l (uncorrected) SD = 0.88		4	Angerer 1976
6	0.4 g/24 hours (uncorrected)		5	Von Oettingen et al., 1942
46	Median 0.36 g/g creatinine		6	Gossler et al., 1977

Methods 1 Spectrophotometry or colorimetry without previous chromatography
2 Spectrophotometry after chromatography
3 Gas chromatography
4 Densitometry after thin layer chromatography
5 Titration after precipitation
6 High pressure liquid chromatography

N.B : Since methylhippuric acid is not normally present in urine of non exposed persons, control values for hippuric acid should be approximately identical whatever the method used

Quantitative evaluation of hippuric acid (and methylhippuric acid) by densitometric analysis of the thin layer plates has also been attempted but is probably less precise than gas chromatographic methods (Van Kerckhoven et al., 1974; Angerer 1976).

Gas chromatographic methods offer the advantage of rapidity, specificity and can also permit the simultaneous determination of hippuric and methylhippuric acids (Buchet and Lauwerys, 1973). High pressure liquid chromatography has also been used for the quantitative determination of hippuric acid in urine (Gossler at al., 1977).

Hippuric acid is a normal constituent of urine, originating mainly from food containing benzoic acid or benzoates. Values found in persons non exposed to toluene have been summarized in Table 1.

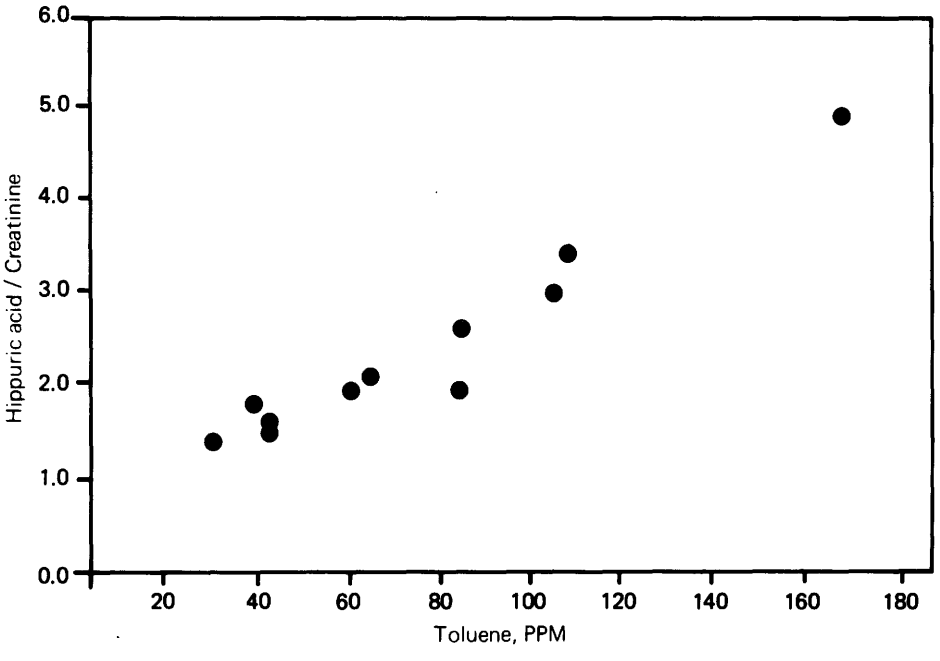


Figure 2 - Comparison of postexposure hippuric acid - creatinine ratio with toluene exposure (from Pagnotto and Lieberman, 1967)

The majority of authors found that in non-occupationally exposed workers, the concentrations of hippuric acid in spot urine samples rarely exceed 1.5 g/g creatinine.

Results of studies which have attempted to correlate hippuric acid excretion in urine with levels of toluene exposure are summarized below.

In 1942, von Oettingen et al. reported that exposure to concentration of 50 to 800 ppm of toluene is associated with and followed by an increased excretion of hippuric acid which is roughly parallel to the intensity of the exposure.

The results obtained by Pagnotto and Lieberman in 1967 are presented in Fig. 2.

Each point on this figure represents the average hippuric acid content of samples collected at the end of the usual eight-hour work shift from small groups of individuals with nearly similar toluene exposure. Pagnotto and Lieberman indicated in their paper that in most cases the percentage of deviation of individual urine values from the mean did not exceed 15% and even less deviation was found when values were reported as a hippuric acid-creatinine ratio.

According to their data an exposure to 100 ppm toluene (current atmospheric TLV proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) in 1975 and by NIOSH in 1973) would on a group basis produce a urinary hippuric acid content of about 2.8 g per liter (adjusted to a S G of 1016) or per g creatinine, in samples collected at the end of the work shift. It should, however, be noted that Paghotto and Lieberman have used a non specific spectrophotographic technique which may give higher values than chromatographic techniques if xylene was also present in ambient air.

The data obtained by Ikeda and Ohtsuji in 1969 (b) are summarized in Fig. 3 taken from their publication and in Table 2.

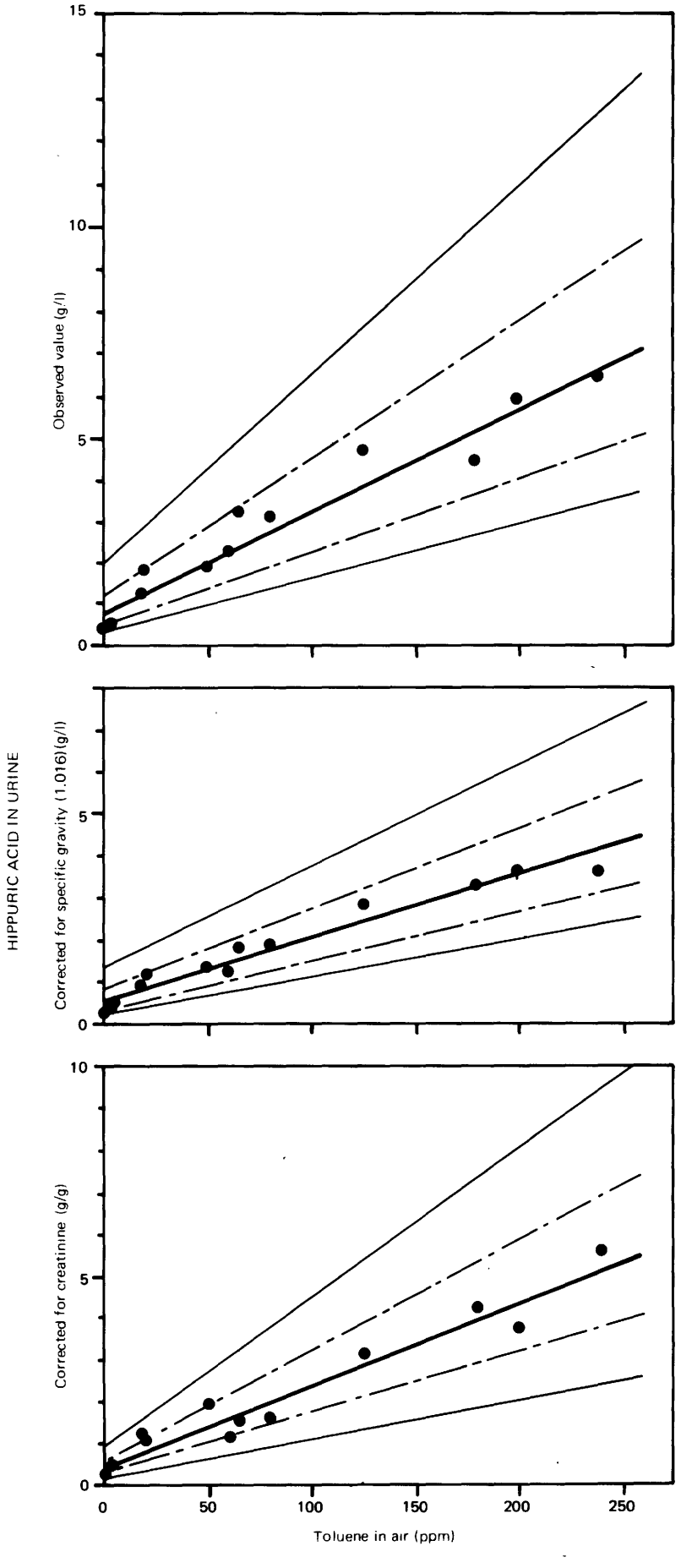


Figure 3 - Comparison of postexposure hippuric acid excretion with toluene exposure. The lines are weighted calculated regression lines for the means (——) together with those for the S.D. ranges (— · — · —) and fiducial ranges (P = 0.05) (–) (from Ikeda and Ohtsuji, 1969 b)

Table II - Hippuric acid in urine of workers exposed to toluene

Number of subjects	Toluene conc. ppm	Duration exp. (hrs)	Total exposure	Concentration hippuric acid in urine			Time of urine sampling	Method	Remarks	Ref.
Group (N ?)	50	± 8	200	± 1.5 g/g creatinine ± 1.46 g/liter (b) (SG. 1016)			End of shift	1	· possible simultaneous exposure to xylene · quantitative determination of toluene in air (10 min. samples)	Pagnotto and Lieberman, 1967
Group (N ?)	100	± 8	800	± 2.5 g/g creatinine ± 2.66 g/liter (SG 1016)			End of shift	1		
Group (N ?)	200	± 8	1600	± 5.0 g/g creatinine ± 4.6 g/liter (SG 1016)			End of shift	1		
				Geometric mean						
				g/liter (a)	g/liter (b)	g/g creatinine				
8	4	± 8	32	0.45 (0.25 - 0.79)	0.49 (0.28 - 0.86)	0.43 (0.36 - 0.53)	End of shift	2	Toluene in air measured by detector tubes	Ikeda and Ohtsuji (1969 b)
5	18	± 8	144	1.23 (0.97 - 1.56)	0.90 (0.6 - 1.24)	1.23 (0.72 - 2.11)	End of shift			
10	20	± 8	160	1.84 (1.22 - 2.79)	1.18 (0.85 - 1.64)	1.06 (0.81 - 1.40)	End of shift			
9	50	± 8	400	1.92 (1.26 - 2.93)	1.40 (1.03 - 1.89)	1.96 (1.53 - 2.50)	End of shift			
10	60	± 8	480	2.27 (1.33 - 3.88)	1.21 (0.70 - 2.10)	1.14 (0.75 - 1.72)	End of shift			
6	65	± 8	520	3.29 (2.83 - 3.84)	1.87 (1.62 - 2.16)	1.51 (1.15 - 1.99)	End of shift			
8	80	± 8	640	3.13 (2.15 - 4.55)	1.87 (1.33 - 2.63)	1.64 (1.05 - 2.55)	End of shift			
8	125	± 8	1000	4.73 (3.45 - 6.49)	2.84 (2.28 - 3.54)	3.17 (2.34 - 4.31)	End of shift			
22	180	± 8	1440	4.48 (3.23 - 6.20)	3.31 (2.60 - 4.22)	4.21 (3.42 - 5.19)	End of shift			
20	200	± 8	1600	5.97 (4.12 - 8.65)	3.66 (2.62 - 5.11)	3.58 (2.28 - 5.61)	End of shift			
12	240	± 8	1920	6.48 (4.81 - 8.34)	3.59 (2.82 - 4.57)	5.67 (4.38 - 7.34)	End of shift			
4 or 5	100	7	700	3.09 (1.69 - 4.49)	1.87 (1 - 2.75)		Second period of exposure	2	Volunteer experiments	Ogata et al. 1970
4 or 5	200	7	1400	8.19 (3.0 - 13.5)	3.9 (2.25 - 5.6)					
36	27	± 7	189	2.04 S.D.: 1.37			End of shift	4		Angerer 1976

a uncorrected, b corrected for a specific gravity of 1016, Method : see Table 1

According to their results, the average hippuric acid concentration in urine collected at the end of the work shift in a group of workers exposed to an atmospheric toluene concentration of 100 ppm is estimated at 2.0 g/liter (specific gravity 1.016) or 2.35 g/g creatinine. Individual values (5th and 95th percentile) could range from 1.4 to 3.9 g/g creatinine (Imamura and Ikeda, 1973). It should be stressed that their results on toluene concentration in air (photogravure printing industry) were obtained with detection tubes and therefore represent semi-quantitative spot sample determinations. The authors recognize that fluctuations of environmental toluene concentrations in the workshop surveyed are among the possible causes of wide variation in hippuric acid excretion.

Tokugana et al. (1974) have found that in 7 workers exposed for 7 hours to 40.2 ppm toluene (range : 28.6 - 53.7), the excess hippuric acid excreted within 24 hours after the start of exposure (i.e. after substrating the amount of hippuric acid normally present when the workers were not exposed) amounts to 482 mg (range : 364 - 719). Unfortunately they have not indicated the "normal" level of hippuric acid found in these workers.

Angerer (1976) has recently reported that in 36 workers exposed to a mean toluene concentration of 27 ppm, the mean urinary hippuric acid concentration amounts to 2.04 g/l with an SD of 1.37 g/l.

Ogata et al. (1970) have exposed 23 male volunteers for 3 hours in the morning and for 4 hours in the afternoon or just for 3 hours in the morning to toluene vapour. They collected the urine for about a day after exposure. They demonstrated that on a group basis there is an excellent correlation between the total amount of hippuric acid excreted (exposure period + 18 hours after exposure) and the total exposure (C x T). We have reproduced their published figure illustrating this relationship (Fig. 4).

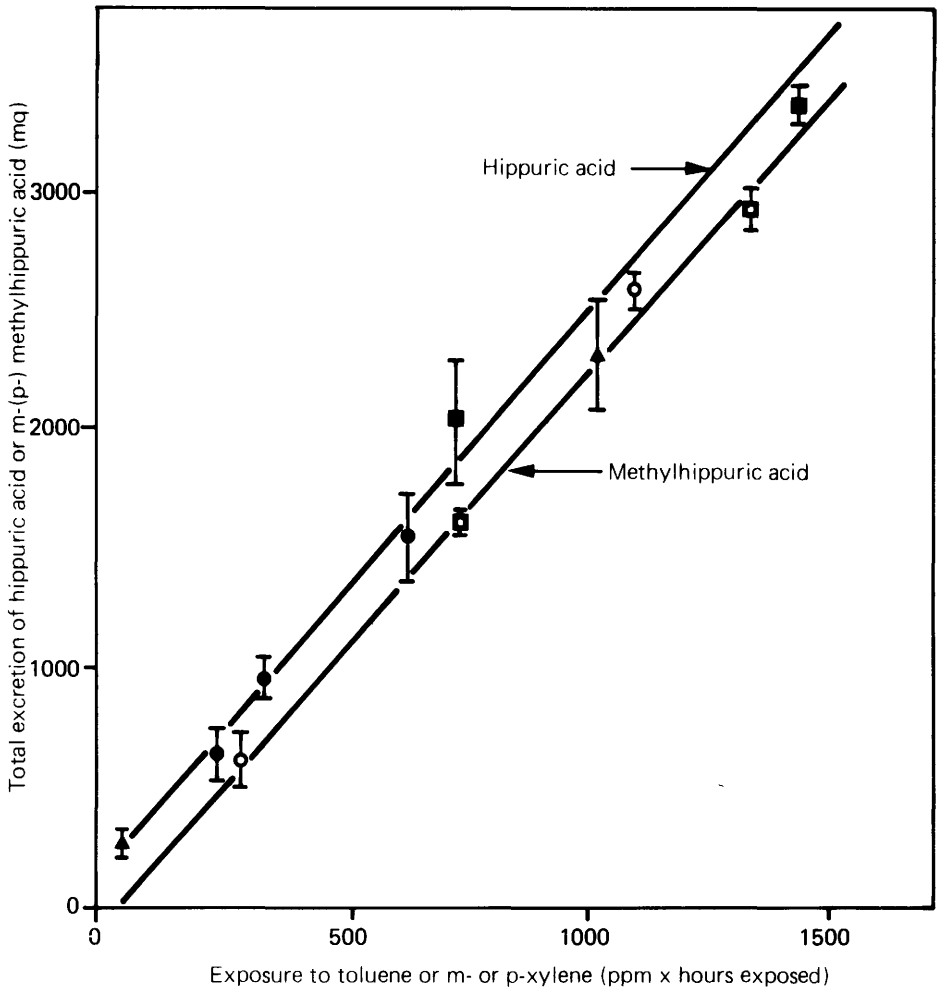


Figure 4 - Relationship between total exposure (ppm x hr) and total excretion of urinary hippuric acid from: ▲ , normal individuals; ● , those exposed 3 hours to toluene (for the lowest point mixed with m-xylene); and, ■ , those exposed 7 hours to toluene; and of urinary methylhippuric acid from: ○ , those exposed 3 hours to m-xylene; □ , those exposed 7 hours to m-xylene; and △ , those exposed 7 hours to p-xylene. Means ± S.E.M.s are shown (from Ogata et al., 1970)

They have also studied the correlation between intensity of exposure (0, 100 and 200 ppm) and the concentration of hippuric acid in urine collected either during the first period of exposure or during the second period of exposure or during the whole exposure period.

The results obtained when urine are collected during the afternoon are shown on the following figure (Fig. 5) and accompanying table.

In a group of 5 persons exposed to 100 ppm toluene for 7 hours the average hippuric acid concentration in urine collected during the second period of exposure would be about 2.81 g/l (corrected for S.G. 1.024) or 1.87 g/l (corrected for S.G. of 1.016). Ninety percent of the values are included in the range 1 to 2.75 (corrected for an S.G. of 1.016). Ogata et al. (1970) have suggested that when the hippuric acid concentration of a worker exceeds a value corresponding to 2 standard deviations less than the average quantity excreted by subjects exposed to the permissible level, one must conclude that he may have been exposed to a concentration greater than that level. According to their data, the screening (5th percentile) concentration of hippuric acid in urine collected during the second period of a shift during which the time-weighted average exposure has been 100 ppm should be 1.00 g/liter (corrected for a specific activity of 1.016).

We have seen in a previous table that the upper normal limit for toluene may exceed 1 g/liter.

Thus on an ***individual basis*** separation of the exposed from the non-exposed can hardly be done regarding single urine analysis for hippuric acid.

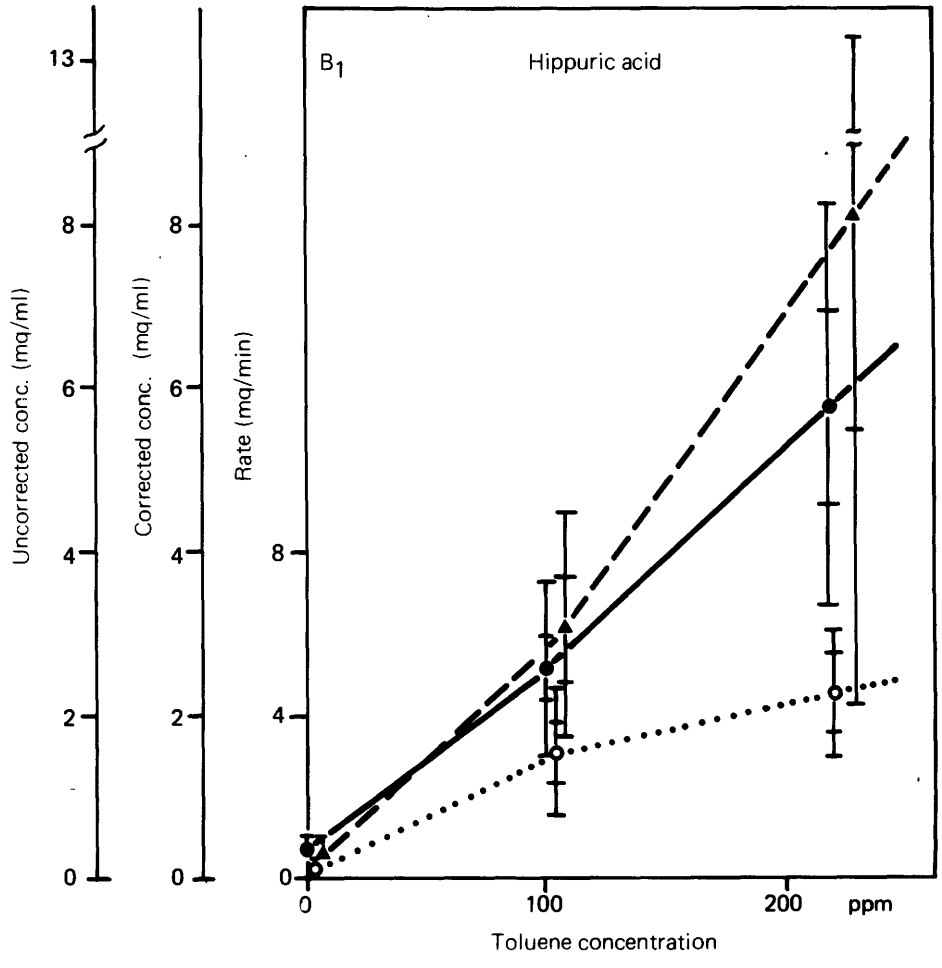


Figure 5 - Relationships between toluene concentrations in the air and concentrations and excretion rates of urinary hippuric acid. Concentration, uncorrected --▲---; corrected to density = 1.024 —O—; rates, ---O---. Means, standard deviations (SD), and 2 x SD are shown. The groups were exposed for 7 hours. The urine specimens were collected during the last 4 hours of exposure (from Ogata et al., 1970)

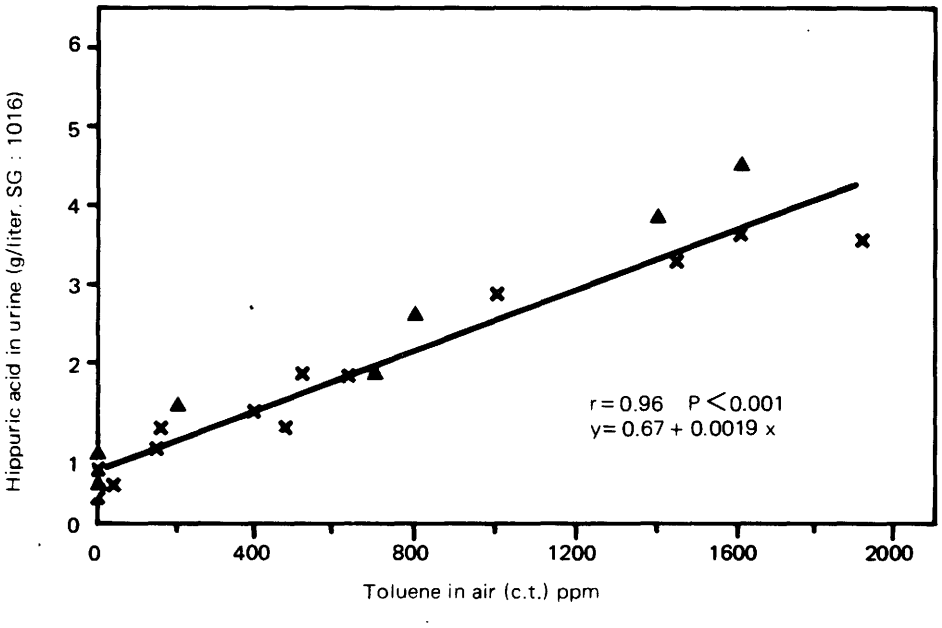


Figure 6 - Relationship between hippuric acid concentration (expressed in g/liter corrected for specific gravity of 1016) in post-shift urine sample and toluene exposure. Each point represents the mean value found in a group of workers (see Table 2).
▲ : arithmetic means; ✖ : geometric means

Imamura and Ikeda (1973) and Engström et al. (1976) came to the same conclusion.

On a group basis, however, this test is sufficiently sensitive. The results of the hereabove cited studies have been summarized in Table 2 and in Figs. 6 and 7.

The excretion rate of hippuric acid at the end of the exposure period is much closely related to the time-weighted toluene load than concentrations alone (Veulemans and Masschelein, 1979; Veulemans et al., 1979; Wilczok and Bieniek, 1978). On a groups basis, a time-weighted average exposure of 100 ppm corresponds to an hippuric excretion rate of 2.6 mg/min according to Wilczok and Bieniek (1978) and to 4 mg/min according to Veulemans et al. (1979). Unfortunately, for practical reasons, the collection of a time-urine sample is frequently impossible.

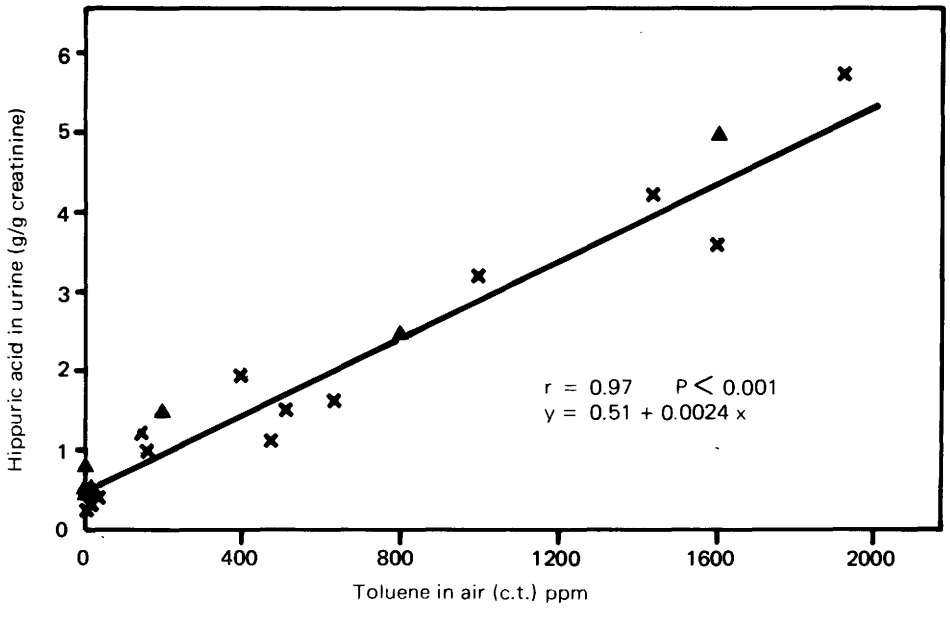


Figure 7 - Relationship between hippuric acid concentration (expressed in g/g creatinine) in post shift urine sample and toluene exposure.
Each point represents the mean value found in a group of workers (see Table 2).
▲ : arithmetic means; ✖ : geometric means

Factors Influencing Hippuric Acid Excretion

Increased urinary excretion of benzoic acid can also occur when food containing benzoic acid precursors of benzoic acid (mainly plums, cranberries, prunes) are ingested (Pagnotto and Lieberman, 1967).

Alessio et al. (1981) have found that in non-exposed persons, the mean urinary hippuric acid excretion is higher in females than in males. Further investigation is necessary to evaluate whether this is due to a sex-linked difference in hippuric acid production or to a difference in the dietary habits of both groups.

In summary, it appears that in a group of workers exposed to 100 ppm toluene for 8 hours, the mean hippuric acid concentration in urine samples collected at the end of the exposure period would amount to around 2.2 g/liter (S.G. 1016) or 2.5 g/g creatinine (Figs. 6 and 7):
The available data indicate that this test can only be used to appreciate exposure of groups of workers because with the proposed biological threshold, the sensitivity of the test is probably limited. A group of experts of the World Health Organization has reached the same conclusion (OMS, 1981).

Benzoic Acid in Urine

Bardodej (1968) has indicated that it is thoeretically preferable to measure total benzoic acid concentration in urine (free + conjugated) because benzoic acid produced after toluene exposure is conjugated not only to glycine but also to glucuronic acid.

It has, however, been found recently by Engström et al. (1976) that there was no difference between the direct determination of hippuric acid and the determination of benzoic acid after alkaline hydrolysis of urine.

O-Cresol in Urine

Only a minor fraction of inhaled toluene vapour is oxidized at the aromatic ring with the production of cresols. Since O-cresol is not a major constituent of normal urine (normal concentration < 0.3 mg/l), its determination has been proposed as a biological monitoring method (Angerer 1979). Woiwode and Drysch (1981) exposed 10 volunteers to approximately 200 ppm toluene for 4 hours and at the end of the exposure period found an average concentration of 1.6 mg O-cresol per liter of urine (S.G. 1017). According to Pfäffli et al. (1979) a time-weighted average toluene exposure of 100 ppm corresponds to a urinary O-cresol concentration of 1 mg/l (urine collected at the end of the workshift). Further studies are required to compare the validity of this test with that of hippuric acid determination in urine.

Hippuric Acid in Serum

Angerer et al. (1975) have described a gas chromatographic method for hippuric acid determination in serum. They found a low but significant correlation between hippuric concentration in serum and in urine (r = 0.37).

Toluene in Blood

Engström et al. (1976) found a low but statistically significant correlation (r = 0.64, n = 20) between toluene in blood and in urinary hippuric acid (blood and urine samples collected at the end of an 8 hr-working day). On the contrary, Szadkowski et al. (1973) found no correlation between toluene concentration in blood and hippuric acid in urine.

von Oettingen et al. (1942) exposed two volunteers to toluene vapour for 8 hours and determined the toluene content of venous blood at the end of the exposure. They found the following relationship between air and blood toluene concentration.

Toluene in air (ppm)	Toluene in blood (mg/100 ml)
200	0.41 - 0.73 mg/100 ml
300	>0.60 - 0.73
400	0.87 - 1.17
600	>0.66 - 0.95
800	1.82 - 2.64

Szadkowski et al. (1973) reported that in a control group the normal ***upper*** limit of the toluene concentration in blood was 15 µg toluene / 100 ml blood (X = 5.3 µg/100 ml, n = 30). In an occupationally exposed group they found a correlation between the toluene concentration in air and in blood. The correlation was, however, very low (r = 0.29).

On the contrary, Apostoli et al. (1982) found an excellent correlation (r = 0.89) between environmental toluene concentration measured with personal samplers and toluene level in blood during exposure. In addition these authors were unable to detect toluene in blood of non-exposed persons. This data suggest that in workers exposed to less than 50 ppm toluene (< 188 mg/m^3) toluene concentration in blood during exposure is about 3 times higher than in air. A similar ratio was found by Angerer and Behling (see below), but on volunteers at rest, the ratio seems closer to 1.

In six subjects exposed at rest to 100 ppm toluene, Astrand et al (1972) found a mean value for venous blood concentration during exposure of 0.045 mg/100 ml (SE = 0.015). As expected this concentration increased during exercise (0.0135 ± 0.013 mg/100 ml after an exercise of 50 watts during 20 minutes). For an exposure to 200 ppm, the concentration at rest amounted to 0.064 mg/100 ml (SE = 0.010).

Under steady state conditions, Veulemans and Masschelein (1978) found also a constant relation between uptake rate of toluene and toluene concentration in venous blood. During exposure to 50, 100 and 150 ppm toluene at rest, the average venous blood concentration reaches a plateau value of about 0.02, 0.04 and 0.06 mg/100 ml respectively. Under non-steady state conditions, however, no simple relation exists between uptake and venous blood concentration of toluene. According to Angerer and

Behling (1981), an atmospheric concentration of 200 ppm toluene corresponds to a blood concentration higher than 0.26 mg/100 ml. Gas chromatographic techniques for toluene determination in blood have been described by several authors (Angerer et al., 1973; Engström et al., 1976).

Toluene in Expired Air

By exposing volunteers to xylene, Astrand et al. (1972) have shown that the content of solvent in alveolar air samples collected during exposure is related to the intensity of exposure. At rest the mean value for alveolar air concentration found during exposure to 100 ppm of toluene is 18.1 ppm (SE = 1:41 ppm; n = 15).

The corresponding value for an exposure to approximately 200 ppm at rest was 37.5 ppm. The correlation between environmental exposure to toluene and its alveolar concentration measured in workers during the workshift seems to agree with these estimates (Brugnone et al., 1980).

Astrand et al. (1972) are of the opinion that "neither alveolar air samples (nor venous blood samples) taken at given intervals after the conclusion of a period exposure can provide sufficiently accurate information on the average amount of solvent in inspired air at a working place or on the magnitude of an individual's uptake".

Conclusion

Not enough investigations have been performed to evaluate whether analysis of expired air (or blood) collected 16 hours after the end of exposure (i.e. before the next shift) can provide, like for benzene, an estimation of the magnitude of previous day intake of toluene.

Analysis of expired air and/or blood during exposure reflects current intake.

During exposure to 100 ppm toluene at rest, the average concentration of toluene in venous blood and in air will amount approximately to 40 μg/100 ml and 70 mg/m^3 respectively. During light physical exercise, the blood concentration may amount to 110 μg/100 ml.

Determination of the average hippuric acid concentration in urines collected at the end of the workshift is still the most practical method to evaluate whether the overall hygiene conditions are satisfactory. A group average below 2 g/liter (S.G. 1016) or g creatinine suggests that the atmosphere was probably contaminated by less than 100 ppm toluene. On an individual basis, one must recognize that this threshold has probably a good specificity, but a very low sensitivity. Currently, the determination of O-cresol in urine does not seem to offer additional advantages over the analysis of hippuric acid.

Research Needs

The following are recommendations for further research:

- further investigation on the possibility of determining toluene concentration in exhaled air and in blood for evaluating the integrated exposure during the previous day
- study factors influencing toluene metabolism and toxicity
- comparative study of the validity of hippuric acid and O-cresol determination in urine.

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Biological Monitoring is one of the most promising tools for performing an effective program of prevention from the potentially toxic effects of chemicals in occupational environment; its most interesting feature involves the possibility of predicting toxicological diseases at a very early stage by means, of measurement of indicators of dose and effects.
For industrial chemicals such as benzene, lead, manganese, titanium, toluene, alogenated hydrocarbons and cadmium a description of the types and characteristics of indicators is given.
The limitation and difficulties inherent in biological monitoring are analyzed, indicating the goals for further research in this field.