Rapport

Biologiske eksponeringsmarkører (IFKAN)

Forord

Alt er kjemi, og vi er omgitt av kjemikalier. Noen av disse er helsefarlige og kan gi sykdom og skade. Daglig dør det personer i Norge som følge av kjemikalieeksponering. Sykdommen kommer som regel snikende etter mange år og forårsaker mye lidelse. Mye av dette kunne vært unngått. Vi har et felles ansvar for at dette ikke skjer.

Prosjektet Kjemisk arbeidsmiljø i olje- og gassindustrien ble opprettet i 2007 for å gi et helhetlig bilde av den nåværende og tidligere eksponeringssituasjonen, beskrive og tette kunnskapshull og bidra til at næringen blir bedre til å håndtere risiko rundt kjemikalier i arbeidsmiljøet.

Arbeidet har skjedd i et samarbeid mellom Oljeindustriens landsforening, Norsk Industri, Rederiforbundet, Landsorganisasjonen i Norge (LO), Lederne og SAFE. Petroleumstilsynet og Arbeidstilsynet har deltatt som observatører.

Prosjektets hovedfokus har vært å samle, skape og spre kunnskap. Mye informasjon har blitt samlet inn og presentert i rapporter og foredrag. Prosjektet har stått bak forsknings- og utviklingsarbeid, og det er blitt arrangert mange aktiviteter for å øke kunnskapsnivået i bransjen. Mye av denne informasjonen er tilgjengelig på www.olf.no

Prosjektarbeidet har resultert i flere rapporter. Disse rapportene står selvsagt for forfatters regning, men er blitt til i nær tilknytting til kjemikalieprosjektet. Noen rapporter gir et brett oversyn, andre er smalere og kanskje spissere. Det betyr også at målgruppen vil variere fra rapport til rapport.

Denne rapporten er en del av denne porteføljen av rapporter, som er gitt ut i tilknytting til kjemikalieprosjektet.

Vi som har arbeidet med prosjektet, har et ønske om at kunnskapen vi har opparbeidet oss tas i bruk, ikke bare i den norske petroleumsindustrien, men alle steder der kjemikalier blir benyttet.

Jakob Nærheim
Prosjektleder kjemikalieprosjektet

Biomarkers of exposure

Critical review of literature data regarding relevant analytical methods, international and national standards

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1 Summary

The overall reasons to perform measurements of chemicals in the industry are performed to improve or minimise risk for workers. The strategy for the measurements of various chemicals may be different for the purpose of the sampling and what chemical that are involved. In the product stewardship (related to HMS) all possible exposure situations need to be considered for each individual involved company. Different sampling strategy may be involved depending on where in the life cycle of the product sampling is to be performed. Biological monitoring for exposure assessment on workplaces provides information about the internal dose, i.e. the entire dose of a given substance, which has been taken up by an individual. Biological monitoring has the great advantage over air monitoring to take all routes of exposure into account, as well as individual differences regarding uptake, metabolism etc. In addition, non-volatile compounds can be monitored as biomarkers that are not revealed by air monitoring. The sampling does not interfere with work tasks and samples can be obtained after cessation of work.

This report lists biomarkers suggested as relevant for the petroleum industry. These include metals -arsenic, beryllium, cadmium, chromium, cobalt, copper, lead, mercury (organic and inorganic), molybdenum, nickel, vanadium, titanium and zinc - which may be emitted during hot work or cutting of coated metal parts. Polymeric coatings are commonly used to prevent corrosion of metal parts. Polyurethanes, epoxy, zinc epoxy, acrylates, polyvinyl chlorides (PVC), polyester, zinc silicate, siloxane systems and alkyd paints are coatings frequently used within the petroleum industry for this application. Degradation of such polymers through for instance welding or cutting emits isocyanates, aldehydes, organic anhydrides, amines and bisphenol A. In addition, biomarkers for passive smoking, mental stress and oxidative stress have been identified as relevant, the latter rather being an overall biomarker for a number of compounds. Exposure to nitrogen oxide (NO), nitrogen, dioxide (NO_2), sulphur dioxide (SO_2) and hydrogen sulphite (H_2S), organophosphates and a number of solvents (chlorinated solvents, aliphatic hydrocarbons, benzene, toluene, xylene, ethyl benzene, styrene and polyaromatic compounds (PAHs) have also been identified as significant within the petroleum industry, which biological monitoring would be relevant for. Urine and blood are considered to be the most relevant assay materials for biological monitoring. Cortisol in saliva has been proposed as a biomarker for mental stress. The inter- and intra-variability of cortisol limits its use as a suitable biomarker for mental stress.

Today, biological monitoring guidance values (the maximum recommended concentration in the biological fluids) differ greatly between various countries, being mandatory for some compounds such as lead, while only recommendations for most other compounds. Occupational exposure limit values (OELs) generally exist for airborne metals, solvents, isocyanates, organic anhydrides, some amines, NO_2, NO, H_2S and SO_2. Biological guidance or tolerance values, generally only exist for some metals and solvents in Germany, the UK and the US. At laboratories which perform analyses of biomarkers located in Norway, Sweden and at Health and Safety Laboratory (HSL) in U.K., routine analyses are generally available for metals, some isocyanates, formaldehyde, cotinine, some organic anhydrides and some solvents.

Routine analysis is available today for biomarkers for around 100 substances, mostly organic compounds, metals and some solvents. Metal analysis is commonly performed using atomic absorption spectroscopy or inductive coupled plasma mass spectrometry (ICP-MS). Detection and quantification of organic compounds is preferably performed by high performance liquid

chromatography mass spectrometry (HPLC-MS/MS) or gas chromatography mass spectrometry (GS-MS).

Biomarkers may either be the presence of the chemical itself, its metabolites or indicators of effects exerted by the chemical in the human body. Biomarkers of exposure are required to be chemically specific, detectable in trace quantities and quantitatively relatable to a prior exposure regimen. Biological monitoring may be carried out in order to evaluate the efficiency of personal protection equipment (PPE), to validate that workers haven't been exposed to harmful substances and for epidemiological studies in order to establish correlations between exposure and health effects. Biological monitoring has a particular role for substances that are absorbed through the skin and are systemically toxic. Biological monitoring can help to assess the individual exposure. By informing workers the results can help them to improve work habits.

However, the internal dose also takes potential non-occupational exposures into account as well. Such exposure, for instance smoking, may greatly influence the development of adverse effects such as asthma and chronic obstructive pulmonary disease (COPD) and it is difficult to distinguish occupational diseases from diseases from other causes. In several countries there are data bases of high quality regarding e.g., cancer in the population. In order to relate the exposure to disease it would be a great advantage to have access to exposure data from biomarkers. Today, the exposure is often only poorly estimated from a limited number of air measurements. In most countries there are claims that the workers have had their disease caused by exposure at work. Biomarkers can be of assistance in learning about the relation between the disease and the work place exposure. Skin patch tests enable diagnosis of allergic contact dermatitis.

In order to establish a program for biological monitoring, the most appropriate substances should be selected with regard to toxicity and occurrence at the workplace. In addition, informed consent, procedures for sampling, transportation, storage, analysis, compilation of results and reporting of results need to be established.

In some cases measurement of all possible degradation products including the parent compound and metabolites gives a more correct picture of the exposure situation. For this type of substance, further development of methods for determination of different protein adducts emerging from chemical exposure. For instance, isocyanates are able to form such adducts together with proteins. Analytical methods should be developed for isocyanate-protein conjugates and nitroso compounds in order to assess isocyanate exposure at workplaces. In other cases a simple method for a marker compound may be sufficient to help control exposure.

All polymers liberate very reactive free radicals at hot work or by mechanical stress of the polymeric coating e.g., grindings. No systematically investigation has been conducted on the exposure of free stable radicals from the thermal decomposition of polymers to human.

2 Contents:

3 Abbreviations

1-Hydroxypyrene (1-OHP)
3-Dimethylaminopropylamine (DMAPA)
4,4'-Methylenedianiline (MDA)
4,4’-Methylene bis(2-chloroaniline (MbOCA)
8-Hydroxy-2’-deoxyguanosine (8-OHdG)
Acetic aldehyde (AAL)
Administrative Norm (AN)
Airway Hyper Reactivity (AHR)
American Conference of Governmental Industrial Hygienists (ACGIH)
American Thoracic Society (ATS)
Arsenic (As)
Arsenobetaine (AsB)
Atmospheric Pressure Chemical Ionization (APCI)
Benzylmercapturic acid (BMA)
Beryllium (Be)
Biological Exposure Indices (BEI)
Biological Monitoring Guidance value in UK (BMGV)
Biological Tolerance value (BAT)
Biologische Leitwert (BLW)
Biologischer Arbeitstoff-Referenzwert (BAR)
Bisphenol A (BPA)
Bisphenol A diglycidylether (DGEBA)
Bisphenol F diglycidylether (DGEBF)
Bronchoalveolar Lavage (BAL)
Cadmium (Cd)
Carbon mono oxide (CO)
Central Nerve System (CNS)
Chromium (Cr)
Chronic Beryllium Disease (CBD)
Chronic Obstructive Pulmonary Disease (COPD)
Cobalt (Co)
Control of Substances Hazardous to Health (COSHH)
Control of Lead at Work (CLAW)
Copper (Cu)
C-reactive protein (CRP)
Cyanide (CN)
Dehydrated glucuronic acid (DHGlcA)
Detection limit (LMT)
Deutsche Forschungsgemeinscaft (DFG)
Diethyldithiocarbamate (DDC)
Diethylene triamine (DETA)
Diethyldithiocarbamate (DDC)

Diglycidylether bisphenol A (DGBA)
Dimethyl arsenic (DMA)
Direct Analysis in Real Time (DART)
Electron Impact Ionization (EI)
Electro Spray Mass Spectrometry (ES-MS)
Electrospray Ionisation (ESP)
Endothelin (ET)
Environmental Protection Agency (EPA)
Enzyme-linked immunosorbent assay (ELISA)
Eosinophil cationic protein (ECP)
Ethyl isocyanate (EIC)
Exhaled breath condensate (EBC)
Expositionsäquivalente für Krebserzeugende Arbeitsstoffe (EKA)
Finnish institute of occupational health (FIOH)
Food and Drug Administration (FDA)
Forced expiratory volume (FEV)
Forced vital capacity (FVC)
Formaldehyde (FA)
Gas chromatography (GC)
Glucose (Glc)
Glucuronic acid (GlcA)
Glutathione (GSH)
Health & Safety Executive (HSE)
Hexamethylene diamine (HDA)
High Performance Liquid Chromatography (HPLC)
High Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC)
High Performance Liquid Chromatography with Fluorescence Detection (HPLC-FD)
Hydrogen sulphide (H_2S)
Immunoglobulin (Ig)
Indicative Occupational Exposure Limit Values (IOELVs)
Inductive Coupled Plasma Mass Spectrometry (ICP)
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Inhaled Corticosteroids (ICS)
Interferon γ (IFN- γ)
Interleukin (IL)
International Agency for Research on Cancer (IARC)
Isocyanic acid (ICA)
Isophorone diamine (IPDA)
Isophorone diisocyanate (IPDI)
Lead (Pb)
Limit of detection (LOD)
Limit of quantification (LOQ)
Liquid Chromatography (LC)
Liquid Chromatography Mass Spectrometry (LC-MS)

Liquid Chromatography -Negative Ion Atmospheric Pressure Chemical Ionisation-tandem mass Spectrometry (LC-APCI-NI-MS/MS)
Maleic anhydride (MA)
Matrix Assisted Laser Desorption Ionisation (MALDI)
Maximale Arbeitsplatz-Konzentration (MAK)
Medical guidance note No 17 in UK (MS17)
Mercury (Hg)
Metallothioneins (MTs)
methacrylic acid (MAA)
Method detection limit (MDL)
Methyl ester of acrylate acid (MEMA)
Methyl isocyanate (MIC)
Methylene diphenyl diisocyanate (MDI)
Molybdenum (Mo)
Monomethyl arsenic (MMA)
Multiple Chemical Sensitivity (MCS)
m-xylene α,α diamine (XDA)
National Institute for Environmental studies (NIES)
National Institute for Occupational Safety and Health (NIOSH)
Natural killer (NK)
Negative ion Chemical Ionization (NCI) mode
Neutrophil elastase (NE)
Nickel (Ni)
Nitrogen dioxide (NO_2)
Nitrogen oxide (NO)
Nivågränsvärde (NGV)
Nuclear factor –κB (NF–κB)
Occupational exposure limit (OEL)
Occupational Safety and Health Administration (OSHA)
Odds ratio (OR)
O-Methylbenzyl mercapturic acid (MBMA)
Peak expiratory flow (PEF)
Perchloromethane (CCl_4)
Permissible Exposure Limit (PEL)
Personal protective equipment (PPE)
Phenyl isocyanate (PhI)
Phenylglycidylether (PGE)
Phtalic anhydride (PA)
Polychlorinated biphenyls (PCB)
Polycyclic aromatic compounds (PAHs)
Polyetheretherketone (PEEK)
Polymethylmethacrylate (PMMA)
Polyurethane (PUR)
Polyvinyl chloride (PVC)

Positive ion Chemical Ionization (PCI)
Proliferating Cell Nuclear Antigen (PCNA)
Propionic aldehyde (PAL)
Propyl isocyanate (PIC)
Radio Allegro-Sorbent Test (RAST)
Radio immunoassay (RIA)
Reactive Airways Dysfunction Syndrome (RADS)
Registration, Evaluation, Authorisation and Restriction of Chemicals European Union (REACH)
Scientific Committee on Occupational Exposure Limits (SCOEL)
Short-Term Exposure Limit (STEL)
Statens Arbeidsmiljöinstitutt (STAMI)
Solid phase extraction (SPE)
Sulphur dioxide (SO_2)
Tetraethylene pentamine (TEPA)
Thiazolidine-4-carboxylate (TZCA)
Threshold Limit Values (TLV)
Time-of-flight (TOF)
Titanium (Ti)
Toluene diamine (TDA)
Toluene diisocyanate (TDI)
Tri(dimethylaminomethyl)phenol (TEPA)
Triethylene tetramine (TETA)
Triglycidylisocyanurate (TGIC)
Tumour necrosis factors (TNF)
Tumour protein 53 (p53)
Turbo ion spray (TIS)
Vanadium (V)
Vascular endothelial growth factor (VEGF)
Volatile organic compounds (VOC)
Zinc (Zn)

4 Foreword

There is a need for industry to assess and control chemical exposure to minimise risk for workers. The strategy for the measurements of various chemicals may be different for the purpose of the sampling and what chemical are involved. In the product stewardship (related to HMS) all possible exposure situations need to be considered for each individual involved company. Different sampling strategy may be involved depending on where in the life cycle of the product sampling is to be performed.

There is no single general method for the monitoring of all possible airborne compounds that may occur in the work environment. It is necessary to understand the compounds that are in use, what compounds that may be formed during the process and any possible degradation products. Safety data sheets may contribute to the sampling strategy, but the quality is often too poor for the occupational hygienist and the laboratory that will analyse the samples. The safety data sheets, in most cases, only contain information regarding major components and not necessarily the ones that may be the most harmful ones. Analytical procedures for the material samples, relevant air samples or possible biomarkers are missing. The availability of such information would be of great advantage and would save cost for involved companies.

It needs to stress that chemicals used in the industry in many cases contain complex mixtures of various components. The chemicals are mainly manufactured for a special purpose and to make a product with adequate properties and often to the lowest possible cost.

In many cases measurements focus upon chemicals that are listed in occupational exposure limit (OEL) lists. The list may look long and the reader may get the impression that many or most relevant compounds are listed. In fact, there are several orders of magnitude many more compounds present in working life that are not listed. The OEL values are not necessarily levels that ensure exposed workers intrinsically safe working conditions. Many of the OEL levels are set based on social and economic factors together and scientific data regarding health risks. Many of these aspects are mentioned in e.g., Agenda 21: Chapter 19 1992 Rio de Janeiro and the Registration, Evaluation, Authorisation and Restriction of Chemicals, 2006, European Union (REACH).

"19.8. The broadest possible awareness of chemical risks is a prerequisite for achieving chemical safety. The principle of the right of the community and of workers to know those risks should be recognized. However, the right to know the identity of hazardous ingredients should be balanced with industry's right to protect confidential business information. (Industry, as referred to in this chapter, shall be taken to include large industrial enterprises and transnational corporations as well as domestic industries.) The industry initiative on responsible care and product stewardship should be developed and promoted. Industry should apply adequate standards of operation in all countries in order not to damage human health and the environment.

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19.11. Assessing the risks to human health and the environment hazards that a chemical may cause is a prerequisite to planning for its safe and beneficial use. Among the approximately 100,000 chemical substances in commerce and the thousands of substances of natural origin with which human beings come into contact, many appear as pollutants and contaminants in food, commercial products and the various environmental media. Fortunately, exposure to

most chemicals (some 1,500 cover over 95 per cent of total world production) is rather limited, as most are used in very small amounts. However, a serious problem is that even for a great number of chemicals characterized by high-volume production, crucial data for risk assessment are often lacking. Within the framework of the OECD chemicals programme such data are now being generated for a number of chemicals. "

When using chemicals a risk assessment is necessary. Risk assessment can be helped by air monitoring and/or biological monitoring and comparing data with OELs, BEILs and other toxicological data. The Health & Safety Executive (HSE) in UK says (2006 06) *"The law does not expect you to eliminate all risk, but you are required to protect people as far as 'reasonably practicable'. This guide tells you how to achieve that with a minimum of fuss".* HSE have defined five steps:

1. Identify the hazards;

2. Decide who might be harmed and how;

3. Evaluate the risks and decide on precautions;

4. Record your findings and implement them;

5. Review your assessment and update if necessary.

For the petroleum industry one suggestive approach can be:

1. Identify what materials, compounds, chemicals, tools, personal protection equipment (PPE) and clothing that are present and used. Investigate the composition of the used materials etc. from safety data sheets. If the data is insufficient request further details or try to perform a relevant material test. In addition, investigate properties of the different materials etc. with regards to the expected life cycle of the product(s). Individual components may be harmless, but in a mixture they can form harmful compounds. It is necessary also to take into account possible degradation products from the used materials etc. that may be formed in a different part of the life cycle of a product. If there are indications that a material etc. may be harmful in the life cycle, exchange it to a less harmful one if it is possible.

2. Study work procedure documents and find out what personnel that may be involved, exposed and harmed when handling the material etc. Take into account the performance of used PPE and the whole life cycle of the product. Find out if who and under what circumstances a worker might be exposed via the air ways, skin or other routes of exposure.

3. From the objective data evaluate the risks. If compounds are released to the work environment, air sampling can be performed to study the emission to the air and swipes can be used to study contamination on surfaces. If levels are not well below e.g., OELs, precautions regarding ventilation and/or as a last solution recommend satisfactory PPE. In addition, find out a solution to ensure that preventive measures are correct and adequate. Such checks can be difficult to perform with air samples as the efficiency of the PPE is not often checked. Here, biomarkers have a great advantage to study the uptake into the body.

4. Document details and implements findings in the work documents. Set up a protocol for measurements (biomarkers or air samples) to ensure that the work procedure is performed as expected according to the work protocol.

5. If an unacceptable exposure still occur or changes is made in the working conditions, review the assessment and update the work procedure documents.

Clearly exposure assessment is necessary for the risk assessment. The exposure assessment of workers can be performed by air sampling or the monitoring of biomarkers, if the natures of the chemicals are known. If this is not the case, the used materials need to be analysed. If PPE are used the exposure can only be estimated with the monitoring of biomarkers. In many cases the exposure is predominantly dermal. Dermal uptake (exposure) can only be estimated by biological monitoring.

In the petroleum industry, there are safety measures and PPE are widely used. Air levels outside PPE do not contribute to the knowledge regarding the actual exposure among workers. It is the chemicals that evade the PPE that may result in exposure among workers and this exposure can only be estimated by biomarkers. Still, today the monitoring of biomarkers as compared to air sampling is sparse. The reason for this is unclear, but it may be explained by tradition and lack of sufficient number of reference data or that proper methods for biomarkers are missing.

The collection of exposure data basically starts with the study of the materials used. In many cases there is a need for the user to get independent data from the chemical analysis of the material and in some cases degradation products thereof. From this information the occupational hygienists and the analytical laboratory will get data, reference compounds and standards that will enable appropriate measurements.

If the nature of the exposure is not known, advanced sampling with regards to gas and particle size distribution followed by analysis of a wide spectrum of e.g., metals and inorganics, and screening for a variety of relevant organic compounds must be carried out. The chemical composition can be revealed using modern analytical equipment. Compounds such as aldehydes, epoxy compounds, phenols, isocyanates, organic-nitrogen compounds, organic-phosphorus compounds and amines etc. may be involved.

Once the natures of the compounds are known the monitoring can be performed using robust and simplified sampling. Sampling and analysis can then focus on selected relevant groups of compounds.

Real-time or almost real-time measurements can be performed and some are even recommended or required by governmental agencies e.g., Occupational Safety and Health Administration (OSHA) (US). Direct-reading instruments give valuable complementary information when studying airborne compounds.

The true individual exposure may be revealed by the monitoring of biomarkers. Biomarkers have the great advantage that compounds that evade the PPE can be monitored. Biomarkers will take into account the individual breathing rate, metabolism and all routes of exposure. For some biomarkers the individual metabolism may affect the resulting concentration in biological fluids.

There are complementary aspects between the analysis of chemicals/materials, air samples or biomarkers. All these techniques to study the exposure and risks are necessary and it is impossible, for a satisfactory estimation of the risks, to be without access of any of these.

There are many purposes for measurements such as: Measurements to fulfil demands by governmental bodies, measurements to check if work procedures are satisfactory, measurements to ensure that protection devices are meeting demands, measurements to ensure safe zone, measurements to reveal individual exposure, measurements to reveal the exposure of a group, and measurements for epidemiological studies.

What to measure is mainly governed by the purpose of the occupational measurement? It is not feasibly to measure all compounds. If there is a lack of information on what to measure, one approach is to first use a package of "less" selective methods to reveal the nature of compounds released. The package must contain methods that can show the presence of various metals and inorganic compounds. Furthermore, the presence of organic compounds should be monitored with methods that reveal the total content. It is also important to measure the presence of airborne particles. Once this picture is clear, there is a need to do a specification of individual components by selective methods.

5 Introduction

5.1 Why biological monitoring?

Biological monitoring has many advantages compared to air monitoring of the exposure. Air samples may be used to estimate the inhalation (or dermal etc.) dose which is taken up by workers on a worksite. Monitoring of exposures by measurements of air levels may be difficult and may not give a relevant picture of the real short or long term systemic dose. In addition, the concentration may differ depending on the location of the sampler as can be seen in Figure 5.1. The conditions in the oil industry are exceptional. Therefore, the use of biomarkers may be more relevant as will be discussed below. Furthermore, compounds such as 4,4'-methylene bis (2-chloroaniline) (MbOCA), which is released during degradation of polyurethane (PUR) polymers, are non-volatile and rarely found in air but exposure can be assessed by biological monitoring.

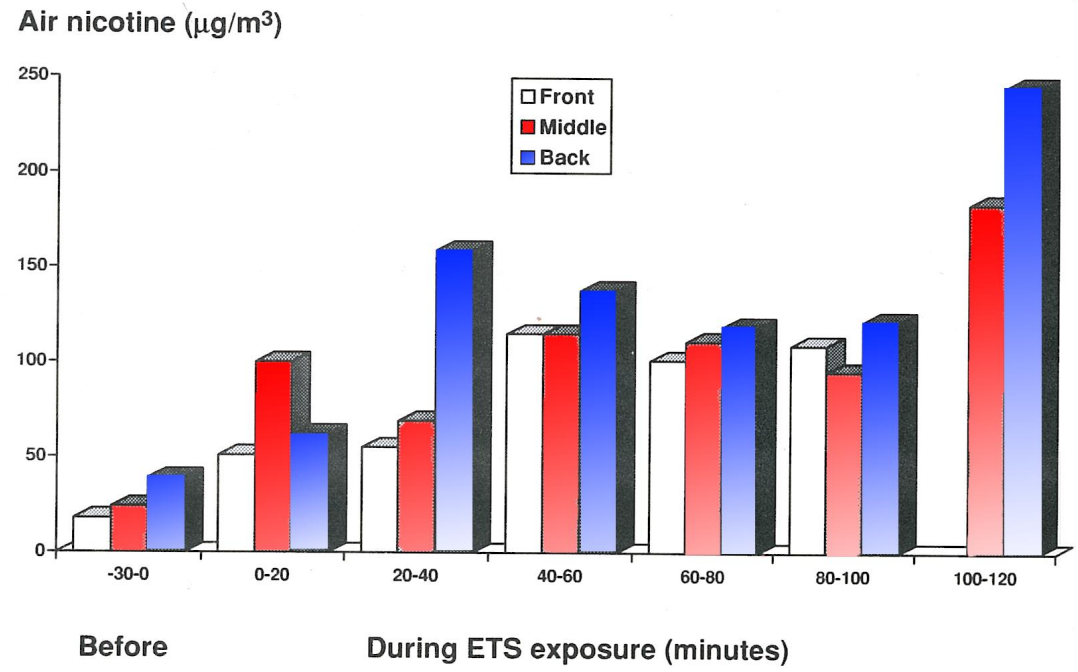


Figure 5.1 Air monitoring of nicotine in a bus displaying, at times, large variations in measured concentrations depending on the location of the sampler [1].

One major advantage with biological monitoring is that all routes of exposure are taken into account. The sampling does not interfere with work tasks and for many substances samples can be obtained after cessation of work. The number of samples that can be taken during a certain period of time are many more for biomarkers as compared to air samples. When workers are using personal protective equipment, biomarkers reveal the efficiency of the PPE – air samples taken outside the PPE does not reveal anything with regards to actual worker exposure. As for air samples it is essential to avoid contamination during sampling. The robustness of methods for the biological monitoring at workplaces is basically the same as the ones used at hospitals. Biomarkers are not novel in medicine. In principal, clinical chemistry is the science of overall biomarkers, but mainly with regards to disease and not for exposure. In fact, biological monitoring occurs at a very large scale in hospitals, both for metabolites and for unmetabolised pharmaceutics. It is performed in clinical trials, but also among patients to verify that a given dose of a pharmaceutical results in an appropriate concentration inside the body in order to have an effective cure without side effects. When taking into account the ethics, biological monitoring is straightforward and in routine. The company physicians should have a list, as for other clinical samples, of relevant biomarkers for the occupational exposure from which he/she can order samples to be taken and have analysed.

Biomarkers in routine analysis contribute to assessment of the exposure among the workers. When it can be presented that the work environment is of good quality and that the exposure is kept to a minimum the recruitment of personnel is facilitated.

For an ideal biomarker there is a need for basic knowledge regarding: The uptake in the body, the distribution in different pools in the body and the toxicokinetics (e.g., elimination half-life). Data regarding the metabolism can be obtained from animal and human studies. Detailed knowledge may rarely be available from studies with radio labelled compounds [4-7]. To know the relation between the biomarker and the uptake, the dose and response relation needs

to be established [5, 6]. For some metabolites it is known that the different genotypes and/or phenotypes affect the metabolites and typically discussed phenotypes are fast and slow acetylators. These aspects will be discussed in further detail in following sections in Appendix.

5.2 Biomarkers

5.2.1 Definitions and applications of biomarkers

A biomarker has been defined and an alteration in a biological system, that is related to a pathogenic process, such as cancer, or to exposure to a xenobiotic compound. This paper, when using the term "biomarker", will mainly refer to biomarkers as those indicating exposure to xenobiotic compound. Biomarkers of exposure may either be the parent molecule itself, its metabolite, or a quantitatively measurable effect as a result from exposure to this compound (see Figure 5.2).

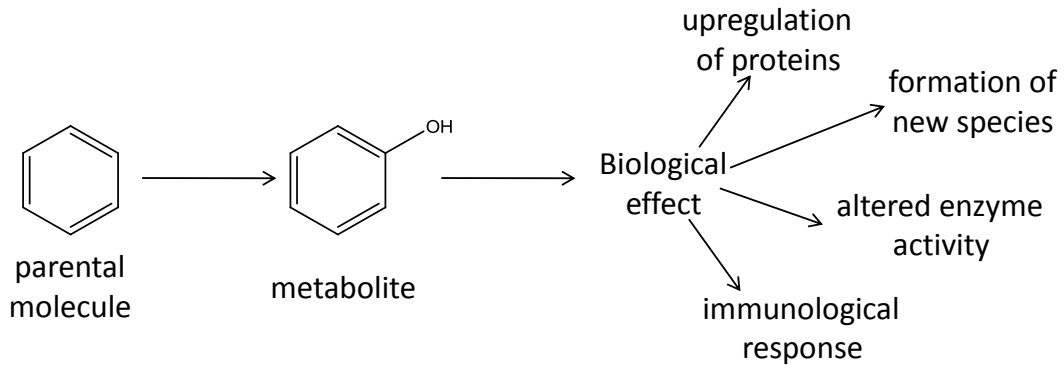


Figure 5.2 Possible biomarkers from exposure to a certain chemical.

Biomarkers can be quantitatively measured in biological matrices, such as in tissues, blood and urine. They are primarily used to predict the dose that an individual has been exposed to. Determination of biomarkers may also enable evaluation of compound's potency for various physiological responses and carcinogenicity in humans.

There are a number of requirements for a biomarker for exposure assessment of a certain chemical. A biomarker ideally should be chemically specific for a xenobiotic, detectable in trace analysis and quantitatively related to the extent of exposure. The relation between a substance and its biomarker should be linear. Biomarkers provide a measure of the internal dose of an individual of a given substance. The internal dose is the total dose an individual is subjected to through exposure. The internal dose includes all routes of exposure. In the cases with offshore applications, exposure through inhalation and dermal absorption is the main routes of administration. Biomarkers as a measure of exposure also takes into account individual factors, such as metabolism, breathing rate, work load etc. Ideally, a biomarker should be easy to sample, in that regard urine is often preferred as collection is non-invasive and risks of infection are eliminated compared to blood.

Strong regulation of the pharmaceutical industry requires lots of efforts in order to characterize and measure biomarkers for documentation during pharmaceutical development. However, when it comes to exposure of chemical substances in the industry, there are not much data available in terms of biomarkers, nor is there any strict guidelines regarding the tests necessary. For example, there are only biological tolerance values listed for xenobiotic compounds as lead and mercury in Norway. In this study, OELs have been summarized as recommended in the US, Germany, Norway and Sweden. However, out of the compounds for which OELs are available, only a few are complemented with biomarkers and biological tolerance values. Measurement of biomarkers is an easy and efficient way of determining exposure. Consequently, more research is needed within this area.

5.2.2 References values

Biological monitoring requires reference values and limit values in order to interpret the results from the samples. Reference values are obtained from a reference group that is not occupationally exposed. The reference interval is the interval between two reference limits obtained from the statistical evaluation of the internal doses of the reference group [2]. Reference values are available for several compounds with listed BEIs. The reference values represent the upper margin of the current background exposure of the general population [6]. However, reference values have only been evaluated for six metals (arsenic, lead, cadmium, mercury, nickel and platinum) and polychlorinated biphenyls, chloro organic compounds, polyaromatic hydrocarbons, phthalates, organophosphates and pyrethroids [6].

5.2.3 Interferences during biological monitoring

When studying occupational respiratory diseases air pollution (indoor and outdoor) have to be taken into account. Air pollution is defined as the presence of gases or particles of matter in the air that are not natural to the atmosphere at such concentrations. Although air pollution as such does not appear to increase the risk of developing asthma, it is a known trigger for asthma exacerbation, particularly in severely asthmatic patients. It is generally man-made, but could also arise from natural events such as eruption of volcanoes. Air pollution is mainly caused by combustion fuel exhaust from motor vehicles and factory fumes. Diesel exhaust is a major source of particulate matter pollution, which also contains chemicals such as nitrogen dioxide and sulphur dioxide (SO_2), all of which trigger asthma. Diesel particles have also been shown to absorb allergens from grass pollen into their surface, thereby enhancing the antigenicity of pollen and its deposition in the lung. SO_2, nitrogen dioxide (NO_2), and ozone (O_3) are the common gaseous pollutants known to trigger asthma. The concentrations of chemicals i.e. volatile organic compounds (VOC) are generally higher indoors as compared to outdoor levels [3].

However, the interpretation of a certain level of a biomarker however must be done with caution as several non-industrial factors may influence the levels and possible associations with risk/ disease.

Non-industrial factors:

- Home exposures e.g., from the stove/fireplace
- outdoor personal exposures e.g., car engines and petrol,
- ambient air
- Personal life style e.g., smoking behaviour, food intake
- Age, sex,
- Airway infections
- Genetics e.g., α-1 antitrypsin deficiency

Especially, smoking (active) significantly raise levels of most of relevant biomarkers and is associated with very high risk / disease.

Advantages and disadvantages with biomarkers

Biological markers (biomarkers) have been defined as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids." More recently, the definition has been broadened to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. In practice, biomarkers can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease [4].

The advantages and disadvantages of biomarkers are shown in Table 5.1. Several points should be considered before using biomarkers in clinical studies of any type. Such points are:

- Measurements errors
- Bias
- Confounding
- Cost Acceptability

Table 5.1 Some Advantages and Disadvantages of Biomarkers

Advantages	Disadvantages
Objective assessment	Timing for sampling is critical
Reveals exposure that evade PPE	Normal range difficult to establish
Reveals dermal uptake	Ethical responsibility
Individual exposure assessment	Less helpful for technical improvements
Individual awareness of exposure is improved	For many compounds there missing guidance values (BAT, BEI, PEL)
Samples can be taken after exposure	Affected by age, sex, phenotype
Reveals average exposure during a certain time	Affected by e.g. exposure other than from the work place

5.3 Routes of uptake

Foreign compounds entering the body are called *xenobiotics*. Xenobiotics may be present as additives in food, in the ambient air and as work-related substances, the latter under some circumstances being a complex mixture of different compounds at high concentrations.

The body eliminates xenobiotics through metabolism, during which compounds are converted into more water-soluble derivatives in a process known as *biotransformation*. The lipophilicity of xenobiotic compounds enables absorption through the skin, through the lungs or in the gastro intestinal tract but also prevents them from being excreted from the body. Without biotransformation, lipophilic xenobiotics would accumulate inside the body due to the slow excretion rate and would eventually be fatal.

Chemical reactions during this process are catalysed by enzymes in the liver and in other tissues. Biotransformation leads to excretion of xenobiotics in urine or in faeces. Biotransformation may lead to the formation of harmful metabolites that can cause severe damage in organs, poisoning and chronic injuries or be carcinogenic [5].

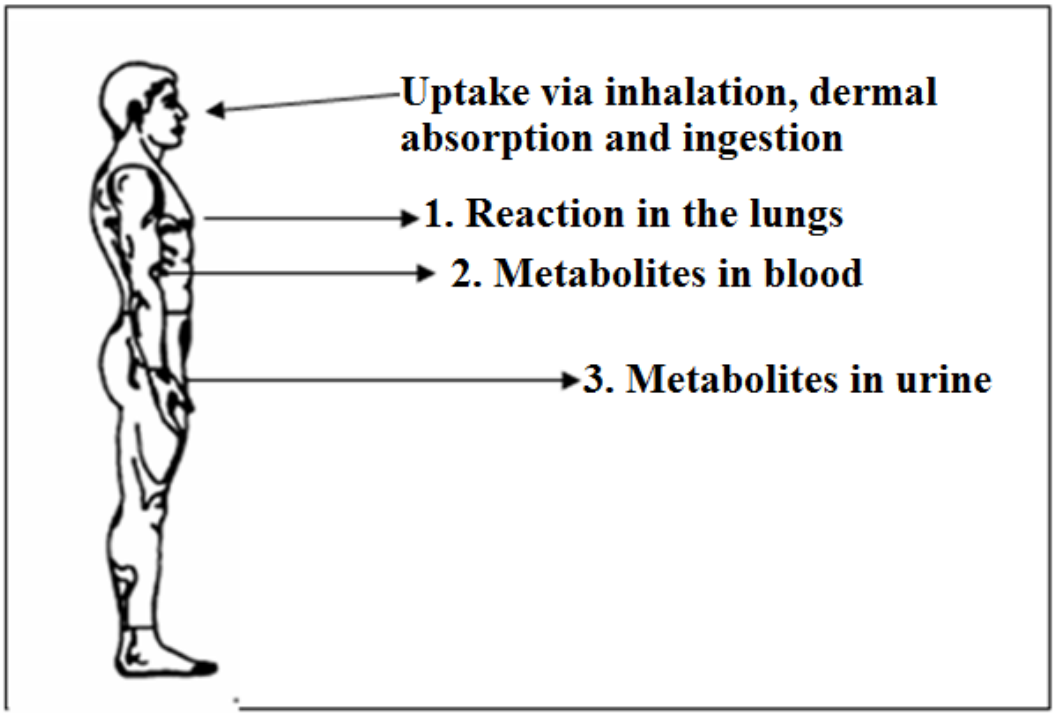


Figure 5.3 Route of uptake and excretion of xenobiotic compounds. Intake occurs dermal or through inhalation during occupational exposure.

To eliminate risks for workers exposed to chemicals in a work environment, it is of great importance to know which chemicals they may be exposed to and to have information regarding the toxicity of these chemicals. It is also crucial to have methods in order to determine whether workers have been subjected to exposure or not. The extent of exposure of a given chemical substance can be determined by measurement of biomarkers [5].

6 Biological samples

6.1 Biological samples

Biomarkers can be detected in assay materials, or biological matrices, such as urine, blood (including serum and erythrocytes), nasal lavage, fingernails, hair, faeces, exhaled air and saliva. In assessment of occupational exposures, urine and blood are the most appropriate choices. The choice of assay material requires easy sampling under routine conditions. Samples can generally not be collected in the target organs, which would most preferable.

Very seldom samples can be collected in the target organs for the compound to be studied and sampling is in most cases accomplished to take samples from blood or urine and these kinds of samples will basically only reflect the up-take. To avoid invasive sampling, urine samples are most common.

Other samples relevant for biomarkers are: Blood (Plasma, serum, erythrocytes, lymphocytes, and immunoglobulins), saliva, hair, exhaled air, nasal lavage, lung lavage, faeces, spinal fluids and tissue samples.

6.1.1 Urine

Urine is an excretion fluid containing numerous compounds that are leaving the body. Some of these compounds are certain break-down products from macromolecules in the body. These break-down products (peptides) may originate from albumin or haemoglobin. For some compounds e.g., isocyanates, these peptides also contain the isocyanate adducts, that were present in e.g., albumin, and the concentration in the blood can be calculated, from urine samples, without invasive sampling. But here there is a need for the improvement of methodology to isolate and enrich urine samples for the determining break-down products of adducts to interesting macromolecules in the body.

Urine is most commonly used for sampling of metals and hydrophilic substances or their metabolites and corresponds well with plasma levels of these compounds. However, Chromium and Cadmium can accumulate in the kidneys and as a result influence the urine level [6]. Substances that have a molecular weight < 5 000 Da in size readily diffuses across the membranes during renal excretion. Any molecules larger than that can be prevented or restricted. Renal clearance is a measure of how efficiently compounds are excreted. Coupling of compounds in the blood to plasma proteins inhibits excretion. For instance may metals such as Cadmium, bind to metallothioneines in the renal cortex and no levels can be detected in the urine until these binding sites have been saturated.

Due to varying grade of ionization, compounds are excreted with different rate depending on pH in urine, since ionic substances are less prone to cross membranes.

Very diluted or undiluted urine samples cannot be used even though the biological limit value is based on the relationship to the mass of creatinine present in the urine. Creatinine is a product or creatinine phosphate in skeletal muscles and is excreted in a constant rate throughout the day.

For example: $\frac{3.6\ \mu g / L\ Cadmium}{1.8\ g / L\ creatinine} = 2.0\ \mu g\ Cd / g\ creatinine$

The creatinine content of the urine should be between 0.5 and 2.5 g/l [7] in concentration in order for the urine sample to be usable. According to the World Health Organization, urine samples with a creatinine content of between 0.3 and 3.0 g/L urine are appropriate for bio monitoring [6]. In the cases with volatile compounds such as ketones, toluene, methanol etc., no adjustment for creatinine concentration in urine is necessary, since these compounds are excreted readily in the kidneys according to equilibrium of partial pressures between urine and plasma (tubular diffusion). Adjustment for creatinine is not either essential for morning urinary samples [1]. The concentration of creatinine in urine is measured by colorimetric methods using the Jaffe Reaction. The Jaffe reaction refers to the process when creatinine reacts with alkaline picrate resulting in a red coloured complex, which can be quantitatively measured. Creatinine is determined routinely at most hospitals. Results are generally accurate to ±10% [6]. Hydrolysis is also generally performed for biomarkers present in urine in order to ensure that no protein binding of the target molecule has occurred. Urine sampling is inappropriate for workers with impaired renal function.

6.1.2 Blood

Almost all compounds can be measured through sampling of blood. However blood sampling is invasive and requires medically trained staff and has a risk of infection. Hence, sampling can only be performed by medical personnel. The concentration of a compound may differ between various blood vessels in the body. For instance a higher concentration may be obtained in a blood sample from an arm which has been dermally exposed [6]. In blood, xenobiotic substances may either exist freely in blood plasma, or conjugated to plasma proteins or erythrocytes for example aromatic amines and aromatic nitro compounds able to do this [8]. This is especially true for carcinogenic compounds with electrophilic properties. When conjugation occurs of the target compound to a protein in blood, the conjugates need to be hydrolysed prior to work-up and analysis. Formation of conjugates with haemoglobin in blood of a compound reveals its ability to cross cellular membranes in other cells as well besides erythrocytes.

When blood is used as an assay material, special considerations must be made regarding the handling of the samples for work-up etc.. Personnel involved in handling of the samples need to be educated how to behave in order to minimize the risk of infection.

6.1.3 Exhaled air

Exhaled air or alveolar air can be sampled for analysis of volatile chemicals. These chemicals are able to readily pass the membranes of the alveoli into the lungs from the blood. Even if the up-take of the chemical was through the skin the chemicals can be monitored in exhaled air. In most cases the metabolites of the chemicals cannot be measured in the exhaled air. Hence, another biological fluid should be used for compounds that are readily metabolised in the body. Non-volatile chemicals that are inhaled in the form of particles are however not measurable in this way. Volatile compounds that are converted when in contact with body fluids or very soluble in water are not either suitable for analysis in exhaled air [6].

7 Adverse health effects as a result of occupational exposure

Occupational exposure normally occurs through inhalation, ingestion or dermal absorption of chemicals and can cause occupational-related diseases. Inhalation is often (not always) the major route of exposure at a work place. Chemicals can be inhaled in the form of gases, vapours, liquid aerosols, particulate aerosols, fumes and mixtures of these. Dermal uptake is however also of concern. Exposure through the skin may occur from chemicals present in the ambient air, from liquid splashes, through immersion, or handling of material. The internal dose refers to the intake of a given chemical which a worker has been exposed to [5]. The internal dose is of greatest importance for development of disease and can be measured by biological monitoring. The concentration of the chemical in the target organ is the critical concentration, i.e. the biological effective dose which results in biological effects such as altered function and finally development of disease. The association between the biomarker and the following biological effects shown in the figure is however not always clear. There are of course other factors involved in causing diseases besides chemical exposure. In fact in most cases the cause is unknown. Figure 7.1 shows a theoretical model of the pathway from exposure of a chemical to disease. Table 7.2 shows some data on correlation of toxicants versus occupational diseases.

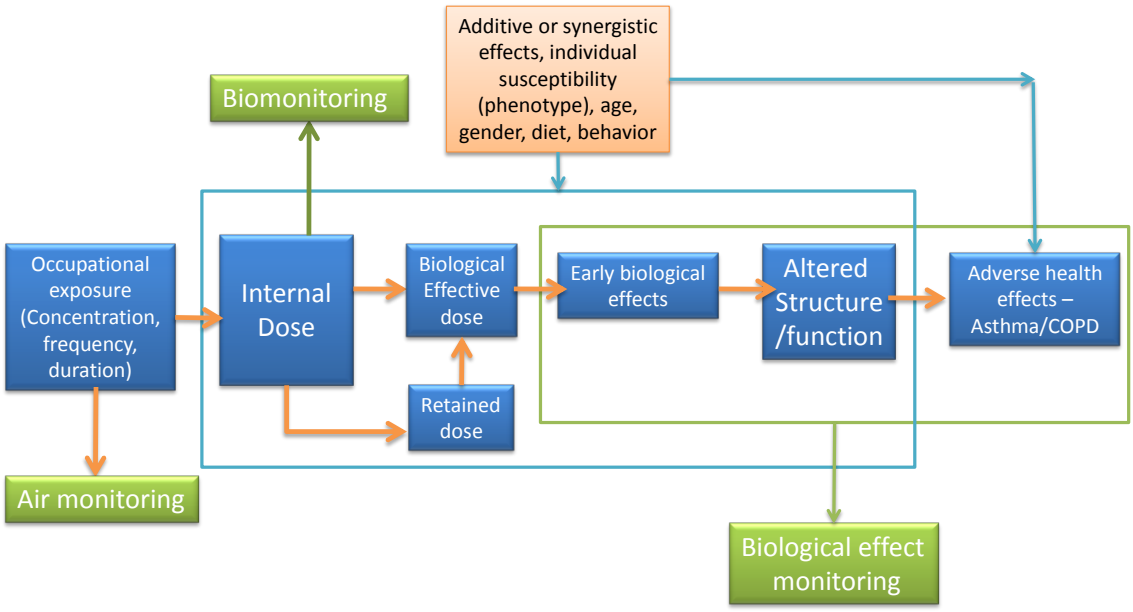


Figure 7.1 Schematic pathway from exposure to disease, showing modifying factors and opportunities for intervention.

Table 7.2 Example of occupational diseases and the toxicants that cause them [5]

Organ system or disease group	Disease	Causative agent
Lung and airways	Acute pulmonary oedema, bronchiolitis obliterans	Nitrogen oxides, phosgene
	Allergic rhinitis	Pollens, fungal spores
	Asthma	Toluene diisocyanate
	Bronchitis, pneumonitis	Arsenic, chloride
	Metal fume fever	Zinc, copper, magnesium
Cancer	Acute myelogenous leukaemia	Benzene, ethylene oxide
	Bladder cancer	Benzidine, 2-naphthylamine, 4-bephenylamine
	Hepatic hemangiosarcoma	Vinyl chloride
	Mesothelioma, lung carcinoma	Asbestos, arsenic, radon, bis-chloro methyl ether
	Skin and Lung cancer	Polycyclic aromatic hydrocarbons
Skin	Allergic contact dermatitis	Natural rubber latex, nickel
Immune system	Autoimmune disease	Vinyl chloride
	Immunosuppression	Lead, mercury,
Renal disease	Indirect renal failure	Arsine, phosphine, trinitrophenol
Cardiovascular disease	Arrhythmias	Acetone, toluene, methylene chloride, trichloroethylene,
	Cor pulmonale	Beryllium
Liver disease	Fatty liver (steatosis)	Carbon tetrachloride, toluene
	Cirrhosis	Arsenic, trichloroethylene

7.1.2 Allergic reactions

Chemical related allergy is an immunologically mediated adverse reaction, sometimes named late adverse reaction, to a chemical resulting from a previous sensitisation to that very chemical or to a structurally similar one. The term hypersensitivity is most often used to describe this allergic state, but *allergic reaction* and *sensitisation reaction* are also used to describe this situation when pre-exposure of the chemical is required to produce a toxic effect.

Chemicals which are able to bind to proteins in the body, forming protein adducts may be able to cause an immunologic response. If the complex is recognized as foreign by the T-cells, these T-cells with receptors specific for this chemical epitope will multiply. As the number of T-cells increases, a threshold is eventually reached (a cascade effect) which results in clinical symptoms. The late adverse reaction is typically not immediate, but may occur after some hours or days. The sensitisation process is due to various degrees of phosphorylation in the T-cell affording a cascade of phosphorylated species in the cell.

There are a number of mediators which may be used as markers for the adverse allergic response, such as interferon γ (IFN-γ), Interleukin (IL-13), Interleukin 5 (IL-5) and ZAP-70 (a protein kinase). IFN-γ, IL-13 and IL-6 can be determined through antibody-based analyses

(immunoassays). ZAP-70 is detected through enzymatic degradation in combination with high performance liquid chromatography – mass spectrometry (HPLC-MS) [9].

7.2 Biological effect monitoring

Since the definition of biomarker is a change in a biological system that can be quantitatively measured and provide information regarding the exposure to certain compounds or a severity of the disease, the use of biomarkers also applies to diagnosis of certain diseases and their progression, such as chronic obstructive pulmonary disease (COPD), asthma and cancer. Biological effect monitoring includes monitoring of early biological effects as a result from exposure [2], i.e. effects which are detectable prior to adverse effects such as cancer or COPD actually have developed. Examples of such effects are sister chromatid exchanges (exchange of DNA between two sister chromatides which indicates mutagenicity), chromosomal aberrations (alterations in the structure of a chromosome), formation of protein adducts in vivo or formation of DNA adducts, sensitisation or elevated levels of inflammatory cells in biological fluids [2].

In later chapters a range of exposures that may be of importance for the oil industry will be described. These substances will be discussed in further detail elsewhere, while this chapter will mainly focus on occupational respiratory disease and cancer. When occupational respiratory diseases are studied, air pollution (indoor and outdoor) has to be taken into account.

It is important be aware of that biases, confounders (risk factors for the studied disease that co-vary with the occupational exposure) and effect modifiers may exist, when assessing causality. This is especially important when looking at disease states which mainly have a non-occupational origin. For lung cancer and COPD about 90% of disease (relative risks RR of 10-15) is attributable to smoking. On the other hand 7% of the smokers` COPD could be attributable to the work environment.

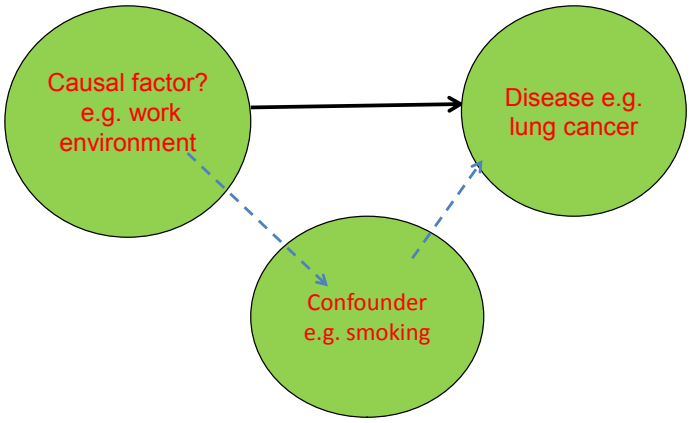


Figure 7.2 Influence of possible confounders in occupational disease development.

Another difficulty is that occupational diseases do not differ from non-industrial disease. Many respiratory diseases (e.g., asthma and COPD) are common in the general population. Although, it is well-known that men who work in dusty trades, especially coal miners, have had a higher incidence as estimated by American Thoracic Society, 2002 (ATS).

A problem using biomarkers is of course that it is for many diseases the total exposure during many years that is of importance for the risk not the current exposure. Current exposure is of importance for hypersensitivity diseases.

7.3 Carcinogenesis

Biomarkers for carcinogenesis are generally alterations in the expression pattern of proteins by the cells. Such variations between healthy cells and cancer cells may be assessed through genomics – which is the evaluation of the genomic content of the cells- or proteomics – the characterization of which proteins are expressed. Evaluation is enabled using microarrays.

Occupational exposure has been coupled to increased incidence of lung cancer in a number of studies of thousands of workers within various industries [10, 11] and is considered to be the cause of 5-20% of all cases of lung cancer [10]. For instance, occupational exposure to Asbestos, Cr(VI), Beryllium, Nickel, Cadmium and Silica has been associated with lung cancer [10]. However, smoking is also a large factor, considered to underlie 85-90% of all cases [10], which makes it harder to assess whether lung cancer in a worker is due to occupational exposure or to smoking. Hence, the connection between smoking and development of lung cancer is better established than any other cause of lung cancer.

The low doses of substances that are able to induce cancer do not cause any other true toxicological effects and usual toxicological analytical methods are not able to detect early effects from carcinogens. During exposure to carcinogenic substances, the nuclear chromatin is damaged, and if it is not repaired inducing mutations in the genome [12]. Cancer may not break out until several years after exposure has occurred, since it takes more than one mutation for the disease to develop. Thus, mutations accumulate in the body throughout the years, which explains why cancer incidence is much higher in older individuals. It is primarily mutations of genes involved in mitosis which lead to cancer development. Consequently, it would be of interest to have biomarkers for carcinogenic effects that are detectable before the disease has started. One example of such a biomarker would be biomarkers of oxidative stress –8-hydroxy-2'-deoxyguanosine (8-OHdG), which also indicates DNA changes due to oxidative stress.

Biomarkers are widely used within cancer research and a vast number of biomarkers for this application, indicating cancer progression are used today in vitro. For instance, up-regulation of proteins involved in proliferation, elevated levels of proliferating cell nuclear antigen (PCNA), Ki-67 etc. [25-27] or indicators of increased level of apoptosis for tumour progression, such as Vascular endothelial growth factor (VEGF) [25] are commonly used biomarkers for cancer progression in vitro.

Lung cancer has been associated with mutations of for instance tumour protein 53 (p53) and the Kras gene, which are both involved in the cell cycle, hence increased levels of mutations of these genes implies exposure to carcinogenic compounds. Mutations of these genes would imply an elevated risk of developing cancer [13].

Carcinogenic substances have been detected to exert immunosuppressive effects and elevated rates of DNA damage [12]. However, smoking and medications exert these effects as well, which makes biological monitoring more difficult. In addition, interferences of carcinogenic effects may be due to carcinogen intake from the diet. Heating during cooking (frying / grilling) of food such as meat or fish may produce carcinogens. For instance, the presence of

heterocyclic amines (HCAs) has been detected in smoke formed during cooking or heating of meat and fish, which have been reported to exert mutagenic effects [14].

The ability of a compound to interfere with DNA is related to its carcinogenicity. Such reactions result in formation of DNA adducts, i.e. complexes of carcinogenic substances coupled to DNA that are appropriate biomarkers for carcinogenesis [8, 13]. Levels of DNA adducts may be measured in urine but the method doesn't distinguish between adducts from DNA, RNA or free nucleotides [8]. DNA adduct monitoring can also be used to measure DNA adducts of specific compounds, such as polycyclic aromatic hydrocarbons (PAHs) [8]. The number of reports of measurement of such DNA adducts in humans are however limited. For epidemiological studies, the number of DNA strand breaks can be measured by comet assays (single-cell electrophoresis assay) [8] by electrophoresis of lymphocytes. The method is based on the ability of DNA, which contains strain breaks, to migrate through the gel [15]. Chromosome aberrations and sister chromatid exchanges are also markers of DNA damage as a result to exposure [8]. However, for routine biological monitoring, urinary or blood levels of DNA breakdown products would be a more suitable alternative. Whether 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a biomarker for oxidative stress, would be a suitable biomarker for carcinogenicity as well is a somewhat debated issue [8]. The role of 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a biomarker for oxidative stress is discussed in more detail in section 7.5.

7.3.1 Concluding remarks

A biomarker for carcinogenicity would indicate exposure to carcinogenic substances which can be observed several years before the disease has actually been developed. The DNA repair product 8-OHdG has been proposed as a biomarker for carcinogenicity as well as for oxidative stress, but some reports contradict its usage for the former alternative. Comet assays are more suitable for epidemiological studies than routine analysis. Urine may not be a suitable assay material from DNA damage products since the origin of these nucleotides may not be all from DNA.

7.4 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by inflammation in the small airways, fibrosis and destruction of alveoli [16]. COPD patients may experience similar symptoms as asthmatics, but don't respond to bronchodilator therapy and the symptoms exhibit less variability [17]. Smoking is one major cause of COPD.

7.4.1 Diagnosis

Diagnosis of COPD is carried out through establishment of a decrease in lung function. The forced expiratory volume (FEV_1), which is the volume in one second divided, and the forced vital capacity (FVC), which is the volume change in the lung between a full inspiration to total lung capacity and maximal expiration to residual volume, are measured. A decrease in the FEV_1/ FVC ration is one of the earliest findings and/ or a significant decrease in FEV_1. Below are described biomarkers which may be useful in diagnosis.

Table 7.3 Correlation between cigarette smoking and the odds ratio (OR), i.e. the number of patients suffering from COPD divided with the number of healthy individuals that are subjected to the same exposure situation [18].

Cigarette smoking/Occupational exposure	N	COPD risk Adjusted OR (95 % CI)
Never/no	549	1.0
Never/yes	210	2.4 (0.9-6.1)
Ever/no	659	7.0 (3.6-13.7)
Ever/yes	454	18.4 (9.3-36.4)

7.4.2 Analytical methods

Pulmonary biomarkers for COPD are currently topics of much interest and much effort is laid on research in order to find appropriate biomarkers for the disease. The aim with pulmonary biomarkers would be to retrieve information regarding the progression of the disease (i.e. diagnosis) and evaluate the effectiveness of new therapies on an early stage. In the future, biomarkers may also be used for prediction of whether the patient will benefit from a potential therapy or not [19]. Currently, there are no biomarkers reported to be available for routine monitoring of COPD [19].

Regarding pulmonary biomarkers, a number of biological fluids have been suggested as assay materials for analysis of biomarkers. The methods used to obtain these fluids differ in their level of invasiveness and reproducibility [16]. Due to the vast number of inflammatory cells, mediators and enzymes involved in the pathologic processes of COPD, there are also many potential biomarkers available [16]. Problems have however been encountered regarding variability, reproducibility and reliability of the methods used to retrieve such biomarkers [16]. In addition, measurement of many of these biomarkers doesn't distinguish between COPD and asthma since these diseases are similar in many ways [19]. Interferences in smokers are also common problems for biomarkers for COPD and asthma, since the levels of many of these biomarkers are also elevated in smokers [16, 19]. There is a need of standardization of these methods and the results must be correlated to the severity of the disease, mortality and exacerbation frequency. Biomarkers of COPD should also be related to clinical outcomes [16].

Bronchial biopsies involve a biopsy performed on lung tissue from the patient. The specimen can be obtained either by washing, which involves a small volume of liquid being put in the area of interest and then removed again, brushing of the epithelial cells or the inner surface [19] or from as a tissue sample from the inside of the lung. However, it is an invasive procedure which cause discomfort for the patient and it cannot be repeated [16].

Bronchial biopsies provide information about the cell composition in the peripheral lung [20] which enables detection of biomarkers of structural damage (apoptosis or uncontrolled proliferation) [16]. For instance, the number of macrophages, activated T lymphocytes (particularly CD8+) has been found to be elevated in bronchial biopsies of COPD patients, expressing IFN-γ, CXCL10 and interleukin 9 (IL-9), which are cytokines involved in the inflammatory process. In addition, the transcription factor nuclear factor -κB (NF-κB) is activated [16], which is involved in regulation of immunological responses.

Bronchoalveolar lavage (BAL) includes a safer procedure which includes a small volume of fluid being put in the area of interest and then collected again. Problems that have been associated with BALs are for instance related to unspecified dilution of the sample, making it harder to determine the levels of the various parameters [16]. Most potential biomarkers found in BALs, such as IL-8 and IL-6, are also associated with smoking [16]. In BALs, increased levels of macrophages [21] and percentage of CD8+ T lymphocytes of the total content of lymphocytes have been detected [22]. Increased activation of elastase and decrease in antielastase activity has been observed in BALs of COPD patients [23]. Elastase is a protease which breaks down elastin, which is as the name indicates an elastic protein in connective tissue. Meanwhile, antielastase is an inhibitor of elastase.

Induced sputum is excreted mucus that is formed in the lower airways as a hypertonic saline is inhaled. A sputum sample provides information about the composition of inflammatory cells. Increased numbers of neutrophils [24] and CD8+ T cells [25] have been found in induced sputum samples of COPD patients. In addition, levels of other inflammatory mediators involved in neutrophil recruitment have also been reported to be elevated [19]. Sputum sampling has become commonly used for COPD and asthma diagnosis [19].

Measurement of exhaled gases to monitor COPD inflammation is a non-invasive method. Today, measurement of nitrogen oxide (NO) in exhaled air is regarded as the most robust and reproducible method to obtain pulmonary biomarkers [19].

Elevated levels of NO in exhaled air is generally related to airway inflammation and correlations between increased levels of NO has been negatively correlated to impaired lung function [19]. In addition, elevated levels of carbon monoxide is associated to COPD patients, but also in smokers due to the content of cigarette smoke [26]. Volatile hydrocarbons, such as ethane and pentane are biomarkers of oxidative stress. In addition, elevated levels of ethane and pentane have been related with severity of COPD in patients [27].

Collection of exhaled breath condensate (EBC) would be another approach for assessment of pulmonary biomarkers in a non-invasive way. This method is however still under investigation [19]. EBC involves collection of warm breath and subsequent condensation of the water in exhaled air into a tube. It has been reported that the levels of prostaglandin E2 and IL-6 are increased in the condensate from patients with COPD [28]. However, it has been proven difficult to measure the levels of most proteins in exhaled breath condensate [16, 19].

Due to problems experienced with the other materials retrieved for analysis of pulmonary biomarkers, of course the most convenient way of biomarker assessment would be through urine and blood. The number of circulating neutrophils in the bloodstream has been reported to be elevated in patients suffering from COPD [29] but also in smokers [30]. Elevated levels of C-reactive protein (CRP) have also been observed in blood samples of COPD patients [31]. In addition, the levels of Eosinophil cationic protein (ECP), MPO and endothelin (ET) -1 are elevated in COPD patients [32]. During exacerbations, fibrinogen and IL-6 levels have been reported to increase in blood plasma [33]. Elevated levels of the cytokine Tumour necrosis factors (TNF) α and leukocytes in plasma are also associated with COPD [19]. Cytokines act as signalling molecules within the immunological system.

7.4.3 Concluding remarks

Bronchial biopsies and BALs are hardly relevant sample procedure for routine sampling. The most reliable biomarkers of COPD which deviate from asthmatic biomarkers are CRP in serum and plasma, fibrinogen, leukocytes and TNFα [34]. No method has however yet been implemented for COPD in common patient management or clinical trials and further investigation is needed [19].

7.5 Asthma

Asthma is present in 10-15% of the adult population [35] and is characterized by wheezing, dyspnea and coughing. Patients are generally diagnosed on basis on such symptoms alone, but diagnosis can be complemented with objective measurements of lung function, such as spirometry [17]. Several hundreds of occupational allergens and chemicals have shown to induce asthma. Asthma induced by isocyanates is the most common type of occupational asthma (OA) [35]. Although there are differences in the mechanism between occupational asthma and allergic asthma, the clinical outcome of diisocyanate asthma and allergic asthma is similar, which could imply a common pathogenic underlying process [35]. The mechanism behind diisocyanate induced asthma has been under investigation, it has for instance been proposed that it is a non-IgE mediated disease [36].

Atopy i.e. the individual capability to produce immunoglobulin (Ig) E antibodies is of importance for high molecular protein allergy but is not proven to be of importance for chemical induced allergy/ asthma. Further, atopic eczema, which have some implications for work selection, is probably not important for pre-employment selection of sensitive individuals who experience chemical exposure in the oil industry.

As employees within the petroleum industry have a low exposure to protein compounds, they run a low risk of type 1 allergy. But they could be exposed to latex, which causes mostly contact allergy. But they run a risk of non-allergic hypersensitivity. Further, smokers have in general an increased incidence of OA probably because the airway mucosa already is damaged by the smoke. Treatment by cortisone inhalation therapy in asthmatics could mask any occupational disease. Therefore it is important to rule out possible OA before starting such therapy.

Possible mechanisms of occupational exposures inducing asthma are:

1. An IgE- mediated mechanism, which means in general that the agent binds to mast cells and basophils and a cascade of mediators activate cells which in turn release other mediators which trigger the inflammatory process. High molecular substances (e.g., wheat, enzymes), but also some low molecular weight chemicals such as anhydrides, platinum may act similar when binding to macromolecules (i.e. haptens).
2. An IgE independent mechanism, of unknown origin may also induce CD4+ activated lymphocytes, which release IL-5, which in turn activate eosinophils. E.g., diisocyanates and amines may use this mechanism.
3. An important mechanism is the direct toxic action on the airway respiratory epithelium (irritant effect) with the resulting damage including neurogenic inflammation, exudation and probably also mast cell effects. This is believed to be the action of e.g., chlorine gas, ammonia, and sulphur dioxide. This exposure is most probably caused by bad handling or accidents e.g., leakages with high exposure levels

(generally much higher than hygiene limits) and with a rapid development often within 24 h after exposure. This gives rise to a condition known as Reactive Airways Dysfunction Syndrome (RADS), which can be considered as a category of occupational asthma developing without a period of latency and is often associated with exposure to high concentrations of irritants [37].

It is important to distinguish the above mentioned diseases, to a disorder called multiple chemical sensitivity (MCS), which is a non-immunological disorder. The mechanism is not believed to be caused by the (occupation) exposure itself and no objective signs of airway disease besides a psycho-social disorder can be detected.

7.5.1 Diagnosis

When diagnosing occupational asthma inhalation provocation is the golden standard. During an inhalation provocation, the patient inhales increasing known amounts of the substance which is suspected to cause the asthmatic symptoms and the pulmonary function is monitored. A decrease of 15 % or more in lung function is considered as a positive test result. It is however difficult to perform and may be dangerous. Instead pulmonary methacholine provocation testing can be done. It measures general hypersensitivity of the airways. It is often of great value but lacks specificity and may be false positive or negative.

In addition, the use of peak expiratory flow (PEF) measurements you can find changes in lung function over time e.g., see differences between holidays and workdays; different work procedures etc. Performed over a long period e.g., 2-6 weeks including holidays it may show work-related pulmonary effects. Further, questionnaires on airway symptoms are valuable especially searching for differences between workdays and holidays.

Several medical tests are available for the diagnosis of pulmonary disease (see below). These include several lung function tests and X-ray methods. Some, but not all cases of asthma are IgE-mediated. Allergy to specific allergens and chemicals can be detected by allergy test procedures such as skin prick tests or measurements of the levels of IgE antibodies in blood analysed by radio allegro-sorbent test (RAST) [38]. The RAST method will be described in further detail in section 15.2.

Spirometry includes measurements during exhalation. The patient exhales heavily into a mouth piece. This method enables determination of the forced expiratory volume (FEV) and the peak flow rate (PEF). FEV is the volume of air exhaled during the first second of exhalation during the spirometry test. The Peak flow rate (PEF) is the maximum flow rate during exhalation. The diagnosis includes observed improvement of these parameters as a result from administration of a short-acting β2-agonist, which is a commonly used therapy for asthmatic symptoms [17].

Smoking has been found to significantly worsen the symptoms of asthma. Approximately 20 % of asthmatics smoke. Smoking in combination with asthma has been related to a higher incidence of hospital admissions, impaired lung function and higher incidence of death as a result from the disease. In addition, smoking has shown to inhibit the anti-inflammatory effect from corticosteroids.

7.5.2 Analytical methods

Pulmonary biomarkers to asthma are much better established than pulmonary biomarkers for COPD.

In biopsies from asthmatics, the number of eosinophils and lymphocytes are higher than in healthy individuals [39]. Eosinophils has been related to lung function [40].

The amount of inflammatory mediators is elevated in BAL fluid from patients suffering from asthma. In addition the number of neutrophils and the levels of neutrophil elastase (NE) are higher in BALs of asthma patients than in healthy individuals [19].

Eosinophils from sputum samples are generally used as biomarkers for asthma. The eosinophil number is related to severity of the disease [41]. Eosinophil cationic protein (ECP) is also elevated in sputum from asthmatics. Additionally, elevated levels of TNF α have been related to airway hyper reactivity (AHR).

The number of eosinophils is elevated in blood plasma, as well as in sputum samples of asthmatics. Using the number of Eosinophils in blood as a biomarker for asthma is however a debated issue, due to reports about the clinical outcome being unaffected by it [19].

In some types of asthma, the level of circulating IgE is elevated. The level of IgE is also used to evaluate whether the patient is susceptible to anti-IgE therapy [19].

7.5.3 Therapies

Asthma is very effectively treated today in a safe manner. This contrasts to COPD; only quitting smoking has shown an effect on the prognosis for COPD. In asthma, bronchodilators relax the smooth muscles in the airways and rapidly inhibit the asthmatic symptoms. β2 – adrenergic agonists is by far the most commonly used bronchodilator today since it's the most effective one and doesn't cause adverse side effects. Bronchodilators administration should however be combined with Inhaled Corticosteroids (ICS) therapy in order to treat the inflammatory process and not only the symptoms. ICS is the most effective type of controller, which is a group of pharmaceutics that inhibit the inflammatory process underlying the asthmatic symptoms.

7.5.4 Concluding remarks

The efforts with biomarkers of exhaled gases are promising and NO measurement in exhaled air is increasingly used. NO is however a better biomarker of asthma than for COPD [19]. The amount of exhaled NO and sputum eosinophils are increasingly are currently used for patient management and in clinical trials for new potential therapies [19].

7.6 Dermal patch testing

About 15-25% in the population have various dermal problems and several chemicals present in the petroleum industry may cause dermal disease and contact eczema. The skin may also be an important route of uptake of chemicals. Well-known diagnostic measures are available

e.g., the patch test consisting of several various occupational suspected substances, can reveal sensitisation at work.

4,4'-metylene dianiline (MDA) can be absorbed through the skin. Figure 7.3 shows an example of the urinary concentration of MDA as a result from dermal exposure to various concentrations of MDA.

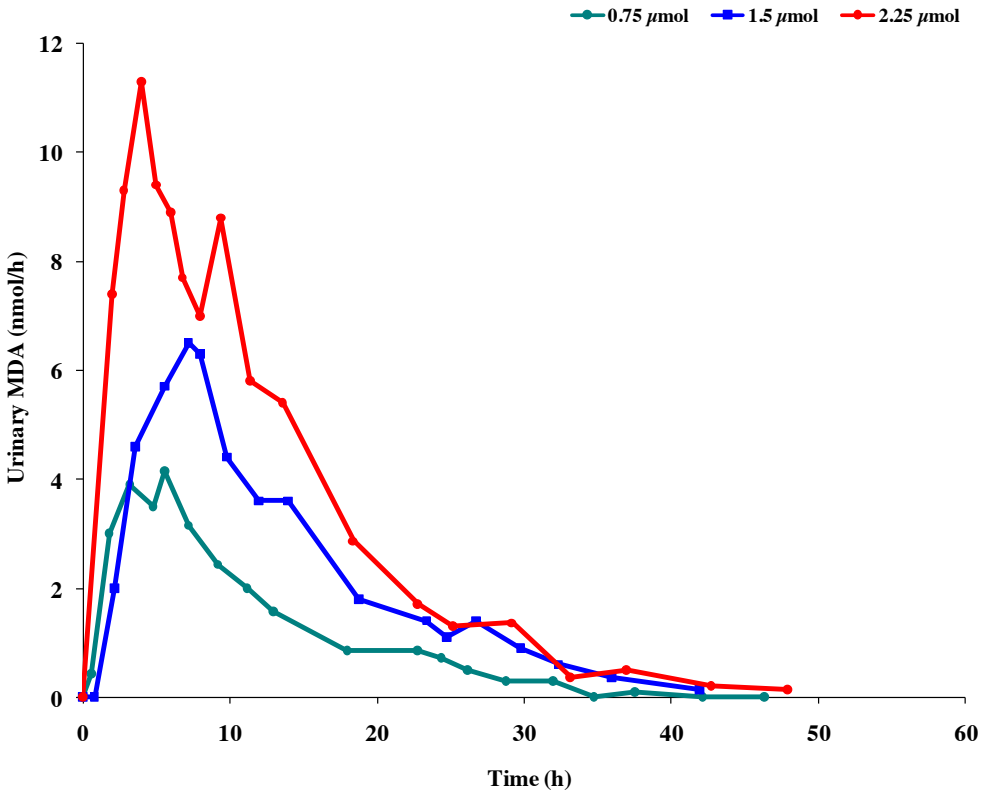


Figure 7.3 The urinary concentration of MDA versus time after dermal exposure of MDA solutions of varying concentration. The urinary concentration declines during excretion.

During patch testing, a small amount of the substance is applied to the skin, resulting in eczema as an indicator of allergic contact dermatitis. Allergic contact dermatitis is a rash of eczema as a result to dermal exposure to a xenobiotic compound, occurring in the upper layers of the skin – the dermis and the epidermis. See Figure 7.4. The test solutions used for this application do however need to be tested prior to use in order to validate the presence of active compounds [55, 56].

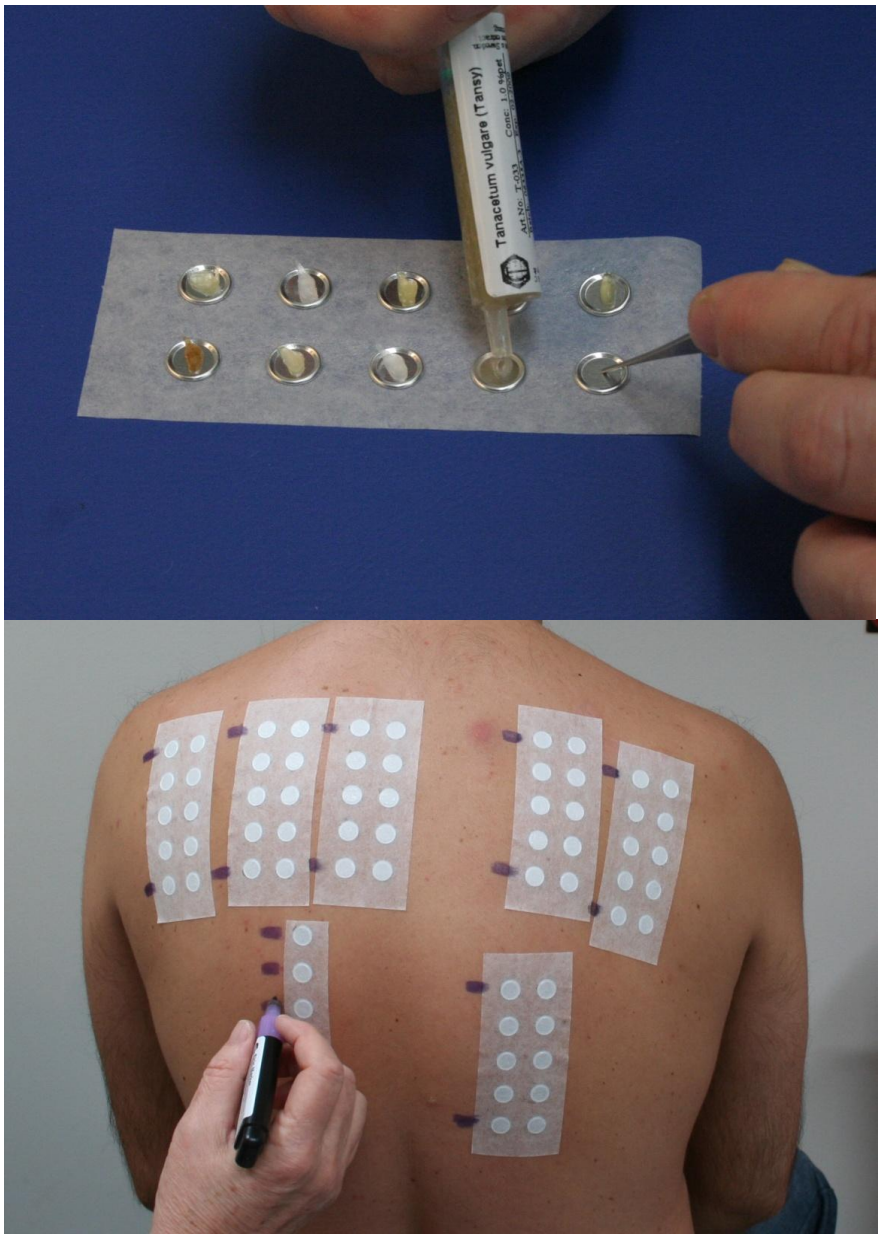


Figure 7.4 Preparation of patch tests for isocyanates [42-45].

8 Governmental rules and guidelines regarding biological monitoring

8.1 Variations between different countries

The regulatory framework for biological monitoring differs in most countries. There is a consensus that biological monitoring is compulsory for workers significantly exposed to lead although the numerical values of the action/limit values may differ slightly. For almost all other substances biological monitoring is optional but in some cases strongly advised and is used widely in practical occupational medicine in many developed countries.

In the EU several Council Directives deal with biological monitoring for selected chemicals, e.g., lead [46, 47] and a Council Directive on the protection of the health and safety of workers from the risks related to chemical agents at work" [48] suggests establishing biological limit values (in addition to airborne occupational exposure limits) on a European level. The Scientific Committee on Occupational Exposure Limits (SCOEL) now considers Biological Limit Values (BLVs) as part of its work proposing Indicative Occupational Exposure Limit Values (IOELVs). However many EU countries have their own regulatory framework for airborne limits and biological monitoring guidance values (see (http://osha.europa.eu/en/good_practice/topics/dangerous_substances/oel/members.stm).

Germany has probably the most extensive system for applying biological monitoring and developing guidance. Biologische Arbeitsstoff-Toleranz-Werte, (BAT, biological tolerance values) are recommended by a scientific expert group of the Deutsche Forschungs-gemeinschaft (German Research Foundation). They are updated annually and published with the Maximale Arbeitsplatzkonzentrationen (MAK) (maximum workplace concentrations) for occupational exposure [49]. The legal implementations of the BAT values are through their adoption by the Federal Ministry for Employment and Social Affairs as Technical Guidelines. For substances with proven dermal absorption under usual working conditions biological monitoring is mandatory [50].

In the UK biological monitoring can be used as part of the Control of Substances Hazardous to Health (COSHH) regulations for either health surveillance (regulation 11) or exposure assessment (regulation 10). The Health & Safety Executive's framework for biological monitoring suggests it should be used where dermal absorption can give rise to systemic toxicity or where control of exposure relies on personal respiratory protection and air sampling alone will not give a complete guide to exposure. HSE has a preference for non-invasive sampling (e.g., urine) and encourages the use of biological monitoring by occupational hygienists as well as physicians [51]. The approach to reducing exposure to hazardous substances is based on good occupational hygiene practice and many of the biological monitoring guidance values published by HSE [52] are based on the 90^{th} percentile of biological values found in workplace with good control.

In the USA the Federal Occupational Safety and Health Administration (OSHA) produce biological monitoring guidance values for lead, cadmium and chromium as part of the Permissible Exposure Limit (PEL) process. Individual states have the right to issue their own standards as long as they are at least as strong as the PELs. However, the American Conference of Governmental Industrial Hygienists (ACGIH) produces more biological

monitoring guidance values [6]. Although BEIs are not considered to represent a legal standard, the ACGIH as an important professional society of industrial hygienists offers guidance for the interpretation of biological exposure data that is widely accepted. Many countries use the BEIs, in whole or in part.

8.2 International standardization of biomarkers

Biological monitoring guidance values produced by the Deutsche Forschungsgemeinscaft (DFG) and ACGIH numerically dominate those of other organisations and they are used, in whole or in part by many countries. Some countries, like the UK, use the DFG and ACGIH guidance values and also develop their own guidance values.

The approach taken to developing guidance values is based on expert critical review of data available (preferably human) in the peer-reviewed literature. Careful consideration is given to the selection of the appropriate analyte, matrix and sampling time taking into account the metabolism and toxicokinetics of the substance as described previously. The bases for guidance values are usually published [6, 53] and some are published for consultation before being adopted.

Where possible the guidance values are health based. However, for substances like carcinogens, mutagens and sensitizers it may not be possible to propose a health-based guidance value and one based on equivalence to an airborne exposure limit or good occupational hygiene practice may be used instead.

Health-based guidance values require an understanding of the dose-response relationship for the hazardous substance (Figure 8.1).

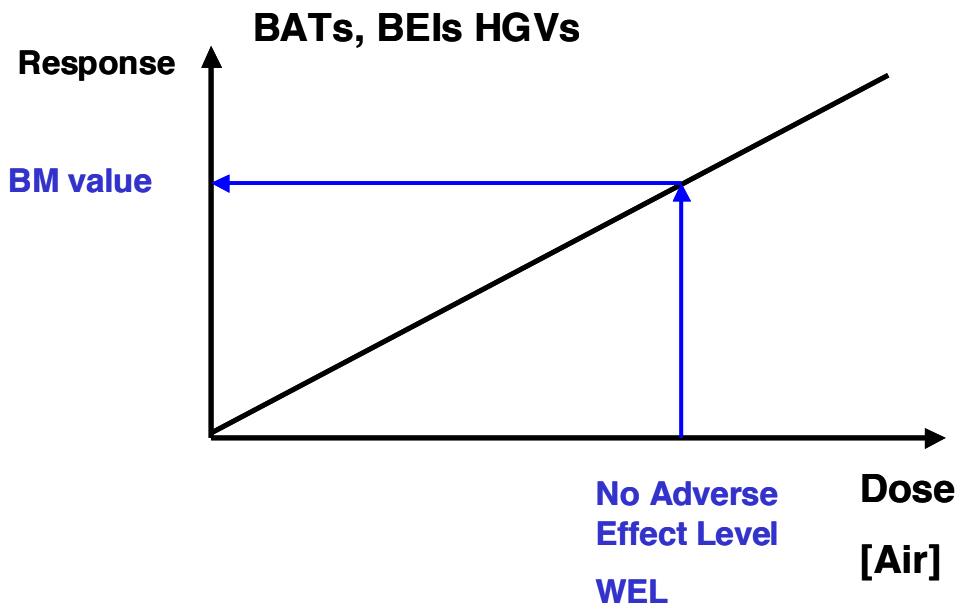


Figure 8.1 Relationship between the dose-response and a biological monitoring guidance value. (BAT, BEI HGV are guidance values produced by DFG, ACGIH and HSE)

As the dose (or airborne concentration) of a substance increases so does the systemic absorption and probability of an adverse effect. If sufficient data are available from volunteer and workplace studies it may be possible to directly determine a biological monitoring guidance value that reflects systemic exposure and is not associated with any adverse effects. An example of this approach is the guidance value for mercury based on inhalation exposures and the absence of neuro-toxic and nephro-toxic effects. More usually the data are insufficient for a direct link and an indirect link is made based on the relationship between a biological monitoring value observed, or predicted, after inhalation exposure at an airborne exposure limit that has been set based on no-adverse-effects. The guidance value is typically the average value that would be found in workers inhaling the substance at the level of the exposure limit for 8h. Examples of this type of value are the ACGIH BEIs and the DFG BATs. Regularly exceeding the guidance value may indicate that an individual's exposure is not well controlled.

Guidance values for substances like genotoxic carcinogens, mutagens and respiratory sensitizers, where a no-adverse-effect level cannot be established, pose a problem. One approach is similar to the health-based approach described above but uses an airborne exposure limit that is not based on health or a no adverse effect level. Examples of this type of guidance value are the Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA) or biological equivalent values published by the DFG. These describe the relationship between inhalation exposure and the level of a substance or its metabolite that might be found in blood or urine. The biological value and the airborne exposure from which it is derive are not health based. The role for this type of guidance value is to aid the control and reduction of exposure.

Another approach to proposing a guidance values that are not health-based is to base the guidance value on the biological values found in workplaces with good occupational hygiene

practice. In the UK the Health & Safety Executive has adopted this approach for carcinogens like hexavalent chromium, 4,4'methylene bis(2-chloroaniline) (MbOCA), methylene dianiline, polyaromatic hydrocarbons, respiratory sensitizers like isocyanates and substances like nitro-glycerine where there is insufficient data to establish a dose-response [52]. The approach involves surveys of workplace exposure and collection of biological monitoring data then simply determining the 90th percentile value of values from those workplaces with good control of exposure.

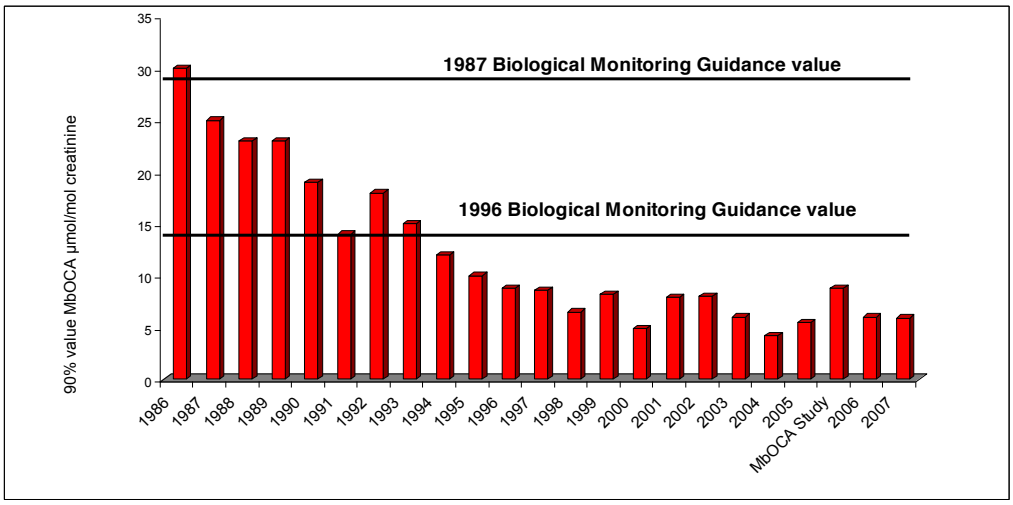


Figure 8.2 The 90th percentile data for urinary MbOCA each year. Each bar is the 90th percentile of between 78 and 1495 samples from 15-27 companies each year.

The guidance value derived is not health-based but serves as an aid to reducing exposure. Exceeding the guidance value should trigger an investigation and improvement of exposure controls. Figure 8.2 shows the gradual reduction in exposure to the aromatic amine MbOCA

This type of guidance values is a pragmatic approach to controlling exposure and works best if the guidance value is periodically revised as exposure controls improve. The approach is also useful for proposing 'in-house' guidance values for substances or situations where there are no external guidance values.

A third approach to biological monitoring guidance values for carcinogens has been used by the ACGIH for polyaromatic hydrocarbons and is effectively based on defining the upper boundary of biological values of 1-hydroxypyrene (1-OHP) found in people not occupationally exposed. This type of guidance value also acts as an aid to reduce occupational exposure.

A fourth approach is the DFG Biologischer Leit Wert (BLW) guidance value. This type of guidance value is proposed where there is concern about occupational exposure to a substance but there is no sufficient data to propose a BAT value. Examples of BLWs are acrylamide, arsenic cadmium, lead, bisphenol A (BPA), cresols, methyl bromide 4,4'-methylene diphenyl diisocyanate and phenol. The BLW is an aid to controlling exposure and also aims to stimulate the collection of more data to aid the setting of a BAT value.

8.3 Proposed changes in MAK and TLVs

In addition to the threshold values listed above, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area and the American Conference of Governmental Industrial Hygienists have decided to establish some new ones which aren't currently listed. For example, a MAK value is to be established for Anthracene [120-12-7], Propionic aldehyde (PAL) [123-386], 2,4-dimethyl phenol [105-67-9], 2,6-dimethyl phenol [576-26-1] and Zinc [7440-66-6] and its compounds [7]. In the U.S., the American Conference of Governmental Industrial Hygienists propose new TWA and STELs for hydrogen sulphide [7783-06-4], Phenyl isocyanate [103-71-9] and for 2,4 - and 2,6–Toluene diamine, Diethylene triamine (DETA) [111-40-0]. A general MAK value shall also be established for the inhalable fraction of dust and the carcinogenic effect and prenatal toxicity shall be examined. Currently, only a STEL value is available for m-Xylene diamine [1477-55-0], but a MAK value is going to be established as well [7] by Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area.

Other investigations are planned regarding investigations of various properties of some substances. The Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area in Germany plans to examine acrylates (monomers and oligomers) for sensitizing effects and Bisphenol A is to be investigated regarding the genotoxic ability to germ cells [7]. Chromium (VI) compounds, all cresol isomers and cumene are to be examined for their carcinogenic potential. A MAK value is going to be examined for Isophorone diisocyanate [4098-71-9] and Maleic anhydride [108-31-6]. A MAK value is going to be established for Manganese and its organic compounds [7439-96-5]. With regard to mercury, (elemental and inorganic mercury compounds) [7439-97-6], a MAK value is going to be established and its prenatal toxic effects examined. Regarding Methyl isocyanate (MIC) [624-83-9], a MAK value is going to be examined and the sensitizing potential of the compounds if going to be examined. Carcinogenic effects from Molybdenum compounds are to be examined. The significance of passive smoking at the workplace is going to be examined for germ cell mutagenicity. For sulphur dioxide [7446-09-5], Toluene [108-88-3] and Styrene [100-42-5], a MAK value is to be examined. Xylene is to be examined for embryo toxic (ability to cause toxic effects of embryos) and/or feto toxic (ability to cause toxic effect on foetuses) effects. The carcinogenic potential of organic Arsenic compounds is going to be examined. The sensitizing potential of glycidyl compounds is going to be examined. Regarding nitrogen oxides, it is going to be questioned whether a common MAK value should be determined. The sensitizing potential of 2,6-Toluenediamine is going to be examined. The sensitizing potential for the latter is also going to be examined.

9 Setting up a biological monitoring program

The **purpose of sampling** may be several, but typically sampling is performed to:

1. Fulfil demands by governmental bodies
2. Check if work procedures are satisfactory
3. Ensure that protection devices are meeting demands
4. Ensure that other personnel at the workplace that are not directly involved, that are not using PPE, are not exposed.
5. Reveal individual exposure
6. Reveal the exposure of a group
7. Get information for epidemiological studies
8. Find out cause of disease

A competent person needs to be responsible for the monitoring in collaboration with an occupational health nurse, an occupational hygienist and a health & safety manager. The input from occupational physician is an advantage for setting up the biological monitoring programme.

The strategy for sampling needs to be decided upon before start-up of sampling. It needs to be evaluated what compounds that need to be monitored. Guidance can be obtained from e.g., from HSE/HSL, ACGIH, DFG, STAMI and experts can be consulted. Depending on the purpose of sampling different strategies may be used. For instance, it may be interesting to follow a few workers over time to see the variation of exposure. Taking samples before and after start-up of work may show the relation of the exposure to work. Sampling before vacation and at the end of the vacation can show the decline in concentration if the exposure is work related.

Aspects regarding ethics and consent need to be identified and discussed. Informed consent with workers and the protection of the rights of the individuals are very important.

Workers need to be informed that the biological monitoring is voluntary and for their benefit, what exposure is being assessed, what sample they will provide, what will & will not be analysed in the samples and when results will be ready and reported. Further, information will be presented on who will have access to the results, what the results mean (exposure) and what will follow up.

The protocol for sampling needs to include certain essential information and procedures necessary for the interpretation of the results.

The sampling is a critical step and the procedure needs to be established for e.g., avoid the contamination of samples and to perform the sampling in a standardised way.

The transportation procedure, to the laboratory, of the samples needs to be outlined. Biological samples may need to be conserved by e.g., acidification or e.g., freezing.

Before transportation the samples may need to be stored in a depository freezer and in the laboratory the samples needs to be stored until analysis.

Before analysis reference samples and/or reference compounds needs to be available.

The choice of analytical method needs to be made. In many cases there is a need for an alternative method to validate the results to minimise the risk of false positive results. Inter laboratory calibrations are of advantages.

It is essential to have means to interpret results. This can be data from the literature (e.g., dose response) or in-house experiences. If means to interpret results are missing it is no meaning to take samples.

All results will have some uncertainty. Uncertainty calculations for all the steps need to be included. In many cases sampling is the most critical step due to the risk of contamination. A typical uncertainty can be expected to be no less than about ± 30%.

Findings from the report may be used for purposes not known when the study started. The means that detailed information needs to be included and the quality of the data need to be described. In addition the report needs a summary containing the major findings and a conclusion that can be understood by the ones who is ordering the study. Essential data on what is measured and what is not measured relevant for the exposure needs to be included.

In many cases one or a few samples does not bring the whole picture. At some stage the results from several reports needs to be compiled. The compilation of results needs to take into account the uncertainty of results from individual included reports.

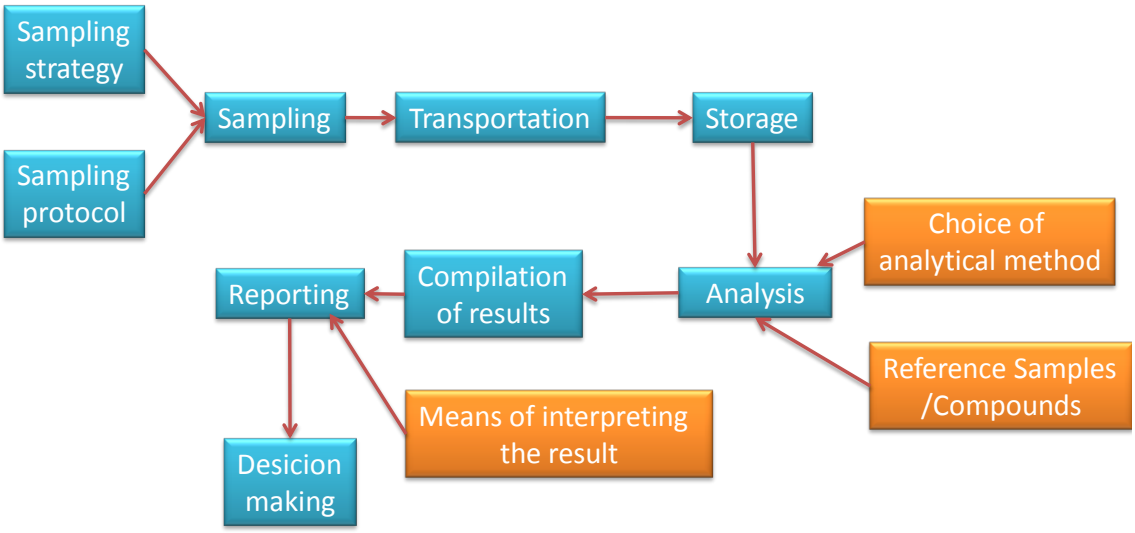


Figure 9.1 Flow chart for the biological monitoring process.

10 A selection of relevant Biomarkers for the petroleum industry

10.1 Introduction

Working in a petroleum industry can result in exposure to a number of various compounds. Biological monitoring can be used to determine the exposure situation in many cases. Compounds have been listed below, that are considered relevant for this industry and to which biomarkers should be defined in order to facilitate biological monitoring of these substances. The list of substances below is based on previous experiences from the petroleum industry.

During hot work, such as welding and thermal cutting of coated metal parts, metals and harmful organic substances are emitted in the form of particles or as gases. During air monitoring on site during hot work, emission of isocyanates, organic anhydrides, aldehydes and amines have been observed [54]. Spray painting of metal structures does also lead to high exposure of these substances on basis of the composition of the paints used [55-57], such as Epoxy [58-60]. Chapter 12 discusses the compounds workers within the petroleum industry may be exposed to due to the use of coatings and polymers in order to prevent corrosion of metal parts, which includes for instance amines and isocyanates. In addition, there are other factors to which the workers within the petroleum industry are particularly prone to be exposed to, such as passive-smoking and leakage of natural gas. Relevant biomarkers for exposure to these parameters are discussed in chapter 13. Workers are frequently exposed to oil mist during drilling, including a number of organic solvents, such as benzene, toluene etc., which are further discussed in chapter 14. In addition, exposure to organophosphate may occur as they are present as additives to hydraulic- and turbine oils. Toxicology and analytical methods regarding organophosphates are discussed in chapter 16.1.

Compounds that have been found relevant for biological monitoring in the petroleum industry on basis of earlier studies have been listed below. The list is complemented with occupational exposure limits (OELs) and biological tolerance values as stated by the American Conference of Governmental Industrial Hygienists (USA), the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (Germany), Arbeidstilsynet (Norway) and Arbetsmiljöverket (Sweden). In Germany, occupational exposure limits are referred to as Maximale Arbeitsplatzkonzentrationen (MAK) and biological tolerance (BAT) value. In addition, the Biologische Leitwert (BLW) is defined as the amount of a chemical substance or its metabolites which serves as an indicator for necessary protective measures [7, 61]. Meanwhile, the Biologischer Arbeitstoff Referenzwert (BAR) refers to the background levels of substances present in a reference population, consisting of individuals that are not thought to be subjected to occupational exposure of the substance [61]. The OELs stated by the American Conference of Governmental Industrial Hygienists are referred to as threshold limit values (TLV) for the ambient air at the workplace and biological exposure indices (BEI) regarding biomarkers in biological matrices [61]. The corresponding OELs for Norway and Sweden are administrative norm (AN) [62] and Nivågränsvärde (NGV) [63] respectively. In Sweden, only biological limits exists for lead and cadmium in blood in terms of limits for when safety precautions needs to be taken [64]. In Norway, there is a biological tolerance value available for mercury and lead. In addition to the set threshold values, suggestions have been made from the American Conference of Governmental Industrial Hygienists and the

Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area regarding new threshold values and other aspects that needs further investigation. Values that are to be changed shortly or substances for which there are no threshold values present at the moment, but new ones are to be investigated, have been marked accordingly.

The OELs are primarily based on epidemiological studies and represent the maximum concentration in air and of the substance that are recommended, to which it is believed that all workers can be exposed during 8 h shifts, 40 h a week without any adverse health effects [7]. However, it should be taken into account that some workers work for more than 40 h during a work week. In some instances a short-term exposure limit (STEL) value is listed either as a complement or instead of OELs. The STEL value refers to a limit which must not be exceeded during a 15 min period of time at any time throughout the day. The corresponding biological limits to the OELs are generally based on the resulting concentration in specified assay material (mostly blood, urine or plasma) in a healthy worker at the threshold limit values in ambient air. In some cases however, such as with lead, the BEI directly related to development of an adverse health affect [7]. The American BEIs are based on exposure of a 70 kg, 170 cm person with a work load of 50 W [7]. It should be noted that due to individual differences, the concentration of a substance or its metabolites in body fluids may vary between individuals. Biomarkers can either be used as a quantitative measure of exposure to a xenobiotic, or just as an indicator of exposure, without information about the concentration of the substance which the worker was exposed to.

It should be noted that protection measures should be based on more than one measurement in a biological media which is above the assigned limit [6, 7] The limit values are based on exposure to a specific compound alone and are not necessarily applicable for the exposure of mixtures of substances [7]. In some cases, threshold limits are also listed for shorted times of exposure.

It has also been noted what biomarker for a specific compound is used when one is available. For many compounds however, there is no assigned limit value due to insufficient information available at this moment and thus neither a biological tolerance value. Generally, more information is available for metals and solvents. For many organic compounds emitted through previously mentioned working operations, data is missing. Both in Germany and USA, the commission responsible for the setting of these recommended thresholds do in some cases plan to lead further investigations in order to provide more data.

10.2 Tables of compounds as interested for the petroleum industry

CAS = Chemical Abstract Service-the largest collection of substance information - as well as indexed references from more than 10,000 major scientific journals and 60 patent authorities around the world [65]. The information in the tables is based from various databases from Swedish Work Environment Authority and its counterpart in Norway Note! If data not found, the column is empty.

Table 10.1 Metals

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
arsenic	As	7440-38-2	10			10	35 µg/L	50 µg/L	Inorganic Arsenic and methylated metabolites	urine	End of workweek
beryllium	Be	7440-41-7	2* (10)	1	1	2			beryllium	urine	
cadmium	Cd	7440-43-9	10		20 (cadmium oxide)	20	5 µg/g creatinine or 5 µg/L in urine	BLW: 7 µg/L urine			Not fixed
calcium	Ca	7440-41-7			2 000 (calcium oxide)	20 (dust), 5 (respirable fraction)					
chromium	Cr(III)	7440-47-3	500		500					urine	
	Cr(VI)		50 (water-soluble compounds), 10 (insoluble compounds)			5	25 µg/L				End of shift at end of workweek
							10 µg/L				Increase during shift
cobalt	Co	7440-48-4	20		20	50	15 µg/L urine				End of shift at end of workweek

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH

Table 10.2 Metals (cont.)

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
copper	Cu	7440-50-8	200 (fume), 1 000 (dust and mist)	100 (as the inhalable fraction of the aerosol)	1 000 (dust), 100 (fumes)	1000 (dust), 2 (respirable fraction)					
iron	Fe	1309-37-1				3 500					
lead	Pb	7439-92-1	50		50	100 (dust), 50 (respirable fraction)	30 µg/L	BLW: 400 µg/L blood, 100 µg/L blood for women <yrs	Lead		Not fixed
mercury	Hg	7439-97-6	10 (alkyls), 100 (aryls), 25 (elemental and inorganic)	100**	20 (biological tolerance value: 30 µg/g creatinine)	10 (organic species) 30 (inorganic		25 µg/g creatinine	Total inorganic mercury	urine	Not fixed
manganese	Mn	7439-96-5	200	500**	100	200 (dust), 100 (respirable fraction)		20 µg/L			End of exposure or end of shift. For long-term exposures: after several shifts

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established

Table 10.3 Metals (cont.)

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
molybdenum	Mo	7439-98-7	500 (soluble compounds), 10 000 (metal and insoluble compounds)		5 000 (solvable compounds) 10 000 (unsolvable compounds)	5000 (solvable compound) 10 000 (insolvable compound, dust), 5 000 (solvable compound, respirable fraction)					
nickel	Ni	7440-02-0	1 500 (elemental), 100 (soluble inorganic compounds), 200 (insoluble inorganic compounds), 100 (nickel sub sulphide)		50	500					
vanadium	V	7440-62-2			200 (dust), 50 (fumes)						
titanium	TiO_2	13463-67-7	10 000		5 000	5 000					
zinc	Zn			**							

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established

Table 10.4 Isocyanates

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
isocyanic acid	ICA	75-13-8				18 (36)					
4,4'-methylene-diphenyldi-isocyanate	MDI	101-68-8	52	50 (100)	12	24 (47)	10 µg/L		4,4'-Diamino-diphenylmethane	urine	End of exposure or end of shift
1,6-hexamethylene diisocyanate	HDI	822-06-0	35	35 (70)	35	20 (30)			1,6-Hexa-methylenediamine		
2,4-toluene diisocyanate	2,4-TDI	584-84-9	36 (145)***			14 (40)			2,4-Toluene diamine		
2,6-toluene diisocyanate	2,6-TDI	91-08-7							2,6-Toluene diamine		
isophorone diisocyanate	IPDI	4098-71-9	46	46 (92)	45	18 (46)			Isophorone diamine		
phenyl isocyanate	PhI	103-71-9		**		20 (50)					
methyl isocyanate	MIC	624-83-9	47	24**** (24)							
propyl isocyanate	PIC	110-78-1			18						
naphthalene diisocyanate	NDI	3173-72-6			40	17 (44)					

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Note that the U.K. has a biological monitoring guidance value (BMGV) for HDI, TDI, IPDI and MDI of 1 µmol isocyanate-derived diamine/mol creatinine.

Table 10.5 Isocyanates (cont. 1)

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
dicyclohexylmethane diisocyanate	HMDI	5124-30-1			50						
ethyl isocyanate	EIC	109-90-0			15						
HDI-biuret											
HDI-isocyanurat											
HDI-uretidon isocyanurat											
HDI-diisocyanurat											
triglycidylisocyanurate	TGIC										
TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)											
*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.											

Table 10.6 Organic anhydrides

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
phtalic anhydride	PA	85-44-9	6 100			2 000 (3 000)					
maleic anhydride	MA	108-31-6	410	410*****		1 200					
Tetra hydro phtalic anhydride		85-43-8									
cis-hexahydro-phtalic anhydride		85-42-7	5								
TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)											
*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.											

Table 10.7 Aldehydes

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
propionic aldehyde	PAL	123-38-6	**								
acetic aldehyde	AAL	75-07-0	(45 700)	91 000 (180 000)	91 000	91 000					
formaldehyde	FA	50-00-0		370 (1 200)	600 (1 200)	600 (1 200)					
TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)											
*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.											

Table 10.8 Amines

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
diethylene triamine	DETA	111-40-0	4 300**		4 000	4 500					
triethylenetetramine	TETA	112-24-3			6 000	6 000					
2,4,6-tri(dimethylamino-methyl)phenol	TEPA	90-72-2									
m-xylene α,α diamine	XAD	1477-55-0	(100)	**	(100)						
Isophorone diamine	IPDA	2855-13-2									
1,2-cyclohexandiamine		694-83-7									
n-amino ethyl piperazine		140-31-8									
Benzyl dimethyl amine		103-83-3									
3-dimethylaminopropylamine	DMAPA	109-55-7									
N, N - diethyl - 1,3 – diamine propane		104-78-9									

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Note that the U.K. has a biological monitoring guidance value (BMGV) for MDA of 50 µmol total-MDA / mol creatinine

Table 10.9 Amines (cont.)

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
4,4'-methylene-dianiline	MDA	101-77-9	820		800			10 µg/L	4,4'-Methylene-dianiline	urine	End of exposure or end of shift
Tetra ethylene pentamine	TEPA	112-57-2									
2,4-toluene diamine	2,4-TDA		******								
2,4-toluene diamine	2,4-TDA		******								
hexamethylene triamine		100-97-0			3 000	3 000					
naphthalene diamine	NDA										
methylene-di(cyclo hexylamine)	HDMA	7560-83-0									
4,4'- methylene bis(2-chloroaniline)	MbOCA	101-68-8									
ethylene diamine		107-15-3	25 000		25 000	25 000					

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Table 10.10 Compounds related to Epoxy

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
cycloaliphatic epoxy resin		5493-45-8									
Phenyl glycidylether	PGE	122-60-1	5		5	60					
Cresyl glycidylether		26447-14-3									
glycidylether of C13- C15 alcohols/epoxide		26761-45-5									
2,4,6- tri(dimethylamino-methyl)phenol		90-72-2									
2,2,4-trimethylhexamethylenediamine		25620-58-0									
epoxy 15/Epoxy resin/-bisphenol A diglycidylether	DGEBA	25068-38-5									
bisphenol F diglycidylether	DGEBF	54208-63-8									
4,4'-methylenediphenol diglycidylether		2095-03-6									
bisphenol F diglycidylether dimer		39817-09-9									

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Table 10.11 Compounds related to acrylates

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
methacrylic acid	MA	79-41-4	71	18	70	70 (100)					
acrylic acid	MMA	80-62-6	6	30	100	200 (600)					
TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)											
*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.											

Table 10.12 Compounds related to acrylates (cont.)

Compound	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
		TLV	MAK	AN	NGV	BEI	BAT			
4,4'-methylene-diphenol diglycidyl-ether	2095-03-6									
bisphenol F diglycidylether dimer	39817-09-9									
phenol	108-95-2	20		4	4	250 mg/g creatinine	BLW: 200 mg/g creatinine	Phenol	urine	End of exposure of end of shift
o-cresol	95-48-7	22 *******			4.5					
p-cresol	106-44-5									
2,4-dimethyl phenol	105-67-9	**								
2,6-dimethyl phenol	576-26-1	**								
trimethyl phenol	26998-80-1									
2-ethyl phenol	90-00-6									
4-ethyl phenol	123-07-9									

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Table 10.13 Compounds related to acrylates (cont.)

Compound	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
		TLV	MAK	AN	NGV	BEI	BAT			
2-isopropyl phenol	88-69-7									
4-isopropyl phenol	99-89-8									
2-allyl phenol	1745-81-9									
bisphenol A	80-05-7		5				BLW: 80 mg/L	Bisphenol A, released after hydrolysis	urine	End of exposure or end of shift
bis phenol F: (2,2', 2,4', 4,4')	2468-02-9									
	2467-03-0									
	620-92-8									
biphenyl	92-52-4	1.3		1	1.3					

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Table 10.14 Other non-metal and organic compounds

Compound	Abbreviation	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
			TLV	MAK	AN	NGV	BEI	BAT			
nitrogen oxide	NO	10102-43-9	31		30	30					
nitrogen dioxide	NO_2	10102-44-0	5.7 (9.5)***		1.1	5 (10)					
sulphur dioxide	SO_2	7446-09-05	5.3 (13)	1.3 ****							
hydrogen sulphide	H_2S	7783-06-4		7.1	15	14 (20)					
TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)											
*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.											

Table 10.15 Other environmental factors

Other environmental factors	Biomarker	Sampling time for biological monitoring
tobacco smoke	Cotinine in urine [1]	
indicator of mental stress	Cortisol	
oxidative stress/ free radicals	8-OHdG	

Table 10.16 Solvents in Oil mist/metal working fluid

Compound	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
		TLV	MAK	AN	NGV	BEI	BAT			
benzene	71-43-2	1.6 (8)		1.5 (3)	3 (9)	25 µg/g creatinine		S-phenylmercapturic acid	urine	End of shift
						500 µg/g creatinine		T,t-muconic acid	urine	End of shift
								Benzene	blood	End of shift
toluene	108-88-3	76	190**** (380)	94	200 (400)	0.5 mg/L	3.0 mg/L	o-cresol	urine	End of exposure of end of shift. For long-term exposures: after several shifts.
						1.6 g/g creatinine		Hippuric acid	urine	End of shift
						0.05 mg/L	1.0 mg/L	Toluene	blood	Prior to last shift of workweek or end of exposure of end of shift
styrene	100-42-5	86 (173)	86 (172)	105	90 (200)			T,t-muconic acid		
						400 mg/g creatinine	600 mg/g creatinine	Mandelic acid plus phenyl glyoxylic acid	urine	End of exposure of end of shift. For long-term exposures: after several shifts.
						0.2 mg/L		Styrene	blood	End of shift.

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Table 10.17 Solvents in Oil mist/metal working fluid (cont.)

Compound	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
		TLV	MAK	AN	NGV	BEI	BAT			
ethyl benzene	100-41-4	440 (550)		20	200 (450)	0.7 g/g creatinine		Mandelic acid and phenyl glyoxylic acid	urine	
								2- and 4- Ethyl phenol	urine	
								Ethyl benzene	exhaled air	Not fixed.
glycol ethers								Phenotoxic acetic acid and methoxy propionic acid		
xylene	1330-30-7	440	440 (660)	200	108 000			Xylene	blood	End of exposure of end of shift.

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Table 10.18 Polyaromatic hydrocarbons

Compound	CAS	Occupational exposure limits (short term exposure limit) ($\mu g/m^3$)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
		TLV	MAK	AN	NGV	BEI	BAT			
anthracene oil	65996-91-0							1-Hydroxypyrene	urine	End of shift at end of workweek
benzo[a]pyrene	50-32-8				2 (20)					
naphthalene	91-20-3	53 000 (80 000)		50 000	50 000 (80 000)					
PAHs				40						
pyrene	129-00-0									
cumene	98-82-8	150 000	250 000 (500 000)	125 000						

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

Table 10.19 Chlorinated hydrocarbons

Compound	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
		TLV	MAK	AN	NGV	BEI	BAT			
methyl chloride	74-87-3	105 (210)	100 (200)	50	20 (40)					
methylene chloride	75-09-2	180		50	120 (250)	0.3 mg/L		Methylene chloride	urine	End of shift
carbon tetrachloride	56-23-5	32 (64)	3.2 (6.4)	13						
chloroform	67-66-3	50	2.5 (5)	10	10 (25)					
1-chloroethane	75-00-3	270		270	1 300 (1 900)					
1,1-dichloroethane	75-34-3	410	410 (820)	200	412					
1,1,1- trichloroethane	71-55-6	2 000 (2 500)	1 100 (2 200)	270	300 (500)					
TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)										
*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.										

Table 10.20 Chlorinated hydrocarbons (cont.)

Compound	CAS	Occupational exposure limits (short term exposure limit) (mg/m³)				Biological limit value		Biomarker	Biological fluid	Sampling time for biological monitoring
		TLV	MAK	AN	NGV	BEI	BAT			
1,1,2-trichloroethane	79-00-5	55	55	54						
1,1,2,2-tetrachloroethane	79-34-5	7	7 (14)	7						
pentachloroethane	76-01-7		42 (84)	40						
hexachloroethane	67-72-1	9.8	9.8 (19.6)	10						
vinyl chloride	75-01-4	2.6		3	2.5 (5)					
1,1-dichloroethylene	75-35-4	20	8 (16)	4	20 (40)					
1,2-dichloroethylene	540-59-0	800	800 (1 600)	395						
trichloroethylene	79-01-6	54 (140)		50	50 (140)	15 mg/L		trichloroacetic acid	urine	End of shift
Perchloro ethylene	127-18-4	170 (690)		40	70 (170)	5ppm		Perchloro-ethylene	alveolar air	Prior to last shift of the week

TLV = Threshold limit value (US), MAK = Maximum Concentration (Germany), AN = Administrative Norm (Norway), NGV = Nivågränsvärde, BEI = Biological exposure indices (US), BAT = Biological tolerance value (Germany), BLW = Biologische Leitwert (Germany)

*Another threshold limit value is proposed by the ACGIH **A MAK value is going to be established ***New TWA and STEL values are suggested. ****The MAK value is going to be examined. *****A MAK value will be examined. ******TWA and STEL values will be suggested shortly. *******The TWA value will be changed soon.

10.3 Biomarkers available as routine analyses today

In Sweden and Norway, routine analyses of biomarkers are limited (listed below). In addition to laboratories in Sweden and Norway, routine analyses performed by HSL in the UK are presented. The list of substances is based on the pricelists from each laboratory available on the website. Out of the routine analyses available, most are analyses of metals and solvents. Some routine analyses are also available of isocyanates - and then of course also of their corresponding amines. Routine analysis of cotinine as a biomarker for nicotine and organic acid anhydrides do also exist.

In some cases, instead of specifying a particular compound, a group of substances is mentioned as available for routine analyses by a laboratory. In such case, of course not all substances within that group are available for analysis. The choice of method for analysis of each biomarker have not been listen since in many instances, there if no information about the methods in most pricelists. Note that only substances relevant, according to the list above, to the petroleum industry have been listed here. There are some additional substances available for routine analyses of biomarkers as well, such as several pesticides, nitrous oxygen etc., but these have been left out due to the lack of relevance.

In summation, the routine analyses of biomarkers that are available on the market today is incomplete, especially when it comes to organic substances, such as isocyanates, amines, organic anhydrides and other organic compounds present in Epoxy.

Laboratories in U.K., Norway and Sweden that perform analysis on biomarkers:

1 = HSL Biological monitoring services, U.K.

2 = Department of Occupational and Environmental Medicine in Lund, Sweden

3 = Institutet för Kemisk Analys Norden in Hässleholm, Sweden

4 = Sahlgrenska University Hospital in Gothenburg, Sweden

5 = ALS Laboratory Group in Stockholm Sweden

6 = Analyslaboratoriet Arbets- och miljömedicinska kliniken in Örebro, Sweden

7 = Landstinget i Östergötland, Sweden

8 = The national institute of occupational health, STAMI, Norway

9 = Sykehuset i Telemark, Norway

10 = St. Olavs Hospital, Trondheim University Hospital, Norway

In the following tables it is shown what kind of analyses different laboratories can perform on various chemical man may be exposed to.

Metal	Assay material	Laboratory									
		1	2	3	4	5	6	7	8	9	10
aluminium	urine	X				X					
	blood					X					
	serum				X	X					
arsenic	urine					X					X
	blood					X					X
	serum					X					
beryllium	urine	X									X
inorganic arsenic	urine	X							X		X
cadmium	urine	X	X		X	X		X	X		X
	blood	X	X		X	X	X	X	X		X
	serum					X					
cobalt	urine	X				X					X
	blood					X	X				X
	serum					X					X
chromium	urine	X				X		X			X
	blood	X				X					X
	serum					X					X
copper	urine	X			X	X			X		X
	blood					X			X		X
	serum				X	X					X
mercury	urine	X	X		X	X			X		X
	blood	X	X		X	X			X		X
	serum					X					X
	plasma		X								X
manganese	urine	X	X			X			X		X
	blood	X				X			X		X
	serum					X					X

Metal	Assay material	Laboratory									
		1	2	3	4	5	6	7	8	9	10
nickel	urine	X	X			X		X			**X**
	blood					X					
	serum					X					
lead	urine	X			X	X					**X**
	blood		X		X	X	X	X	X		**X**
	serum					X					
vanadium	urine	X									**X**
zinc	urine	X			X	X					**X**
	blood	X				X					**X**
	serum				X	X					**X**

Compound	Abbreviation	Biomarker	Assay material	Laboratory								
				1	2	3	4	5	6	7	8	9
amines			urine	X	X	X						
			blood			X						
			serum			X						
epoxy resin hardeners		m-XDA	urine	X		X						
formaldehyde	FA	formic acid/formate	urine	X								
hydrogen sulphide		thiosulphate	urine	X								
isocyanates		corresponding amine	urine	X	X	X						
			blood			X						
			serum		X							
		isocyanate specific	serum		X							

	IgE antibodies										
4,4'-methylene bis(2-chloroaniline)	MbOCA		urine		X		X				
methylene dianiline	MDA	MDA	urine		X						
nicotine		cotinine	urine		X	X	X				X
			saliva		X						
organic anhydrides		metabolites of organic anhydrides	urine				X				
		organic anhydride specific IgE antibodies	serum			X					
organophosphate pesticides			urine		X						

Solvent	Biomarker	Assay material	Laboratory								
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
benzene	S-phenylmercapturic acid	urine	X		X						X
dichloromethane	carbon monoxide	exhaled air	X								
	dichloromethane	urine	X								
ethyl benzene and/or Styrene	mandelic acid	urine	X	X					X		
	mandelic acid plus phenyl glyoxylic acid	urine				X					
		exhaled air									X
naphthalene	naphtols	urine	X								
1,1,1-trichloroethane	methyl chloroform	blood	X								
	1,1,1-trichloroethane	exhaled air	X								
trichloroethylene	methyl chloroform	blood	X								
	trichloro acetic acid	urine	X					X	X	X	

PAHs	1-hydroxypyrene	urine	X		
solvents, single or multiple		urine	X		
		blood	X		
		exhaled air	X		
toluene	o-cresol	urine	X		
		exhaled air	X		X
VOCs (alkanes, aromatics, ketones/aldehydes, alcohols, common acetates, acrylates, PAHs, PCB)		exhaled air			X
xylene	methylhippuric acid	urine	X		

HSL in UK analyses around 8 000 – 10 000 samples a year. Out of these analyses, about 3 000 are for lead. The analyses are carried out on behalf by Occupational health providers, hygienists, hospitals and H & S staff. The most common analyses carried out by HSL are listed in Table 10.21.

Table 10.21 The most common biomarkers analysed during 2007 at HSL Biological monitoring services (Crown Copyright 2009). Guidance values: Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA), Biological Monitoring Guidance value in UK (BMGV), Control of Lead at Work in UK (CLAW), medical guidance note No 17 in UK (MS17), Biological Exposure indices (BEI), Biologische Arbeitsstoff Toleranz Werte (BAT), Biologischer Leit Wert (BLW).

Rank Order	Analyte	Guidance
1	Isocyanates	**BMGV**
2	Blood Lead	**CLAW**
3	PAHs	**BMGV**
4	Benzene	**BEI/BAT**
5	Drugs of Abuse	
6	Chromium	**BMGV**
7	MDA	**BMGV**
8	Nickel	**EKA**
9	Arsenic	**BEI/EKA**
10	Cholinesterase	**MS17**
11	Furoic Acid	**BEI**
12	Mercury	**BMGV**
13	Cobalt	**EKA**
14	Thiocyanate	**HSL Data**
15	MbOCA	**BMGV**
16	Styrene/Ethylbenzene	**BEI/BAT**
17	Cadmium	**BEI/BLW**
18	Naphtols	**HSL Data**
19	Blood Cadmium	**BEI**
20	Antimony	**EKA**

11 Metals

11.1 Introduction

Occupational exposure to metals is a common issue within the industry. Exposure to heavy metals is of special concern, since it affects the central nervous system, the blood composition, lungs, kidneys, liver and other vital organs [66]. In addition, exposure may induce allergies or even cancer. Exposure to heavy metals during a long period of time may cause symptoms similar to Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis [66]. Out of the metals selected as relevant biomarkers within the petroleum industry, which were presented in chapter 10, arsenic, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, nickel, vanadium and zinc are classified as heavy metals.

Due to the previously experienced problems with metal exposure, a number of methods have been developed for biological monitoring of metals as well as routine analyses. Both qualitative and quantitative analysis can be conducted according to some basis instrumentation principles, see Appendix. Both qualitative and quantitative analyses are preferable conducted today with ICP-MS or high resolution ICP-MS.

11.2 Metal administration/Exposure

Airborne metals occur most frequently as gas and metal particles. They can be inhaled into the respiratory tract causing adverse health effects both to the lungs and other systems to which the metals are transported. Recently new insights on the particle size distribution have to be taken account for e.g., ultrafine particles including nano-particles. The deposition of ultrafine particles in the lung is now a new research area of interest [67].

11.3 Sample preparation

For biological samples, such as blood and urine, there exist several methods but the method of choice is microwave digestion which is highly recommended, ref
Biological and organic samples are generally digested using a closed-vessel microwave digestion procedure that is appropriate to the matrix of the sample. The high temperature and pressures reached in the microwave digestion system can dissolve most biological samples, including fatty tissues. This is also the best method for digesting organic samples, including crude oils [68].

When performing elemental analysis in urine, the value obtained must be corrected for the creatinine. Very diluted or undiluted urine samples cannot be used even though the biological limit value is based on the relationship to the mass of creatinine present in the urine. Creatinine is a product or creatinine phosphate in skeletal muscles and is excreted in a constant rate throughout the day.

For example: $\frac{3.6\ \mu g / L\ Cadmium}{1.8\ g / L\ creatinine} =$ 2.0 µg Cd / g creatinine

11.4 Metal analysis

11.4.1 Arsenic (As)

11.4.1.1 Biological relevance

Several epidemiological studies have shown that, long-term occupational exposure to in-organic arsenic can increase the risks of cancer in the skin, bladder and kidney. The toxicity and carcinogenicity of arsenic depend on its species [69]. Arsenic can occur in an in-organic form or in an organic form. In-organic arsenic is methylated in mammals into monomethyl arsenic (MMA), dimethyl arsenic (DMA) and arsenobetaine (AsB). DMA or its derivatives are believed to be the most active carcinogens.

In humans, chronic arsenic ingestion is strongly associated with an increased risk of skin cancer, and may cause cancers of the lung, liver, bladder, kidney, and colon; chronic inhalation of arsenicals has been closely linked with lung cancer. The precise mechanism of arsenic-related carcinogenicity is unknown. Arsenic does not induce genetic mutations in most test systems, but chromosomal damage has been reported in cultured mammalian cells, possibly as a result of arsenic's effects on the enzymes involved in DNA replication and repair. Paradoxically, cancer associated with arsenic exposure has not been observed in experimental animal studies [70].

As toxicity, mobility, and bioavailability can differ greatly between the various chemical species in which an element occurs, reporting only the total concentrations can often be misleading. Arsenic is one such example where the various species differ; the inorganic trivalent form (As III) is the most toxic, followed by the inorganic pentavalent form (As V). Other common forms of arsenic include monomethyl arsenic (MMA), dimethyl arsenic (DMA) and arsenobetaine (AsB), which have significantly reduced toxicities (**Error! Reference source not found.**). Therefore, separation and detection of these species can greatly assist risk-based toxicity assessments.

11.4.1.2 The metabolic pathway of As V (arsenate)

Both inorganic and organic forms leave your body in your urine. Most of the inorganic arsenic will be gone within several days, although some will remain in your body for several months or even longer. Measurement of arsenic in urine is the most reliable means of detecting arsenic exposures that you experienced within the last several days. Most tests measure the total amount of arsenic present in your urine. This can sometimes be misleading, because the non-harmful forms of arsenic in fish and shellfish food can give a high reading even if one have not been exposed to a toxic form of arsenic. For this reason, laboratories sometimes use a more complicated test to separate “fish arsenic” from other forms. Because most arsenic leaves your body within a few days, analysis of your urine cannot detect if you were exposed to arsenic in the past. Tests of your hair or fingernails can tell if you were exposed to high levels over the past 6–12 months, but these tests are not very useful in detecting low-level exposures.

11.4.1.3 Analytical method

A study to determine analysis of arsenic in urine has been conducted, 2007, of Japanese subjects [69]. The method used was HPLC-ICP-MS.

When Liquid Chromatography (LC) is interfaced with Inductively Coupled Plasma Mass Spectrometry (ICP-MS), species elute one by one from the LC column directly to the ICP-MS

for detection by elemental speciation. The coupling of LC to ICP-MS is a straight forward task; the LC column is connected to the nebuliser of the ICP-MS by a piece of polyetheretherketone (PEEK) tubing, and no hardware changes are required to either the LC or the ICP-MS. Coupling an LC to a Varian ICP-MS (Table 11.1) has the added advantage of offering high sensitivity, with over 80% of the analyte ions passing through the skimmer cone being transferred to the mass spectrometer [71].

Table 11.1 Typical detection of limits values from Varian (ICP-MS) of arsenic compounds

Arsenic species	Detection limit (ng/L)
Arsenobetaine	2.2
Arsinite	3.9
Dimethyl arsenic acid	4.4
Monosodium acid methane arsenate	2.6
Arsenate	2.3

To certify the method the National Institute for Environmental studies (NIES) no 18 for human urine was used. The results were within the allowed errors for certified values, which mean that the method can be used for the determination of as of humans in urine. The American Conference of Governmental Industrial Hygienists (ACGIH) and Deutsche Forschungsgemeinscaft (DFG) use the urinary excretion of the sum of in-organic As, MMA and DMA for biological monitoring of occupational exposure to in-organic As.

A typical separation is shown in Figure 11.1.

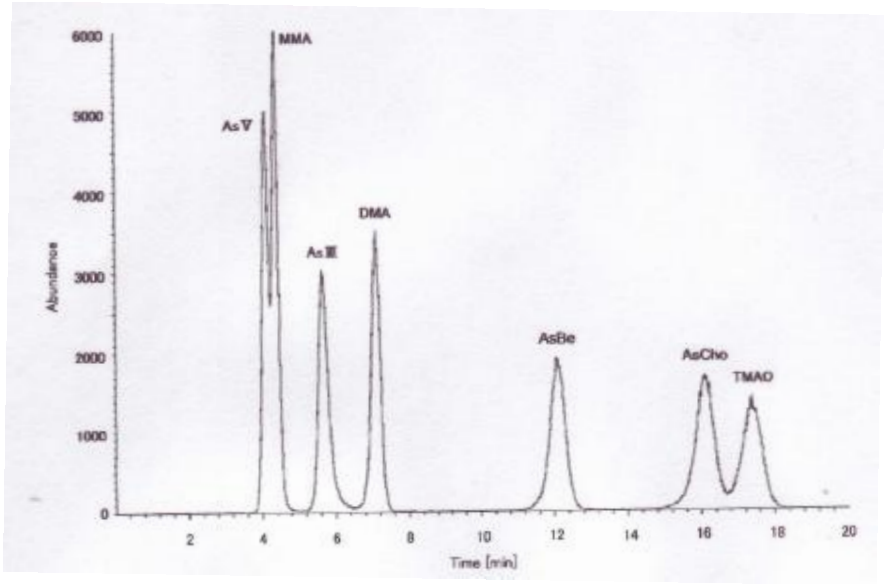


Figure 11.1 HPLC-ICP-MS chromatogram of standard solutions containing seven arsenic compounds (10µg As / L). As III (arsinite), As V (arsenate), MMA (monomethyl arsonic acid), DMA (dimethylarsenic acid, AsBe (arsenobetaine, AsCho (arsenocholine) and TMAO (thrimethylarsine oxide. From reference [69] with permission from J. Occup. Health

11.4.1.4 Concluding remarks

HPLC-ICP-MS seems to be method of choice in the analysis of arsenic compounds both as in-organic- and as organic species and is preferential on urine samples. The work referring to above seems to be the latest work performed in this area. The work also demonstrates all the requirements against established certification of analysing As compounds from human.

The biological limit value is 50 µg/L [7] or 35 µg/L [6] in urine in Germany and the U.S., respectively for the total As and the detection limit of the ICP-MS instrument is in the order of <10 ng/L.

11.4.2 Beryllium (Be)

11.4.2.1 Biological relevance

Chronic beryllium disease (CBD) is a potentially debilitating and fatal lung disease [72]. It is a significant occupational health concern in the manufacturing and processing of beryllium-containing materials. Previous medical and epidemiological data collected from workers at nuclear weapons facilities and beryllium manufacturing facilities have reported facility prevalence rates of beryllium sensitisation as measured in the blood. These studies have raised several key issues, including whether CBD can result from exposures of less than the occupational exposure limit (OEL) of 2 µg/m^3 in the U.S., whether a dose-response relationship exists between total airborne beryllium concentration and CBD, and whether the chemical form or particle size of beryllium is more predictive of CBD risk than total air mass concentration is [73, 74].

The danger of beryllium is its sensitization of the airways and the skin, e.g., an immune system response to beryllium exposure. A person can develop beryllium sensitivity soon after exposure or years later (late adverse reaction). Not all people who are sensitized will develop a beryllium related disease (late adverse reaction). The ACGIH have proposed reducing the TLV for Beryllium to 0.05 µg/m^3.

11.4.2.2 Analytical method(s)

Methods used for the analysis in biological materials are reported [75] Several methods (11) of Be in urine are published, but out of date the newest found from 1999, however the method of choice is ICP-MS.

Traditionally, trace-level analysis of beryllium by ICP-MS has been difficult. However, developments in the technology now allow detection limits in routine analysis in the sub ppt level. Long term precision and accuracy data recorded over 8 hours of continuous analysis of a highly diluted certified reference material (NIST 1640) is also presented. Recoveries were greater than 97% and precision was in the order of 1-2%. The detection limit was found to be 0.052 ng/L in urine.

11.4.2.3 Concluding remarks

No recent publication utilising ICP-MS for biological samples on beryllium is found, thus there is a need to develop new methods with using modern equipment. HSL has developed a method for beryllium in urine and is collecting data from occupationally exposed workers and controls.

11.4.3 Cadmium (Cd)

11.4.3.1 Biological relevance

Exposure to cadmium may cause kidney damage. The cumulative exposure to cadmium and its concentration in the kidneys can be assessed by measuring cadmium in urine. All studies of exposure to cadmium and tubular dysfunction have been cross sectional. The tubular damage may have occurred a long time before the study was performed. If the concentration of urinary cadmium at the time of onset of tubular damage was higher, the dose-response curve would shift to the left in subsequent studies. This problem, with a variable shape of the dose-response curve, is probably less relevant for environmentally exposed people, who have a continuous relatively low level of exposure compared with workers who have experienced high exposure to airborne cadmium in the past. It is, therefore, particularly noteworthy that tubular proteinuria occurs at relatively low concentrations of cadmium in urine and in the environmentally exposed group, in which the tubular damage appears at even lower concentrations than in the occupationally exposed group [76]. Proteinuria refers to an excess of serum proteins present in urine.

Cadmium is a normally occurring environmental pollutant. Cadmium accumulates in the kidney cortex and causes end-stage renal disease. The metal has been found carcinogenic in both humans and animals and can be related to renal and lung cancer. Cadmium may also be involved in transitional cell carcinoma of the bladder. Biopsies of liver, kidney, bladder, blood, serum and urine have been made in order to assess the association of Cadmium to cancer.

Cadmium can be transported and metabolised in different ways in the body. Metallothioneins (MTs) are low molecular, cystein-rich proteins that bind metals such as zinc, copper, mercury, lead and Cadmium [77]. Cadmium can be metabolised in different ways in the body and may induce production of MTs. Cadmium is excreted through the kidneys with a very slow excretion rate with an elimination half-life of 10-40 years. Thus, most Cadmium excreted through the urinary system is probably in the form of a Cd-MT complex.

11.4.3.2 Analytical methods

For the urine analysis of the Cd-MT complex a combination of size exclusion chromatography and ICP-MS is used. The limit of determination (LOQ), calculated with the 6^{th} criterion and the highest value of the limit of detection (LOD) range of Cd, was calculated to be 0.46 µg/L.

For the urinary Cd-containing fraction the BLW is 7 µg/L in urine in Germany [69].

Another published method [78] utilisation solid phase extraction (SPE) purification before the ICP-MS experiments. The reason for this is the interference of molybdenum (Mo) which is an essential element for nutrition. Limit of quantification (LOQ) was 0.012 mg Cd/ L = 12 µg/L which is > than the MAK value. However, utilising a new chelating agent (poly (hydroxy-methacrylate)-based porous resin) which successfully minimized the amount of interfering molybdenum lowered the detection limit for cadmium to 0.05 µg/L.

In a new publication [79] it was possible to eliminate the interference of molybdenum oxide in urine samples. The method is based on inductive coupled plasma dynamic reaction cell mass spectrometry, ICP-DRC-MS.

11.4.3.3 Concluding remarks

The method to analyse Cd in biological samples is new (2008) but has not yet been proven for routine samples.

11.4.4 Chromium (Cr)

11.4.4.1 Biological relevance

Chrome (Cr) exists in two oxidation states, Cr^{III} and Cr^{VI}. Cr^{III} has limited toxic effect [80]. Both Cr^{III} and Cr^{VI} are environmentally stable, and their toxicological profiles are well known. Epidemiologic studies have consistently shown that human exposure to Cr^{VI} compounds (chromates) are potent carcinogens in animal, as well as mutagens in bacterial and mammalian cell-based mutagenicity assays [81].

The respiratory tract is the main target organ of Cr^{VI} toxicity associated with both acute (short-term) and chronic (long-term) inhalation. Chronic exposure leads to ulcerations and perforations of the nasal septum, chronic bronchitis, decreased pulmonary function, pneumonia, and other respiratory effects. On the basis of experimental and epidemiologic evidence Cr^{VI} has been classified as a *class 1 carcinogen* (recognised human carcinogen).

Therefore, the respiratory tract is the primary target organ for Cr^{VI} compounds. Chromate, Cr^{VI}, is iso-structural with phosphate and sulphate ions, which is the basis for its rapid transport into cells and penetration into many tissues. Intracellularly, Cr^{VI} undergoes reductive metabolism, ultimately forming Cr^{III}. Because of the extremely tight binding of Cr (III) by intracellular macromolecules, a significant portion of Cr^{III} becomes trapped inside the cell for long periods of time. Cr^{III}-mediated cross-links of DNA with peptide glutathione or single amino acids were mutagenic in human cells, with a relationship of higher molecular weight of the peptide/amino acid correlating with a more potent mutagenic response.

Current biological monitoring approaches for assessing occupational exposure to Cr compounds are typically based on measurements of Cr in urine or serum. Although urinary Cr screening can sometimes reveal environmental exposures, these measurements can be significantly influenced by dietary factors, which are difficult to account for. A contribution of diet-originated nontoxic Cr^{III} to serum and urine Cr levels can be significant leading to difficulties in assessing low-level exposure to toxic Cr^{VI}.

11.4.4.2 Analytical methods

Numerous papers have been published over the years to measure Chromium in biological fluids [82, 83]. The current conception is that ICP-MS is the method to be employed. However, some sample pre-purification must be executed prior to ICP-MS analyses. Some of the issues addressed below, may be instrument dependent, whether skimmer de-clustering can be used or high resolution ICP-MS, e.g., TOF-MS.

During analysis of samples such as urine, sodium- and chloride- ions may be a problem and thus needs to be eliminated. The isotopic composition of natural chromium is ^{50}Cr (4.3%), ^{52}Cr (84%), ^{53}Cr (9.5%) and ^{54}Cr (2.4%). Various chlorine-, nitrogen-, and carbon-containing molecules in the solution delivered into the plasma typically result in spectral interference; these signals include $^{38}Ar^{14}N^{+}$, $^{40}Ar^{12}C^{+}$ to mentioned some. However, these effects can be minimized by correct sample handling and use of correct instrumentation parameters and equipment.

With ICP-MS is may be possible to achieve a detection limit of (LMT) 4.4 ng/L. The method was applied on Cr in and plasma samples [82, 84].

11.4.4.3 Concluding remarks
The determination Cr with ICP-MS is well proven but further improvement regarding sample handling and minimising interfering compounds in the biological samples.

11.4.5 Cobalt (Co)

11.4.5.1 Biological relevance
Like nickel, cobalt hypersensitivity is expressed as contact dermatitis and asthma [85, 86]. Cobalt-induced dermatitis in the general population is rare and its prevalence does not exceed 1%. However, it is often found in association with nickel sensitivity in females and chromium sensitivity in males and a prevalence of 7% of positive reactions to cobalt has been reported in eczema patients. Asthma-like symptoms are also associated with hard metal disease, along with hypersensitivity pneumonitis and interstitial lung fibrosis. Cobalt is classified by the International agency for research on cancer (IARC) as a possible human carcinogen (group 2B). Toxicological profile for cobalt, U.S. Department of Health and Human Services, 2004, 486 pages, has been published.

Cobalt is administrated mainly as dust via the repertory tract, in which the particle size is of importance in terms in dissolving in the blood via lungs. Once cobalt enters your body, it is distributed into all tissues, but mainly into the liver, kidney, and bones. After cobalt is inhaled or eaten, some of it leaves the body quickly in the faeces. The rest is absorbed into the blood and then into the tissues throughout the body. The absorbed cobalt leaves the body slowly, mainly in the urine. Studies have shown that cobalt does not readily enter the body through normal skin.

11.4.5.2 Analytical methods
There are two methods for cobalt: ICP-MS and electrospray mass spectrometry (ES-MS), published 2008.

11.4.5.2.1 ICP-MS
Biological monitoring of 30 trace elements in urine [83] the limit of quantification was 17 ng/L in urine, the corresponding biological tolerance value is 15 µg/L in urine in the U.S. [61]. Using ICP-MS analysis for blood samples and urine has been demonstrated [87], with method detection limit (MDL) value 0.8 ng/L using a high resolution ICP-MS instrument.

11.4.5.2.2 ES-MS
This method describes ES-MS-MS for the determination of cobalt in urine. In the sample, cobalt is complexes with diethyl dithiocarbamate (DDC) and extracted with isoamyl alcohol in the presence of citric acid. The quantification is performed using selecting reaction monitoring at m/z 291 of the product ion which is produced by collision-induced dissociation from the precursor ion at m/z 355. ES-MS-MS data is obtained in less than 10 min with a limit of detection (LOD) of 0.05 µg/L (biological tolerance value is 15 µg/L) [88].

11.4.5.3 Concluding remarks
The determination of Co is performed either with ICP-MS with high sensitivity or with ES-MS-MS, which are both well proven techniques.

11.4.6 Copper (Cu)

11.4.6.1 Biological relevance

Relevant biological effects associated with mild to moderate copper deficiency and copper excess are unknown. It is difficult to identify markers of these early changes because limits of the homeostatic range are still undefined and early changes may represent adaptive responses that do not imply necessarily risk of damage [89]. Exposure to copper fumes, dusts, or mists may result in metal fume fever with atrophic changes in nasal mucous membranes. Chronic copper poisoning results in Wilson's disease, characterized by a hepatic cirrhosis, brain damage, demyelisation, renal disease, and copper deposition in the cornea.

11.4.6.2 Analytical method

ICP-MS is the method used. Limit of quantification in urine 0.30 µg/L.

11.4.6.3 Concluding remarks

Copper seems not to cause any serious environmental problem in the petroleum industry.

11.4.7 Lead (Pb)

11.4.7.1 Biological relevance

Lead is a very toxic element, causing a variety of effects at low dose levels. Brain damage, kidney damage, and gastrointestinal distress are seen from lead exposure. Although lead is absorbed very slowly into the body, its rate of excretion is even slower. Thus, with constant exposure, lead accumulates gradually in the body. It is absorbed by the red blood cells and circulated through the body where it becomes concentrated in soft tissues, especially the liver and kidneys. Lead can cause lesions in the central nervous system and apparently can damage the cells making up the blood-brain barrier that protects the brain from many harmful chemicals. Biological monitoring of workers exposed to lead by regular analysis of lead in blood is compulsory in many EU countries.

11.4.7.2 Analytical method

Lead in blood is measure by electro thermal Atomic Absorption Spectroscopy or, more usually, by ICPMS. Lead in urine was used as an indicator of exposure to organic lead but with the removal of lead from gasoline is rarely used now. Lead in urine can be analysed by ICP-MS [90, 91]. The limit of detection around 8 ng/L

11.4.7.3 Concluding remarks

Even though the references regarding ICP-MS for Lead analysis are about ten years old, the technique which is described is sufficient to determine the concentration of lead well below the biological tolerance value of 300 µg/L [61] (US) and 400 µg/L (Germany) [69].]. See also the compulsory guidance values for lead in blood in the EU (action levels and suspension levels depend on age and sex).

11.4.8 Mercury (Hg)

11.4.8.1 Biological relevance

There are substantial differences in toxicity of elemental mercury metal, in-organic mercury salts and organo-metallic mercury metals [92].

11.4.8.1.1 Metallic mercury

Metallic mercury is a liquid metal, having a low vapour pressure at room temperature. The pressure approximately doubles for every 6 °C increase in temperature, so that heating metallic mercury greatly increases the associated risks. Inhalation of mercury is the important route of exposure. Inhaled mercury vapour accumulates in the body and in particular in the central nervous system, which is the site of its major toxic actions. Once absorbed, a proportion of mercury is taken up by the red blood cells, whilst some remains in the bloodstream, allowing its rapid distribution around the body, including the central nervous system. Within the red blood cells, liver and central nervous system the metal is oxidised via the catalase-peroxide pathway to mercury^{II} oxide. Mercury excretion from the body starts almost immediately after absorption, following a variety of routes, through principally by the kidneys. There is a relatively fast initial phase, during which a third of the inhaled mercury is rapidly concentrated in the liver and kidneys and excreted in the bile of urine. This phase has an estimated half-life of 2-16 days. Another slower second phase, reliant on renal accumulation and is probably responsible for the excretion of the majority of the body's mercury load via the urine with a half-life of 30-60 days. A slower third phase of excretion, via the kidney, has been postulated accounting for about 15% of the mercury load.

Mercury deposited within the brain has an elimination half-life that may exceed several years.

11.4.8.1.2 In-organic mercury

The toxicity of mercury salts varies with their solubility. Usually mercury^{I} compounds are of low solubility and significantly less toxic than mercury^{II} compounds. Mercury salts are toxic but usually non-volatile solids and poising by inhalation is rare. However, if the salts are particle borne they may be deposited in the lungs.

Mercury occurs inorganically as salts such as mercury^{II} chloride. Mercury salts primarily affect the gastro-intestinal tract and the kidneys, and can cause severe kidney damage; however, as they cannot cross the blood-brain barrier easily, mercury salts inflict little neurological damage without continuous or heavy exposure.[17] As two oxidation states of mercury form salts (Hg^{+} and Hg^{2+}), mercury salts occur in both mercury^{I} (mercurous) and mercury^{II} (mercuric) forms. Mercury^{II} salts are usually more toxic than their mercury^{I} counterparts because their solubility in water is greater; thus, they are more readily absorbed from the gastrointestinal tract [93].

11.4.8.1.3 Organic mercury compounds

Compounds of organic mercury tend to be much more toxic than the element itself, and organic compounds of mercury are often extremely toxic and have been implicated in causing brain and liver damage. The most dangerous mercury compound, dimethyl-mercury^{II}, is so toxic that even a few microliters spilled on the skin, or even a latex glove, can cause death.

Methyl-mercury^{I} is the major source of organic mercury for all individuals. It works its way up the food chain through bioaccumulation in the environment, reaching high concentrations among populations of some species. Methyl-mercury has the ability to cross the blood-brain barrier.

In humans, approximately 80% of inhaled mercury vapour is absorbed via the respiratory tract where it enters the circulatory system and is distributed throughout the body.[14] Chronic exposure generally occurs by inhalation, even at low concentrations in the range 0.7–42 $\mu g/m^3$, has been shown in case control studies to cause effects such as tremors, impaired cognitive skills, and sleep disturbance in workers.[15] [16].

11.4.8.2 Analytical methods

Several direct reading instruments for air monitoring of mercury at workplaces are commercial available. For the analysis of mercury in blood and urine older methods measured 'inorganic' mercury using a cold vapour atomic absorption technique. Modern ICP-MS methods measure 'total' mercury (inorganic and organic). One method has been used to determine total mercury in blood and urine [94]. While the degree of ionisation (ICP) for most elements is > 90%, it is for mercury only around 38%. The assay is linear between 2 to 200 µg/L with a limit of detection (LOD) of 0.2 µg/L. Corresponding MAK 0.1 mg/m^3, 0.01 mg/m^3 (alkyl compounds), 0.1 mg/m^3 (aryl compounds), 0.025 mg/m^3 (elemental and inorganic forms). Mercury has been determined as total trace element of low-biomass biological samples [95]. Determination of methyl-mercury in human blood has been conducted with GC-MS [96-98].

11.4.8.3 Concluding remarks

Mercury compounds were added to paint as a fungicide until 1990. These compounds are now banned; however, old paint supplies and surfaces painted with these old supplies still exist. Mercury is readily spread as aerosol in the air. Care should be taken when handling. If using a method that detects 'total' mercury consumption of seafood 24 hours prior to collecting the samples should be avoided or at least noted.

11.4.9 Molybdenum (Mo)

11.4.9.1 Biological relevance

Little data are available on absorption of molybdenum after inhalation in human. Guinea pigs showed no notice absorption after exposure through inhalation of 285 mg Mo/m^3 as molybdenum disulphide. The source of exposure of Mo to man is through diet. From animal experiments, molybdenum and its compounds are known to be highly toxic. Liver dysfunction with subsequent hyperbilirubinemia has been reported in workmen chronically exposed in a Soviet Mo-Cu plant. Hyperbilirubinemia is characterized by elevated levels of bilirubin in blood plasma, which may cause damages to the CNS.

11.4.9.2 Analytical method

For Mo ICP-MS is the preferred analytical method [83] for analysis of Mo in urine with a limit of quantification (LOQ) 0.090 µg/L corresponding MAK values are 0.5 mg/m^3 (soluble compounds), 10 mg/m^3 (metal and insoluble compounds).

11.4.9.3 Concluding remarks

Molybdenum seems not to cause any serious environmental problem in work related exposure in the petroleum industry.

11.4.10 Nickel (Ni)

11.4.10.1 Biological relevance

The main routes of nickel exposure for humans are inhalation, ingestion and absorption through the skin. Nickel absorption through the skin contact is the major human exposure, with a large portion of the population suffering of contact dermatitis and nickel allergy, contact eczema. Another source of nickel to take account for is; tobacco smoking.

Nickel belongs to *group 1 human carcinogen*. Historically, nasal sinus and respiratory cancers have been reported amongst workers in nickel refineries. Humans exposure to nickel-

containing metal dusts by inhalation are reported to induce mucosal ulcers etc., and in addition sinonasal cancers. When human has developed nickel allergic extremely low levels are sufficient for serious reactions.

Nickel may occur in several forms, nickel monoxide and nickel hydroxide which is potent carcinogens. Nickel sulphide, nickel alloys and nickel salts have limited or no tumour formation in animals test [99].

11.4.10.2Analytical methods
For the analyses of nickel in blood and urine, ICP-MS is most frequently used [87] The method detection limits (limit of quantification) is 28.6 ng/L and corresponding MAK value is 1.5 mg/m^3 (Elemental), 0.1 mg/m^3 (Soluble inorganic compounds), 0.2 mg/m^3 (Insoluble inorganic compounds), 0.1 mg/m^3 (Nickel sub sulphide).

11.4.10.3Concluding remarks
It is of greatest importance not to expose humans to nickel due to its carcinogenic and allergy-inducing properties. The method which includes ICP-MS for analysis of nickel seems to be well proven.

11.4.11 Vanadium (V)

11.4.11.1Biological relevance
There is scarily information on health toxic effects on human, a number of animal's studies have been conducted. However, exposure to high concentration of V_2O_5 is known to cause eye irritation, dermatitis and tracheobronchitis.

11.4.11.2Analytical methods
ICP-MS method is published for vanadium [100] with a method detection limits of 1.5 ng/L

11.4.11.3Concluding remarks
ICP-MS is the method of choice for analysis of Vanadium.

11.4.12 Titanium (Ti)

11.4.12.1Biological relevance
Exposure to Titanium usually occurs in the form of TiO_2. TiO_2 is a powder, on which there are sufficient evidence for experimental studies on animals for the carcinogenicity of titan dioxide but no adequate evidence in humans.

11.4.12.2Analytical methods
Since titan dioxide is a solid substance its analysis in biological fluids can be tricky. However, for the titan oxide powder ICP-MS has been utilised [101]. Prior the ICP-MS analysis the sample has been electro thermal vaporised.

11.4.12.3Concluding remarks
Due to the physical nature of titan dioxide there exists no feasible method to determine titan dioxide in urine.

11.4.13 Zinc (Zn)

11.4.13.1 Biological relevance

Zn is an important metal in the living organism including human. Although excess Zn is not implicated in cancer, Zn deficiency causes single- and double-strand DNS breaks and oxidative modifications of for cancer risk [102]. Exposure to zinc oxide (ZnO) which may occur during welding for instance can lead to a flu-like condition known as metal fever. The condition is an acute allergic reaction with symptoms which arise hours after exposure [103].

The most plausible mechanism accounting for the symptoms involves an immune reaction which occurs when inhaled metal oxide fumes injure the cells lining the airways. This is thought to modify proteins in the lung. The modified proteins are then absorbed into the bloodstream, where they act as allergens.

11.4.13.2 Analytical methods

The method to be used is ICP-MS [83] with a limit of quantification (LOQ) 0.30 ng/L,

11.4.13.3 Concluding remarks

Zn is not a very toxic substance in the petroleum industry.

11.5 Conclusions

The method of choice for elemental analysis in urine or other biological fluids is ICP-MS, a search on "Sci Finder" gave the number of references as is presented in Table 11.2. There are however probably even more references available.

Table 11.2 Number of publications of elements in urine from "Sci Finder", 1067 references.

Element	Number of publications
Arsenic (As)	170
Beryllium (Be)	28
Cadmium (Cd)	133
Chromium (Cr)	66
Copper (Cu)	102
Lead (Pb)	147
Mercury (Hg)	83
Molybdenum (Mo)	59
Nickel (Ni)	86
Vanadium (V)	20
Titanium (Ti)	21
Zink (Zn)	132

12 Biological monitoring of organic compounds from paintings and coatings

12.1 Introduction

For more details see appendix.

When risk assessment regarding "hot work" is performed, one of the main tasks at hand is the identification of materials that could emit harmful air pollutants. A detailed knowledge of the history of the material is invaluable. Detailed material and maintenance records greatly facilitate the identification of materials that may release hazardous compounds during "hot work". Indeed, the exploration of the oil fields in the North Sea was impressive pioneering work. It was also a pioneering work with regards to surface coatings. At the very start methods and procedures were implemented from experiences elsewhere. However, the conditions in the North Sea are extremely demanding. Tough weather conditions, humid environment and saline content make well-suited coatings necessary. Low temperature during application of the surface coatings is also a problem. Obviously, the coatings need to be resistant against oil too. After the initial phase, it was found that new kinds of surface coatings were necessary to fulfil all requirements. Coatings for prevention of corrosion are used on almost all steel structures offshore. Even with the increased use of stainless steel in installations, the use will be extensive in the future. In addition, fireproofing coatings will be present in many locations. Surface coating systems initially used in the Gulf of Mexico was modified and used in the early stages of offshore exploration and development in the North Sea during the late 1960 [104, 105]. The considerably harsher environment in the North Sea demanded new strategies for corrosion protection and many novel and untested coating systems were used. By the late 1970's coating systems started to become the precursors of those that have dominated until recent years, i.e. a three coat system comprising of a zinc rich primer, a high build epoxy and a polyurethane top coat. Interestingly, vinyl, epoxy primers and chlorinated rubbers based on red lead were sold during the 50's and 60's and claimed to give excellent performance. This is likely to be doubtful as red lead react specifically with linseed oil, for example, and is basically inert in other polymer systems. With oxidising polymers such as alkyd, or preferably linseed oil, the basic red lead reacts with the carboxylic acid breakdown products to form lead soaps such as lead azelates. These are the actual inhibitors and function by thickening and repairing the naturally formed oxide layer on steel. This has been demonstrated by formulation of chlorinated rubber red lead primers plasticized with conventional chloro paraffins and linseed oil. The linseed oil-based material gave significantly better performance. Unfortunately, the use of linseed oil in coatings gives other detrimental properties, such as decrease in water resistance and resistance to saponification, which made its use unsuitable to the offshore environment. Early vinyl coatings were not restricted by Health & Safety and Environmental requirements to what type of plasticizer was used [e.g., Polychlorinated biphenyls (PCB)], again contributing to long term stability to weathering, corrosion and thermal stress. There were still a number of anomalies, for example, the use of chlorinated rubbers or vinyls as the topcoat for an epoxy system – to aid aged over coating at maintenance and improve weatherability. This produced many 'strange' epoxies formulated in an attempt to achieve good adhesion thermoplastic finishes. Chlorinated rubber coatings suffered of the same defects mentioned earlier for the vinyl materials, with a greater problem in thermo plasticity, thermal degradation and weatherability, but also being susceptible to contamination by oil/grease etc. Eventually these chlorinated rubber topcoats were over coated with special surface tolerant epoxies, thus allowing an improvement in oil resistance. Chlorine containing polymers now have a problem with waste

disposal where incomplete combustion can cause formation of extremely toxic dioxins. During decommission of offshore installations one can expect to come across many different types of obsolete coating systems. Included in the many technologies that have been tried and abandoned are thick film urethane elastomers, oil displacing modified epoxies, flexibilised epoxy phenolics, zinc coated blasting media, thermally sprayed copper antifouling, and moisture cured urethanes.

12.1.1 Overview of industrial paint systems used in petroleum industry

The commonly used industrial paint systems that will be described here are listed below:

- Polyurethane
- Epoxy
- Zn Epoxy
- Acrylate
- Polyvinyl chloride
- Polyester
- Zn Silicate
- Siloxane system
- Alkyd paint
- Combinations of the above systems

In a survey [106], the four largest coating suppliers in Norway listed their sold volumes of surface coating products for the years 1997 to 2000 volumes of surface coating products for the years 1997 to 2000 as shown in Table 12.1.

Table 12.1 The supplied volume of surface coating products from the four largest coating suppliers in Norway.

Year	Total volume (m^3)	Epoxy based products (%)	Polyurethane based products (%)	Other products (%)
1997	816	66	15	19
1998	1029	67	12	21
1999	666	66	9	25
2000	413	61	9	30

For the degradation of polymers see section 19.3.1 *in appendix.*

12.2 Polyurethanes

12.2.1 Biological relevance

Thermal breakdown of polyurethane (PUR) polymers is thought to proceed by radical reactions and result in a number of degradation products. Thermal degradation occurs even at temperatures less than 250 °C. At these temperatures, diisocyanate monomers, aminoisocyanates and amines will dominate among the break-down products. With increasing temperature, many more different kinds of isocyanates are formed. At yet higher

temperatures, less is known about what compounds are emitted. Low molecular weight isocyanates [isocyanic acid (ICA) and methyl isocyanate (MIC)] have however been found in high concentrations at temperatures above 250 °C. If isocyanates are present in a mixture with primary amines, the isocyanate group (NCO) can be transferred to the amine which then is converted into an isocyanate. This is also done industrially to produce isocyanates. The amines among the emitted compounds are further oxidised and possibly also to very reactive nitroso amines (-NO) and nitro radicals (likely to be genotoxic compounds). Isocyanates can form adducts with biomolecules in humans. For many of the compounds formed during thermal breakdown, there are however no methods available. It can be surmised that oxidation products must be formed. It also needs to be stressed that other ingredients such as biocides and flame retardants present in the polymer may be emitted and thermally degraded, but information is missing regarding these products. At present, there are methods described for the determination of airborne isocyanates, amino isocyanates and some of the amines [57, 107-111]. Methods for the further degradation products are essentially missing.

As isocyanates or a mixture of isocyanates and amines are release from the PUR these compounds can be monitored as corresponding amines in blood, in urine or isolated from blood proteins [112-121]. Recently, it has been indicated that other species are produced during oxidative processes of PUR. Some of these species are supposed to be carcinogenic and thus not be analysed yet [109, 122]. To be able to biomonitor these compounds, e.g., nitroso- compounds will be an important task in the future. Currently, there are however no such methods available for PUR.

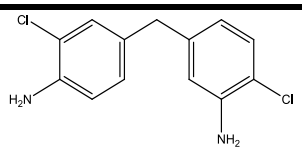
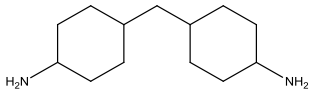
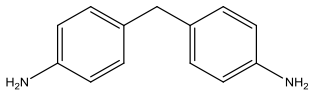
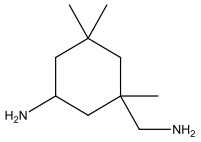
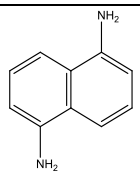
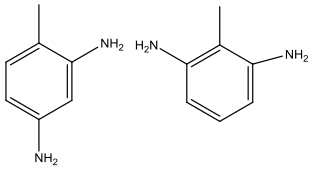
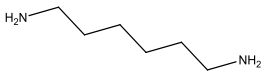
The papers describing carcinogenic properties on nitroso compounds in humans are numerous. Human exposure to environmental arylamines (Aryl = aromatic group) is well established [123], on basis of numerous of epidemiological and laboratory studies on their toxicity, mutagenicity and carcinogenicity [124-131].

For details see section in appendix

12.2.2 Analytical methods

For the following isocyanates listed below in Table 12.2, the corresponding amine (after hydrolysis) can be used as a biomarker in urine, plasma and erythrocytes for determination of the dose of isocyanates a person has been exposed to [132-135]. The levels of the amines are quantitatively measured using HPLC-MS/MS and GC-MS.

Table 12.2 A selection of isocyanates and their corresponding amines (biomarkers) analysed in biological fluids [109].

Isocyanate	Amine	Structure Amine	Biological fluids
MbOCPhI	MbOCA		Urine, Plasma, Erythrocytes
4,4'-HMDI	4,4'-HMDA		Urine
4,4'-MDI	4,4'-MDA		Urine, Plasma, Erythrocytes
IPDI	IPDA		Urine
1,5-NDI	1,5-NDA		Urine, Plasma, Erythrocytes
2,4/2,6-TDI	2,4/2,6-TDA		Urine, Plasma, Erythrocytes
1,6-HDI	1,6-HDA		Urine
Isocyanic acid Methyl isocyanate Ethyl isocyanate HDI biuret HDI isocyanurat HDI uretidon isocyanurat HDI diisocyanurat Triglycidyl isocyanurate			none

12.3 Epoxy

12.3.1 Biological relevance

Epoxy paints are two-component products [136, 137]. The building blocks are normally diglycidylether bisphenol A (DGBA) with a molecular weight of 340 Da (low molecular weight Epoxy). The DGBA is manufactured by reacting Bisphenol A (BPA) with epichlorhydrine (Figure 19.16). Bisphenol A is listed as a contact allergen and has recently been associated with chronic deceases. In a study performed in the U.S the BPA concentrations was examined together with the health status in the general adult population. The study included 1455 adult test subjects of 18-74 years in ages. High urinary BPA concentrations adjusted to the creatinine content was related to cardiovascular diseases when adjusted to age and sex of the participants. Increased levels of BPA in urine were also associated with diabetes and clinically abnormal concentrations of liver enzymes. Data from the National Health and Nutrition Examination Survey 2003-2004 was used in the study [138-141]. The European Union did however present in the early 2008 a report describing present protective measures related to BPA as sufficient [142]. DGPA is also a potent contact allergen.
For details see section in appendix.

12.3.2 Bisphenol A (BPA),

For further information see section in appendix.

12.3.2.1 Biomarkers
There are a number of primary markers of BPA and amongst them; the liberation of amines may be one important class of biomarkers, *for details see section in appendix*.

A study describes the biotransformation of bisphenol A in a mammalian model, combined with HPLC-MS/MS [143]. Metabolites were isolated from urine, liver or the digestive tract.

12.3.2.2 Biological relevance
Bisphenol A is a hormonal-related substance [144] with estrogenic characteristics. Only few epidemiological studies have been conducted on its effect on humans. However, a significant relationship between BPA concentration in urine and increased risk of heart diseases and cardiovascular diseases and diabetes has been reported in USA, as discussed earlier. An overview of various BPA analogues, related compounds and their biological effects have been described [145]. It has been concluded, that more extensive studies must be conducted to establish the risk of BPA exposure on humans [145]. However, BPA should be considered as a harmful substance for humans and thus be accounted as harmful for its exposure to human.

12.3.2.3 Analytical methods
GC-MS and HPLC-MS have been used for the determination of BPA in urine [108, 143, 146-161]

The analysis of phenolic compounds from thermal degradation derived from BPA has been conducted using HPLC-MS [162]. In this work it was found that atmospheric pressure chemical ionization (APCI) was better compared to electrospray ionisation (ESP). The compounds were analysed in the form of derivatives. Derivatisation of the phenols was

performed with phenyl isocyanate (PhI). However, this method has some drawbacks, such as the derivatisation of phenols in low concentrations, which need to be studied more extensively.

GC-MS utilising solid phase combined with derivatisation with a method detection limit (MDL) in the range between 0.45 – 2.3 ng/L is one example on GC-MS of phenols [163].

12.4 Amines

12.4.1 Introduction

In the epoxy and polyurethane chemistry, amine based hardeners are used. In water based coating systems amines are frequently used [72, 164]. These polyamine-epoxide-adducts can be analysed after hydrolytic degradation [165]. A number of amines are liberated from epoxy/polyurethane at hot work and may serve as biomarkers. For amines found from hot metals work see appendix.

12.4.2 Biological relevance

Aromatic amines and aliphatic amines are metabolised differently in the body. There also exist differences in the metabolism of primary- , secondarily- and tertiary amines. One example is the formation of secondary amines into corresponding nitrosamines in vivo, which may be carcinogenic [166]. *See appendix for details 19.7*

12.4.3 Analytical methods

In the body the amines/isocyanates are in some cases free but in most cases they occur as metabolites as amides or as other conjugates. A great part of the amines are covalently bonded to macromolecules e.g., haemoglobin or albumin. When the biological sample is hydrolysed under basic or acidic conditions the amines are liberated. The most frequently used method is to derivatise the amines with perfluorofatty acid anhydrides to amides. The amides are stabile compounds and they can be determined at high sensitivity using GC-MS or LC-MS [167]. Detection limits can be obtained in the low pg per mL level. This is well below any suggested relevant level for the biological monitoring.

In the pharmaceutical industry there is a great interest for the amines as many pharmaceuticals are in fact different kinds of amines and there exist a number of analytical methods, amongst them formation of the 2,4-dinitrophenyl derivative combined with HPLC-MS [168]. In this method 2,4-dinitrofluorobenzene is used for the derivatisation of amines, out of which some are listed in Table 19.3. Recently (in 2008), a number of “new” analytical methods based on HPLC-MS have been published [169-176] claiming improved sensitivity. One example is for Benzyl dimethyl amine that is relevant for the building and transportation industry [177]. Methods for the determination of arylnitroso compounds with GSH and mass spectrometry has been published [178].

12.5 Zink Epoxy

Zink Epoxy consists of “ordinary” Epoxy to which zinc is added as an anti-corrosive metal. It is commonly used to cover metal constructions. Its biological relevance and analytical methods are essentially the same as for Epoxy, which were described in Appendix.

12.6 Acrylates

For detailed information see section in Appendix.

12.6.1 Biological relevance

Acrylic polymers are thermoplastics produced by the polymerization of esters of acrylic acid, methacrylates or acrylonitrile. These polymers are recognized for having good UV and heat resistance. Mixed polymers such as urethane and Epoxy acrylates are increasingly used. Acrylates occur both as thermo plastics and rigid plastics. The properties of acrylates differ depending on the ingredients. The application for acrylates is numerous and they are used in all parts of the industry. Acrylate polymers are used in lacquers, glues as well as in dental medicine and at hospitals for several applications. Most acrylates components, such as acrylates and acrylic acid are irritating in the eyes, skin and the airways. They are more or less dermal sensitizers and can induce contact allergic dermatitis. Inhalation of gas, particles or dust may cause occupational asthma. Similar effects can occur from handling of the acrylate polymer as residues of unreacted acrylate components may be present. It has been reported that occupational exposure to acrylates (glues) enhances the risk of adult asthma. About ten acrylates, in addition to acrylic acid and methacrylic acid are listed in the Swedish list of occupational exposure limits (OELs). Among these compounds, 2-Hydroxyethylacrylate has the lowest OEL (1ppm). Similar OELs are recommended by the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) in the U.S.[179]. Methods for acrylic components are essentially based on dry sampling on a sorbent followed by analysis with GC-FID or LC-UV. For many compounds methods are however not available.

Thermal decomposition of acrylate plastics results in the formation of aerosols containing a mixture of different compounds in gas and particle phase. To reveal the exposure both general methods for different groups of compounds and selective methods for certain compounds are necessary. There are missing information regarding the air exposure to acrylic compounds and especially compounds formed during the thermal decomposition. for details see appendic

12.6.1.1 Analytical methods
A HPLC method with UV detection of acrylic acid collected in air from work places [180]. Analysis of a copolymer of methacrylic acid and butyl methacrylate successfully in air using combined chromatographic techniques has been described in literature [181].

A method for measurement of methyl methacrylate in ambient air at work places included direct-reading photo-acoustic infrared spectroscopy, colorimetric test tubes and gas chromatography [182]. The gas chromatographic method is in accordance with the guidelines from NIOSH [183]. However, analytical methods for biomarkers of acrylate in urine and/or in blood need to be developed.

12.7 Polyvinyl chloride (PVC)

12.7.1 Biological relevance

Polyvinyl chloride (PVC) coating is a polymer consisting of elements depicted in Figure 12.1.

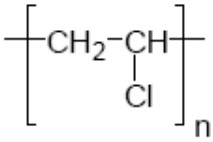


Figure 12.1 Structural elements of PVC

Exposure to PVC degradation products has been related to respiratory tract symptoms [184] and asthma, which has been proposed to be due to occurrence of the plasticizer diethylhexyl phthalate (DEHP) in PVC carpets [185].

The principal component of PVC polymers is polyvinyl chloride (PVC) and di(2-ethylhexyl) phthalate, also called dioctyl-phthalate (DOP). A number of cases of allergic contact dermatitis related to PVC have been reported, but this has often been related to compounds present in PVC such as Bisphenol A, phthalates and phenyl isocyanate rather than PVC itself [186]. See appendix for thermal degradation

12.7.1.2 Analytical method
Compounds such as Bisphenol A, phthalates and phenyl isocyanate is analysed rather than PVC itself [186]

12.8 Polyester

12.8.1 Biological relevance

Polyester paint [136, 137] consists of unsaturated polyester adhesive dissolved in styrene or methylstyrene (vinyl-toluene). In the curing process, a reaction between the aromatic vinyl group and the polyester, takes place when accelerators and peroxide are added.

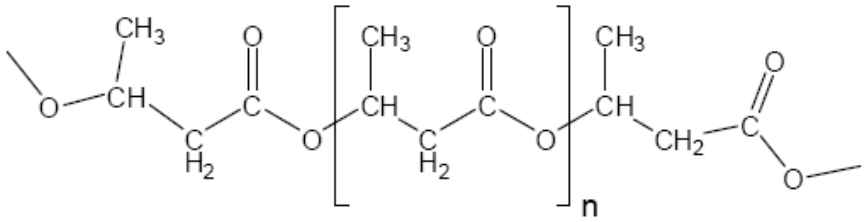


Figure 12.2 Schematic structural formula of polyester polymer

Polyesters often depolymerise and reform the original monomers [187]. This means that in the event of thermal degradation of styrene-based polyester, styrene or methyl styrene (vinyl toluene) will be important components in the low-temperature zone. The metabolic pathways of styrene in man are shown in Figure 19.26, revealing possible biomarkers for styrene [188]. In radical reactions, oxidation products will assumingly form e.g., styrene oxide (see Figure 19.26), a well-known toxicant as well as many other compounds. As a result from styrene exposure, styrene will be present in blood and can hence be used as a biomarker itself, while Mandelic acid and Phenylglyaxylic acid can be detected in urine. Mandelic acid and Phenylglyaxylic acid are the most commonly used biomarkers for styrene exposure*[7, 61]. For further details see section 19.10 in appendix*

12.8.2 Analytical methods

There exist numerous publications on the separation and determination of mandelic acid and phenylglyoxylic acid which serves as biomarkers for styrene. Some resent methods are described here, such as derivatisation under supercritical carbon dioxide determined by gas chromatography [189]. Both compounds has been determined in urine by derivatisation with HPLC separation combined with UV detection including respective enantiomeric forms [190]. A more comprehensive separation and determination of various metabolites from styrene has been determined with HPLC-MS/MS [191].

12.9 Zinc silicate

Zinc ethyl silicate is an inorganic coating where zinc powder and the adhesive ethyl silicate are mixed into a solvent just before application [192]. The coating cures in contact with water and moisture in the air. During combustion of ethyl silicate [193, 194], it has been shown that the most important components formed are aliphatic alcohols (C1 - C4) and butyraldehyde.

12.10 Siloxane systems

The chemical structural networks produced are complex due to multi cross-linking reactions. Both the acrylated urethane and Epoxy modified polysiloxanes are cured with an amino alkoxysilyl functional silane [195].

12.11 Alkyd paints

The adhesive resin in alkyd paints cures in contact with oxygen in the air through a radical reaction involving unsaturated fatty acids [192]. The resin is formed in a reaction between multifunctional alcohols, di-acids and fatty acids to form highly branched molecules. Usually, the pigment and filler (e.g., red lead against corrosion) in the paint give the most important properties of the product. The literature [193, 194] indicates aliphatic aldehydes (C_6 - C_9), acrolein, phtalic acid anhydride and aliphatic acids as the main components. In *Applied Pyrolysis Handbook* [187], it is shown that in connection with thermal degradation of alkyd-based enamel paint, iso-butanol, vinyl toluene (methyl styrene), phtalic acid anhydride and phthalimide are formed. There is no reason to believe that this describes the whole picture and more information is necessary. The biological relevance and analytical methods for Alkyd paints have been described in a previous section.

12.12 Organic anhydrides

Organic anhydrides are irritating and oxidising agents that are formed by a reaction between two carboxylic acids by the elimination of water. They are able to induce airway diseases and act sensitizing to a variable extent. Anhydrides react readily with water, alcohols and amines etc. Anhydrides may be further converted into their corresponding di-carboxylic acids, which may be irritants, or to esters be reaction with alcohols or amines.

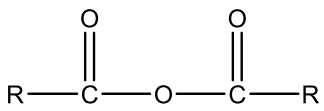
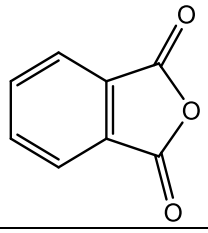
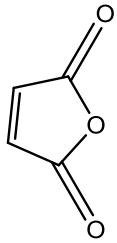
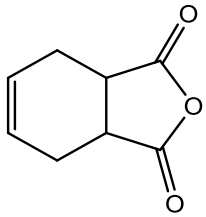
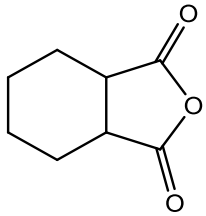
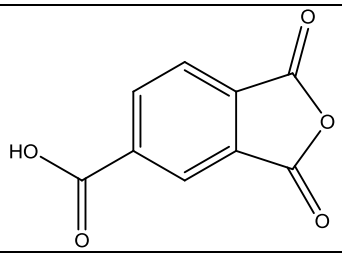


Figure 12.3 General structure of an anhydride.

Anhydrides are formed by thermal degradation of polymers, such as Epoxy polymers. Anhydrides of interest are listed in Table 12.3.

Table 12.3 Carboxylic anhydrides [196]

Anhydride	Abbreviation	CAS number	Structure
Phthalic anhydride	PA	85-44-9	
Maleic anhydride	MA	108-31-6	
Tetrahydro phthalic anhydride	TA	85-43-8	
Cis-Hexahydro phthalic anhydride	HA	13149-00-3	
Trimellitic anhydride	TMA	552-30-7	

12.12.2 Biological relevance

Because of their reactivity and oxidising properties, organic acid anhydrides are irritating to airways, eyes, skin and mucous membranes. Besides from direct toxicity, exposure to acid anhydrides may also cause hypersensitivity which for instance leads to the development of occupational asthma [197]. This has been shown for exposure to PA [198] and HA [199]. Rhinitis and chronic bronchitis are other symptoms associated with workplace exposure. Trimellitic anhydride causes influenza-like symptoms, or cause pulmonary haemorrhage [197]. Trimellitic or phthalic anhydride may also cause allergic alveolitis, i.e. inflammation in the alveoli [200]. Anhydrides are also suspected to cause contact dermatitis [201] and urticaria [202].

12.12.3 Analytical methods

Methods have been developed for biological monitoring of organic acid anhydrides. In the human body the anhydrides are hydrolysed to their corresponding di-acids, which can be found in urine or blood as biomarkers of anhydride exposure. Methods for the determination of phthalic acid [203] and hexahydro phthalic acid [204] in urine and hexahydrophthalic acid and methylhexahydro phthalic acid in plasma [205] have been presented [206-209] . Exposure to anhydrides have also been found to cause an immunological response in workers and hence elevated levels of IgE and IgG antibodies. Presence of antibodies shall only be regarded as biomarkers of exposure, since the connection between antibodies and disease is weak[210]. Adducts between amino acids and hexahydrophthalic anhydride inducing both type I (leading to release of IgE antibodies) and IV (release of antigen-specific T-cells) allergy has been determined utilising LC-MS [211].

12.13 Biomarkers of aldehydes

12.13.1 Biological relevance

Aldehydes (R-CHO) are often small molecules that readily react with an amino group (R-NH_2) by the formation of an imine ($R_1R_2C=NR_3$). Unsaturated aldehydes are more toxic as compared to saturated aldehyde, which has been shown in toxicological studies in animals. The relative toxicities of aldehydes decreases with increasing chain length [212].

Formaldehyde (FA) is an industrial chemical found in several materials, in tobacco smoke and in vehicle exhaust. FA is considered to be human carcinogenic and has been linked to increased incidence of leukaemia in some epidemiologic studies [213]. In 2004, the International Agency for Research on Cancer (IARC) concluded that FA is a human carcinogen based on sufficient evidence from studies on humans and experimental animals [214].

FA is metabolised into formic acid by in vivo biotransformation, and the formic acid is further oxidised into carbon dioxide. Since these metabolic processes occur rapidly in the body, exposure to FA does not always result in an increased concentration of FA in blood and urine [213].

Thiazolidine-4-carboxylate (TZCA) is formed by non-enzymatic condensation between L-cysteine and aldehydes or carbonyl compounds including FA, which can be seen in Figure 12.4. TZCA was found to be a biomarker for the exposure of formaldehyde in urine reflecting the degree of FA exposure [215]. This method was also applicable to acetaldehyde.

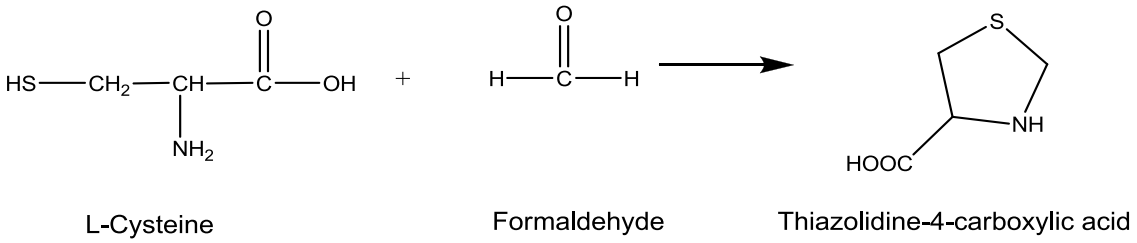


Figure 12.4 Reaction of L-Cysteine and formaldehyde

12.13.2 Analytical methods

The formation of TZCA as a biomarker for formaldehyde was also found to be of value for acetaldehyde by the formation of Me-TZCA. Analyses were performed by derivatisation in combination with GS-MS [215]. This method may be extended to other aldehydes. Other analytical methods on the analysis on TZCA have been published. TZCA and Me-TZCA has been determined in plasma of cancer patients by HPLC [216].

13 Biological monitoring of inorganic compounds

13.1 Nitrogen dioxide (NO_2)

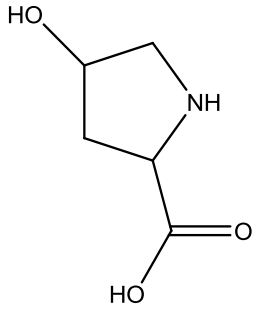
13.1.1 Biological relevance

Emission of NO_x of levels up to 8 ppm has been detected during hot work [54]. The OEL for NO_2 in Norway is listed as 1.1 ppm [62]. Presence of NO and NO_2 in ambient air may hence be a problem. Exposure to NO_2 is also commonly a results from vehicle exhaust in urban areas [217], or from large vehicles on the work site. Exposure to NO_2 has shown to be related by an increasing number of total lymphocytes, T CD3+, TCD4+, T CD8+ cells or IgG antibodies, implying a role of NO_2 in the inflammatory response [218]. Tests performed on experimental animals have also shown associations between NO_2 exposure and lung injury. NO_2 exposure is related to increased prevalence of respiratory diseases [219] and impaired lung function [220]. In other cases, not much research has been performed on NO_2 on humans [217]. NO_2 toxicity may also be due to its oxidative properties, leading to increased oxidative stress [219].

13.1.2 Analytical method

There is not much information in literature regarding biomarkers of NO_2, but rather of biochemical biomarkers of effects as a result from NO_2 exposure. Immunological methods have been used to detect immunological responses as a result from exposure to NO_2.

Today hydroxyproline seem to be the most commonly used biomarker of NO_2 exposure. Hydroxyproline is a biomarker of effect from NO_2 exposure and is a product formed during breakdown of connective tissue matrix of lungs, i.e. a metabolic product of lung collagen [217]. Hydroxyproline is however an unsuitable biomarker for NO_2 exposure assessment in individuals suffering from growth hormone deficiency, Paget's disease, osteoporosis, bone metastasis and metabolic bone disease and other diseases which naturally causes alterations in the urinary levels of hydroxyproline [217]. Due to occurrence of collagen in a diet containing meat or gelatine, hydroxyproline may be an unsuitable biomarker for NO_2 due to high interferences from diet collagen under routine-analysis conditions. During hydroxyproline analysis of patients, the patients are normally put on a collagen-free diet for 24 h prior to urine sampling. Of course this wouldn't be a manageable procedure for workers, which makes the biomarker unsuitable for routine controls. During exertion, skeletal muscles forms hydroxyproline at higher rate, causing additional interferences of hydroxyproline in urine. The level of urinary hydroxyproline can be determined with HPLC after overnight hydrolysis of urine samples [221]. To our knowledge, there exists no study investigating the relationship between hydroxyproline levels in urine and NO_2 levels in ambient air.



Hydroxyproline

Figure 13.1 Structure of hydroxyproline, a breakdown product of collagen.

Elevated levels of NO in exhaled air are commonly a measure of airway inflammation or untreated asthma due to production from L-arginine in the airways [220, 222], but also of impaired lung function as a results from increased levels of air pollution [220]. Hence, increases in NO levels in exhaled air may be a marker of exposure to NO_2 as well as of particulate air pollutants [222]. Elevated levels of urea and uric acid have been coupled to NO_2 exposure [220].

13.1.3 Concluding remarks

The most common analysis of exposure to NO_2 is through measurement of hydroxyproline in urine. This biomarker may however be unsuitable for routine controls of workers for assessment of the occupational exposure situation to NO_2 due to interferences of hydroxyproline from the diet. Hence, no adequate biomarker for NO exists today. Another possible approach would be to continue the investigation in altered immunological response as a result from NO_2 exposure since antibodies and T-cells are relatively easy to measure quantitatively. In addition, biomarkers of oxidative stress would include exposure to NO_2 as well due to its oxidative properties.

13.2 Nitrogen oxide (NO)

13.2.1 Biological relevance

Nitrogen oxide (NO) occurs naturally in the body as a neurotransmitter enabling for instance vasodilation. NO readily diffuses across biological membranes. Due to the instability of the molecule though, NO doesn't exist in the body for long periods of time.

NO is present in vehicle exhaust and is emitted during hot work. Levels up to 13 ppm of NO have been measured during hot work [54]. The OEL for NO in Norway (30 ppm) is considerably higher than for NO_2. However, it is more difficult to protect workers against NO, filters of respirators being permeable for NO rather than for NO_2.

The immunological significance of NO has been investigated in workers exposed to both NO and NO_2. Correlations between NO exposure and increased levels of T CD3+, T CD8+, B CD19+ cells were found. Exposure to NO could also be coupled to reduced levels of IgG, IgA

and IgM antibodies. These results imply that NO seems to acts as a modulating factor of a proinflammatory effect from NO_2 exposure [218].

13.2.2 Analytical method

NO exposure has been related to elevated levels of interleukin -8 (IL-8), nitrite and nitrate in nasal lavage from children during a study where the impact of vehicle exhaust was investigated in children [220]. The concentration of IL-8 was determined by enzyme-linked immunosorbent assay (ELISA). Nitrate and nitrite can be quantified using the method of Griess [220, 223]. The Griess method includes colorimetric quantification at 540 nm from adding Griess reagent (sulphonamide, naphtylethylenediamine dihydrochloride and H_3PO_4) to the sample [223], which results in a coloured complex. The levels of these nitrogen oxide metabolites were however not suspected to be a result from absorption of NO but formation of nitrite and nitrate would occur from endogenous release of NO by the nasal mucosa resulting from a mild inflammatory response [220]. Another study has however measured the levels of NO released from cultured human retinal pigment epithelial cells. The levels of nitrite and nitrate were measured in the culture supernatant [223].

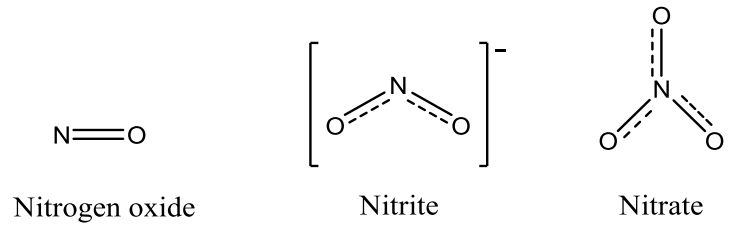


Figure 13.2 Structure of nitrogen oxide nitrite and nitrate.

13.2.3 Concluding remarks

No biomarker currently exists for assessment of NO exposure. Nitrite and nitrate are products from NO and have been used as biomarkers for NO in exhaled air, but whether they truly represent the exposed dose of NO is doubtful.

13.3 Hydrogen sulphide (H_2S)

13.3.1 Biological relevance

Hydrogen sulphide (H_2S) occurs in natural gas and crude petroleum [224]. The gas is toxic and influences the nervous system. The compound inhibits cellular respiration by inhibition of Cytochrome oxidase [225] and thereby exerts its acute toxic effect [226]. At high levels, hydrogen sulphide is lethal and several deaths due to hydrogen sulphide poisoning have been reported [227, 228]. Exposure to low levels can cause headache, nausea and irritation to eyes and throat [226]. When inhaled, H_2S act in a similar way as cyanide (CN) and carbon monoxide (CO) through uptake in the alveoli which blocks the uptake of oxygen [226].

Once brought into the body, hydrogen sulphide is metabolised through oxidation, methylation or reaction with Cytochrome C or other metabolic enzymes. Most of the hydrogen sulphide brought into the body is though oxidised into sulphate and excreted in urine [224].

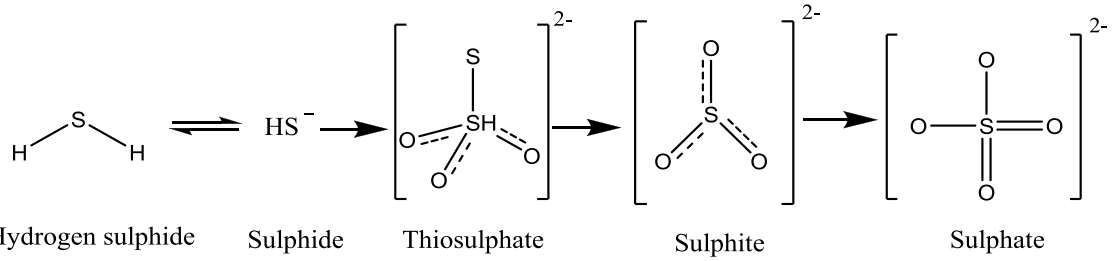


Figure 13.3 Metabolic pathway of hydrogen sulphide [224].

13.3.2 Analytical method

Inhibition of Cytochrome oxidase in target tissue has been found to be a biochemical biomarker of exposure to hydrogen sulphide [224]. The activity of the enzyme was measured by determination of the rate of oxidation of reduced ferricytochrome C, including colorimetric analysis by a UV/VIS spectrophotometer and determination of the A_{550}/A_{565} ratio [224].

Thiosulphate can be used for assessment of occupational exposure to hydrogen sulphide [229]. Increased levels of thiosulphate have been detected in blood and urine of rabbits as a results from exposure to 100 – 200 ppm H_2S for 60 min [230]. This biomarker has also been determined in urine of humans after exposure for 30-45 min of 8, 18 or 30 ppm H_2S [231].

Thiosulphate was analysed in urine as its bromobimane complex by LC with a concentration maximum 15 h after exposure to hydrogen sulphide [231]. Thiosulphate can however also be analysed by GC-MS [227].

13.3.3 Concluding remarks

Hydrogen sulphide is a highly toxic compound. Thiosulphate has mainly been used as a biomarker for H_2S by LC or GC in combination with MS.

13.4 Sulphur dioxide (SO_2)

13.4.1 Biological relevance

Sulphur dioxide (SO_2) is present in petroleum and is released during combustion. The chemical is toxic in large amounts and can cause poisoning when inhaled. Individuals suffering from asthma are especially sensitive to SO_2 exposure [232].

Sulphur dioxide is toxic by inhalation, corrosive to respiratory tract, eyes and skin. Sulphur dioxide is a systemic toxic agent. The compound has been found to induce oxidative stress and DNA damage in mice [233]. In workers which were chronically exposed to sulphur

dioxide, the level of chromosomal aberration, sister chromatid exchanges was increased [234].

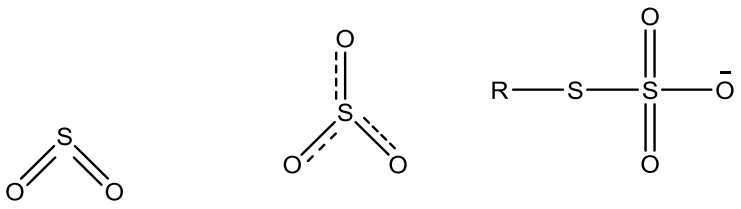
The toxic effects from sulphur dioxide are carried out by the SO_2 derivates bisulphite and sulphite. During metabolism of sulphur dioxide, the compound is hydrated and converted into sulphurous acid when inhaled. Sulphurous acid is further converted into bisulphite and sulphite which are dissociated in the bloodstream [234].

It has also been found that the incidence of chronic obstructive disease (COPD) is higher in populations that are subjected to high levels of SO_x and NO_x [235].

Exposure to sulphur dioxide from vehicle exhaust has been related to increased white blood cell count [236].

13.4.2 Analytical method

It is generally difficult to measure SO_2 once it has been taken up by the body due to its reactive properties. However, since SO_2 is absorbed in the nasal passage during inhalation, breakdown products of SO_2 in nasal lavage have been suggested as a biomarker of short-term (less than 24 h) exposure to sulphur dioxide. SO_2 is converted into bisulphite, which reacts with S-S bonds in proteins present in the nasal passage and forms S-sulphonate [235]. Clearance of sulphite from the nasal passages occurred within 24 h, making S-sulphonate most appropriate as a short-term biomarker of sulphur dioxide exposure. The nasal lavage was performed immediately after exposure. Proteins present in the nasal lavage fluid were treated with cyanide in order to release sulphite. The level of sulphite in relation to the protein content was then measured by ion chromatography. Analysis was carried out using an IONPAC AS4 separator column with conductrimetric detection.



Sulphur dioxide Sulphite S - sulphonate

Figure 13.4 Structure of sulphur dioxide, sulphite and sulphonate.

It was also reported by Bechtold et al [235] that plasma levels of S-sulphonates increased as a result to SO_2 exposure, which would serve as a more suitable biomarker. This would however require a more sensitive method for quantification than the one Bechtold et al [235] used. It was also suggested that occurrence of S-cystein in urine or blood serves and biomarker for SO_2. This method would however include enzymatic cleavage of proteins in blood or urine into individual amino acids [235].

Levels of sulphite have been determined in target organs after exposure to sulphur dioxide in rats. The analysis was carried out by high performance liquid chromatography with fluorescence detection (HPLC-FD) [234]. The method included release of protein bound sulphite from target organs, derivatisation of free sulphite with mono bromo-bimane, separation of sulphite bimane from thiol bimanes by reverse-phase HPLC and quantification of sulphite bimane by fluorescence detection [234]. It this method would be applicable to determine the sulphite levels in blood, sulphite would serve as a suitable biomarker for SO_2

exposure. To our knowledge, there is however no reports about sulphite being measured in blood as a biomarker for SO_2 exposure.

13.4.3 Concluding remarks

Measurement of sulphite in blood plasma would probably be a suitable biomarker for SO_2 exposure, on basis on studies performed in tissues which have used sulphite as a biomarker. In addition, S-sulphonate in blood plasma has been proposed as a potential biomarker. No reports do however exist to our knowledge, where these compounds have been measured in urine or blood for occupational biological monitoring.

14 Solvents

14.1.1 Introduction

14.1.2 Toxic effects of solvents and vapours

The term solvent refers to a class of liquid organic chemicals of variable lipophilicity and volatility. Non-polar organic solvents of low molecular weight readily diffuse across the membranes in the alveoli, thus entering the blood through inhalation. Non-polar solvents may also be absorbed through the skin due to their lipophilicity. The lipophilicity of solvents enables them to accumulate in fatty rich tissues, such as the central nervous system, which is the primal biological effect of solvents on humans. Various solvents cause different toxic effects, such as carcinogenicity and fatal damages to the liver. The blood brain barrier consists of cells surrounding the capillaries in the CNS thus preventing substances to enter the brain directly from the blood. Lipophilic and uncharged compounds are able to cross the barrier. Due to the ability of solvents to readily cross the blood brain barrier, they are able to directly influence the CNS.

Classification of solvents is made according to molecular structure or functional group. Classes of solvents include aliphatic hydrocarbons, many of which are chlorinated, aromatic hydrocarbons, alcohols, ethers, aldehydes, ketones etc., and complex mixtures that defy classification. Generally, the lipophilicity of a compound increases with increasing molecular weight, while large molecules are less volatile.

The main determinants of solvent's inherent toxicity are: Its number of carbon atoms. Whether it is saturated or has double or triple bounds between adjacent carbon atoms. Its structure i.e., straight-chain, branched-chain or cyclic and the presence of functional groups greatly affects the toxicity.

In some cases, solvents within the same class have similar toxicological properties. For example, amides and amines tend to be potent sensitizers while aldehydes are particularly irritating. Hydrocarbons that are extensively metabolised tend to be cytotoxic and/or mutagenic and many unsaturated short-chain halocarbons are animal carcinogens. The toxicity of solvents within the same class may however vary dramatically.

Exposure of solvents often includes exposure to a number of different compounds, rather than one in particular. It is therefore important to have information of the toxicology of solvents when in a mixture. As mentioned earlier, during exposure of mixtures, the resulting toxic effect may be additive of the effect of the individual solvents. Alternatively, they can interact synergistically or even antagonistically. In many cases, reliable risk assessments cannot be made due to too little data available regarding mixtures of solvents. Even though the hazard varies between one solvent to another, all solvents can cause toxic effects, such as narcosis and irritation of skin and mucous membranes as long as the dose is sufficient. Several solvents are classified as animal carcinogens and a handful as human carcinogens. Health effects from solvents are influenced by the toxicity of the solvent, the exposure route, the amount or rate of exposure, the duration of exposure, individual susceptibility and interaction with other chemicals. These factors also influence the effect not only from solvents, but from all other chemicals. It needs to be mentioned, for e.g., dermal uptake, that solvents can act as carriers for other compounds that have difficulties to be absorbed through the skin. *For more information see section in Appendix*.

14.2 Chlorinated solvents

14.2.1 Biological relevance

The biotransformation of chlorinated solvents is quite complicated due to the diversity of such solvents. Chlorinated aliphatic solvents have been associated with numerous health effects on basis of the toxicology of these solvents, animal metabolism and studies which have been conducted regarding occupational exposure [237]. These effects are exerted on the central nervous system (CNS), the liver and kidneys. In addition, some chlorinated solvents are carcinogenic. In Table 14.1, some common chlorinated solvents and their adverse effect is listed. *See also section in Appendix*.

Table 14.1 Some aliphatic chlorinated solvents and their biological effect [237].

Compound	Formula	CAS Number	Target organ and biological effect
Methyl chloride	CH_3Cl	74-87-3	Possible CNS
Methylene chloride	CH_2Cl_2	75-09-2	Possible CNS, liver, carcinogen
Chloroform	$CHCl_3$	67-66-3	Possible CNS, liver, kidney, carcinogen
Carbon tetrachloride	CCl_4	56-23-5	Possible CNS, liver, kidney, carcinogen
1-chloroethane	C_2H_5Cl	75-00-3	Possible CNS
1,1-dichloroethane	$C_2H_4Cl_2$	75-34-3	Possible CNS, liver, kidney
1,2-dichloroethane	$C_2H_4Cl_2$	107-66-2	Possible CNS, liver, kidney, probably carcinogen
1,1,1-trichloroethane	$C_2H_3Cl_3$	71-55-6	Possible cardiac, CNS, liver, kidney
1,1,2-trichloroethane	$C_2H_3Cl_3$	79-00-5	Possible CNS, liver, kidney
1,1,1,2-tetrachloroethane	$C_2H_2Cl_4$	630-20-6	Possible CNS
1,1,2,2-tetrachloroethane	$C_2H_2Cl_4$	79-34-5	Possible CNS, liver, kidney
Pentachloroethane	C_2HCl_5	76-01-7	Possible CNS
Hexachloroethane	C_2Cl_6	67-72-1	Possible CNS, liver, kidney
Vinyl chloride	C_2H_3Cl	75-01-4	Possible circulatory system, liver, spleen, known carcinogen
1,1-dichloroethylene	$C_2H_2Cl_2$	75-35-4	Possible liver, kidney
1,2-dichloroethylene	$C_2H_2Cl_2$	540-59-0	Possible liver,
1,1,2-Trichloroethylene	C_2HCl_3	79-01-6	Possible CNS, liver, kidney, carcinogen
Perchloroethylene	C_2Cl_4	127-18-4	Possible CNS, liver, kidney, carcinogen

14.2.2 Analytical methods

There are numerous of analytical gas chromatography (GC) and GC-MS methods for analysing solvents, most often these can be obtained from the instrument vendors and are fully tested or the method described in following reference(s) [238, 239].

14.2.3 Concluding remarks

Chlorinated solvents are metabolised in the liver and the metabolites of these compounds can be used as biomarkers. Analysis can generally be performed with for instance GC-MS. However it would be impossible do give detailed description of analytical methods for all the hundreds of chlorinated solvents and their metabolites.

14.3 Benzene

14.3.1 Biological relevance

Benzene is a toxic solvent and is classified from the International Agency for Research on Cancer (IARC) as a human carcinogen (group 1) [240]. The toxicity of benzene is also related to individual genotype and personal exposure [241]. A review article on biomarkers of benzene has recently been published [242].

The metabolism of benzene is complex, producing a number of metabolites. Three metabolites, t,t-muconic acid [243, 244], S-phenylmercapturic acid and phenols [241] in urine are used as biomarkers for benzene exposure. *See also section 19.13 in appendix*.

14.3.2 Analytical methods

Benzene in exhaled air can be analysed as such with a reliable field method utilising GC-MS [245]. Estimation of unmetabolised benzene is used for the evaluation of benzene exposure in human in urine [246] and blood [247].

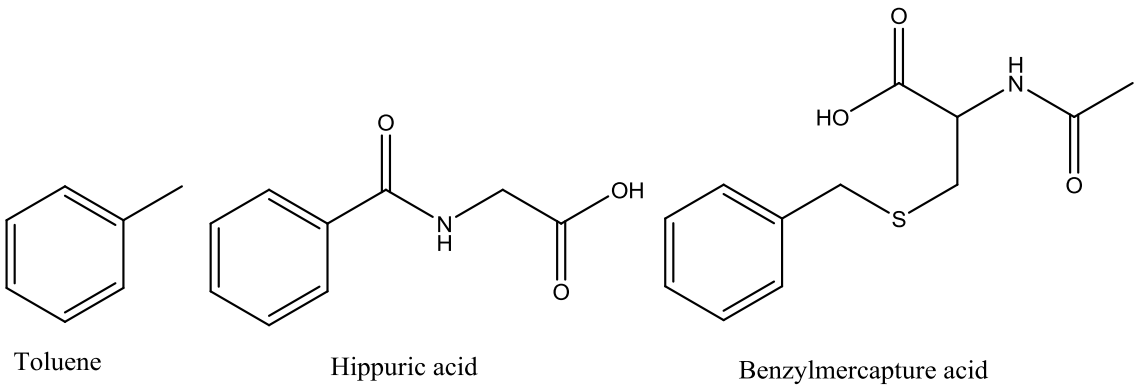
A number of GC-MS and HPLC-UV methods have been described for benzene metabolites [248]. This includes a sensitive and specific HPLC-MS/MS assay for the determination of benzene metabolites such as t,t- muconic acid, S- phenylmercapturic acid, hydroquinone and catechol in urine [249]. In addition, a LC-MS/MS method for the determination phenylmercapturic acid, benzylmercapturic acid and *o*-methylbenzyl mercapturic as biomarkers of benzene, toluene and xylene has been validated [250]. Also GC-MS has been applied [251, 252].

14.4 Toluene

14.4.1 Biological relevance

Toluene can cause brain dysfunction as a result from long term exposure [253]. Currently, no evidence exists which suggests carcinogenic properties of toluene. Nevertheless, exposure to high concentrations of toluene can induce changes in the central nervous system and other neurotoxic effects [254].

One main biomarker of toluene that was commonly used is Hippuric Acid. However, due to the high levels of hippuric acid in urine (from dietary benzoic acid) this is no longer recommended. Another biomarker is of benzene exposure is benzylmercapturic acid (BMA) [250] although there is some doubt about its application to biomonitoring of toluene exposure. Ortho cresol is another metabolite useful for assessing exposure to toluene around the current exposure limits. Toluene itself has also been measured in breath, blood and urine as an indicator of exposure.



Toluene Hippuric acid Benzylmercapture acid

Figure 14.1 The chemical structure of Toluene and its metabolites Hippuric Acid and Benzylmercapturic acid (BMA).

14.4.2 Analytical methods

Saliva can be used as specimen for sampling instead of urine or blood samples for biological monitoring in the workplace utilising GC-MS [255]. The analysis of biomarkers from toluene the method described [249] has been used. A validated LC-MS method validated according to U.S. Food and Drug Administration (FDA) guidance [250], Also GC-MS can be applied on a biomarker from toluene [251, 252].

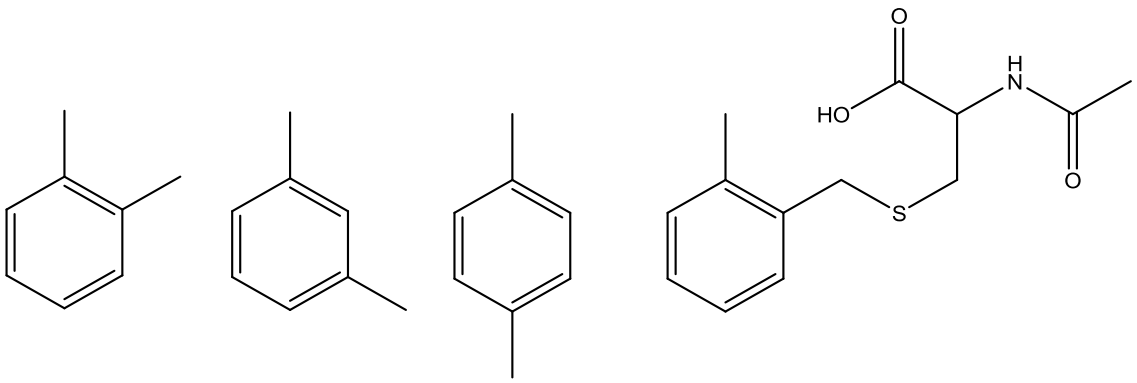
14.4.3 Concluding remarks

Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with a validated GC-MS from urine [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

14.5 Xylene

14.5.1 Biological relevance

In principle as described for toluene, O-Methylbenzyl mercapturic acid (MBMA) is proposed to be a reliable biomarker of the exposure of xylene [250]. A more common and widely used method detects methyl hippuric acid in urine. The ACGIH and DFG have a BEI and BAT for methyl hippuric acid



ortho-xylene meta-xylene para-xylene O-Methyl mercapture acid

Figure 14.2 All xylene isomers and the metabolite O- Methyl Benzylmercapturic acid.

14.5.2 Analytical methods

Methyl hippuric acid can be detected in urine by a simple HPLC-UV method. A validated LC-MS method validated according to U.S. Food and Drug Administration guidance has been reported [250]. Also GC-MS can be applied on a biomarker from xylene [251, 252].

14.5.3 Concluding remarks

Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with a validated GC-MS from urine [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

14.6 Ethylbenzene

14.6.1 Biological relevance

Ethylbenzene is a solvent and its main metabolite is mandelic acid and phenylglyaxylic acid are used as biomarkers in urine for ethylbenzene exposure [251, 252]. Mandelic acid and phenylglyaxylic acid are also biomarkers for styrene exposure.

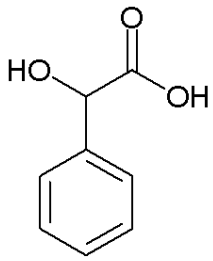


Figure 14.3 Chemical structure of mandelic acid

14.6.2 Analytical methods

Mandelic acid in urine as a biomarker of ethylbenzene can be analysed with a validated GC-MS method [251, 252].

14.6.3 Concluding remarks

Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with a validated GC-MS from urine [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

14.7 Styrene

14.7.1 Biological relevance

Exposure from styrene can be assessed from measurement of styrene in blood or its metabolites Mandelic acid and Phenylglyaxylic acid, which are the same as for Ethylbenzene. Biological relevance and analytical methods was described in earlier chapters (section 12.8) regarding styrene and will not be further discussed here.

14.7.2 Concluding remarks

Biomarker(s) in urine of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with validated GC-MS methods [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

14.7.3 Concluding remarks

Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed in urine using GC-MS [252] [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

14.8 Polycyclic aromatic hydrocarbon (PAHs)

14.8.1 Biological relevance

Polycyclic aromatic hydrocarbons (PAHs) are emitted from oil, coal and tar deposits and are formed during heating of oil products. In addition to polyaromatic hydrocarbons, there are polyaromatic heterocyclic compounds, polyaromatic nitro and amines formed during the heating of oil products.

The toxicity of PAHs is very structurally dependent, with isomers (PAHs with the same formula and number of rings) varying from being non-toxic to being extremely toxic. Thus, highly carcinogenic PAHs may be small or large. One PAH compound, benzo[a]pyrene, is notable for being the first chemical carcinogen to be discovered (and is one of many carcinogens found in cigarette smoke). The EPA (US Environmental Protection Agency) has classified seven PAH compounds as probable human carcinogens: benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]anthracene, chrysene, and indeno[1,2,3-cd]pyrene [257]. *See section 19.17 in appendix*.

14.8.2 Analytical methods

An HPLC-APCI-MS/MS method is used for rapid and sensitive for the determination of 1-hydroxypyrene (1-OHP) and 3-hydroxybenzo[α]pyrene (3-OHPBaP) in urine [258].

The level of 1-OHP in 207 workers was determined and compared to a reference group consisting of smokers and non-smokers, revealing significantly higher levels in the workers as compared to the references. The 1-OHP levels were also higher in smokers as compared to non-smokers, as can be seen in Figure 14.4 [259].

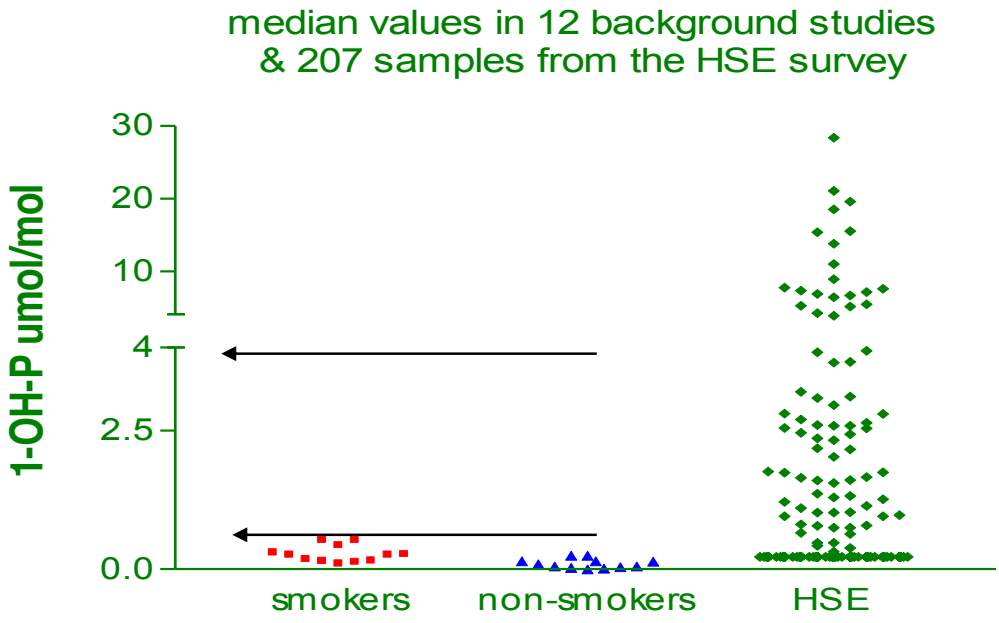


Figure 14.4 Results from an investigation where the 1-OHP level was assessed in a reference group containing both smokers and non-smokers, and a group of workers (Crown Copyright 2009).

Additionally, 1-OHP has been used as a biomarker for PAH exposure in traffic police workers. The occurrence of 1-OHP in urine in test subjects was however also strongly associated with smoking and the 1-OHP level was unaffected by PAH exposure from diesel exhausts in heavy smokers [260].

14.8.3 Concluding remarks

PAHs are highly toxic compounds and should be avoided. The biomarker for pyrene – 1-hydroxypyrene in urine usually serves as a general biomarker for PAHs.

15 Future methods

Most of the current methods described in the literature are developed at different governmental laboratories and at university laboratories in Europe or the US. The funding is in most cases limited and the transfer of research methods into routine methods has great difficulties. At present, the funding in the world for biomarkers and other methods for the monitoring of pollutants is small and declining. The progress in the area of new and improved methods is unsatisfactory. This is remarkable as the available analytical tools have been greatly improved. If there is a great demand for biomarkers there are possibilities for contract laboratories to set up the methods. The contract laboratories struggle however often with competition from governmental financed laboratories and the drive to invest in modern equipment is often low. No doubt, there is a need for several orders of magnitudes more biomarker analyses to ensure that workers exposures (in the world) are monitored in a satisfactory way. The current ambition is in most countries defined by the legislation, labour unions, companies and demands from governmental bodies. Clearly the "business" of method improvement and the development in new ones need to be improved in order to let organisations or companies to have a reasonable financial return. Several factors for the development of new and improved methods need to be stressed. The competition between governmental and private laboratories needs to be fair under equal conditions. The intellectual properties of new methods need to be improved for involved companies or researchers. In some countries there is a belief that the government is best fitted to have the major responsibility for environmental control and laboratory resources. At present the governmental laboratories perform analyses at, in most cases, a low cost. In the longer perspective this may result in a dependence of governmental funding. For some analyses e.g., neutron activation analysis that demands an access to extremely costly equipment the government needs to be involved.

In general many of the methods described in the literature for various analytes are not adopted for analyses of biological samples. For analysis of biomarkers, it is likely that new methods must be developed once the appropriate biomarker has been selected for assessment of exposure to a certain compound. In some cases a combination of analyses may be performed e.g., for quantification of the analyte itself and a selected biomarker thereof to assess the total exposure of a substance to human. Furthermore, even if the substance itself isn’t toxic to humans, the compound may be transformed into another compound or into metabolites which can affect the health. This overall picture has so far not satisfactory been studied. Currently, threshold values are set on basis of the toxicology of the parent molecule and it may be overlooked that the conversion of the compound into another chemical compound and its metabolites may be of relevance for the setting of threshold values.

Since the metabolism may differ between individuals, which was discussed in section 19.1.3, quantification of the parental compound may not reveal the true level of exposure. For instance, if two individuals are considered that are exposed to the same compound. One individual can have a low concentration of a metabolite measured in blood either as results of low exposure or due to slow metabolism. Meanwhile, the other individual, despite being exposed to lower exposure compared to the first one, show higher concentration in the blood of a metabolite due to fast metabolism. By measurement of all possible degradation product including the parent compound and metabolites a more correct picture of the exposure situation should be revealed. In this respect, further development of methods for determination of different protein adducts emerged from chemical exposure is needed, since chemicals may form such adducts together with proteins once they are brought into the body.

15.1 Mass spectrometry

Recently, there has been a technical breakthrough in the mass spectrometry technique. Analytical of Branford (Analytica of Branford, Inc., Branford, CT 06405) has developed instrumentation that greatly facilitates the use of MS. The instrument (TOF) can be used for analysing organic compounds [371], organo-metallic compounds and metallic compounds in the same run. One interesting feature is e.g., the possibility to separately determine different organo-mercury compounds and inorganic mercury, which is an advantage as the toxicity, is different for the different forms of mercury. Detection limits, for metal ions, are in the sub-ppb range and well below the regulation requirements. The resolving power is sufficient to resolve metal ion peaks from organic ion peaks that contain hydrogen. The determination of toxic elements such as arsenic and heavy metals as cadmium, mercury and lead is simplified. It would be essential to further investigate the elemental analysis with this technique. In combination with HPLC both structural information of the organic-metal complex and the elemental determination is made possible.

15.2 Immunological methods

Many reactive organic compounds will form adducts to proteins in the airways and other target organs. They are typically characterized by their half-life in blood or their urinary elimination half-time etc.

A prerequisite for all exposed individuals to have access to exposure assessment is that the analytical procedures are simplified and cheap. However, to develop such methods improved knowledge regarding the metabolism in the body is necessary. Such simplified exposure control is already today in daily use in the medical care in order to e.g., follow the degree of long-term hyperglycemia for control of diabetes or the long term control of ethanol exposure at ethylism. Immunochemical methods are shown to be well suited for routine analysis and have low cost per analysis (usually about 100 - 500 NOK) and can be performed at health care units at different companies.

There are implications that allergy towards reactive low molecular weight compounds (and many other compounds present in the industry) is due to the ability of the compounds to modify proteins in the body. The understanding and characterization of the modified proteins is necessary for the development of appropriate diagnostic methods, but so far, the knowledge is insufficient. Pathogenic important modified proteins need to be characterized. After the characterisation there are great possibilities to identify more relevant modified proteins by immunochemical techniques.

It has been demonstrated a relation between air levels (of isocyanates) and the concentration of modified blood proteins, e.g., haemoglobin and albumin among exposed workers. The levels of modified proteins are about 0.000000005g (5ng) per gram of protein. Only a very small fraction of the proteins is modified. It is well known that proteins are normally modified which is the case for haemoglobin for diabetic patients and such modified proteins are nowadays determined in the clinical routine analysis at all hospitals. By a similar way, simple methods can be developed for isocyanates, anhydrides or other compounds in biological fluids.

Biological markers of exposure to certain compounds have been considered a possible approach for biological monitoring. Chemical compounds that form protein adducts are able

to induce an immune response, resulting in elevated levels of antigen-specific antibodies, lymphocytes and proteins involved in signalling within the immune systems (cytokines). Chemicals that don't form protein adducts are not able to induce an immunological response. In order for a chemical to cause an immunological response, it needs to be taken up by the cell, digested and displayed on the surface of the cells in order to be recognized by antigen specific T-cells that are selected to recognize foreign antigens.

This method for biological monitoring has been adapted to isocyanate exposure. TDI and HDI are common inducers of isocyanate-asthma and exposure to them leads to the formation of primarily IgG and potentially IgE antibodies as well [372]. For instance, TDI is known to induce a broad immunological response as a result of exposure [373]. Increased levels of isocyanate-specific antibodies could either be used for diagnosis of isocyanate induced asthma, or as markers of exposure to isocyanates [374]. Isocyanate specific antibodies can be detected using other antibodies that are specific for them in immunoassay, commonly goat anti-human IgE and IgG antibodies. Such assays must however be thoroughly validated to ensure specificity among the antibodies used. Cross-reactivity among the antibodies used for the assay is a common problem, which means that the antibodies used in the assay also will bind to other molecules than the analyte. In addition, it has been observed that different conjugates of HDI and human serum albumin (HSE) results in variations in selectivity and sensitivity of immunoassays. The conjugates would differ in using vapour monomeric HDI, liquid monomeric HDI or polymeric HDI for conjugation with HSA and were tested by RAST and ELISA for IgE and ELISA for IgG [372]. However, which HDI-HAS conjugate present in vivo is not known [372].

Organic anhydrides are potent inducers of airway diseases and act as sensitizers as stated previously. These compounds are also able to conjugate to proteins in the body and cause an immunologic response. Antibodies specific for these organic anhydrides protein adducts (IgG and IgE) have also been observed in blood serum in exposure-response studies where the test subjects were exposed to hexahydrophthalic anhydride (HHPA) and methylhexahydro phthalic anhydride (MHHPA). Formation of antibodies specific for protein adducts with these anhydrides was observed in the test subjects and related to exposure. The protein adducts of organic anhydrides have also been considered potential biomarkers of exposure to organic anhydrides [375, 376]. The occurrence of Ig E and IgG antibodies have also been related to clinical symptoms as a results from exposure, in eyes and airways [376]. Problems have however been encountered with cross reactivity between antibodies for HHPA and MHHPA, making it more troublesome to differentiate between the two. In addition, the anhydride protein adducts have been considered potential biomarkers of exposure to organic anhydrides. Immunological methods would be applicable for analysis of such conjugates. It has also been noted that most organic anhydrides inhaled are hydrolysed into their corresponding acids while only a small portion are conjugated to proteins [375]. To our knowledge, no similar studies have been conducted regarding occupational exposure to phthalic anhydride, maleic anhydride, tetrahydrophthalic anhydride or cis-hexahydrophthalic anhydride.

15.2.1 Immunoassays

Within medicine, antibody-based assays have been used to a greater and greater extent, for analysis and diagnostics. The basic thought underlying this is that antibodies have the potential to be developed to bind specifically to any molecule or epitope possible. Generally, immunoassays, i.e. usage of antibodies for analysis, are appropriate for detection of proteins, such as other antibodies (IgE etc.) and receptors. Lymphocytes are receptor specific, i.e. they are characterized by occurrence of specific receptors on their surfaces. Antibodies are

commonly used to distinguish between various cells within the immune system, by flow cytometry or fluorescence activated cell sorting (FACS).

Enzyme-linked immunosorbent assay (ELISA) is a common technique today, primarily for antibody detection that is frequently used within self-diagnosis, such as pregnancy tests etc. It is based upon unspecific binding of the antigen to the bottom of wells of a microtiter plate and subsequent binding of a antigen-specific antibody to the antigen (after all other antigens have been blocked). The antigen specific antibody is covalently linked to an enzyme, which will produce a product enabling colorimetric detection and quantification of the antigen [377]. ELISA is commonly used in viral diagnostics, for example for HIV [377].

Microarrays are a further development of the ELISA technique, enabling much higher throughput screening. This technique has proven itself useful for proteomic and genomic evaluation due to its ability to screen for a high number of different antigens simultaneously.

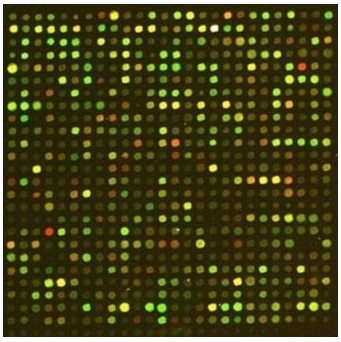


Figure 15.1 An example of a microarray. Each spot represents an individual antigen. Fluorescent light indicates detection.

Radio-allergosorbent test (RAST) is another commonly used technique within diagnosis. The technique resembles ELISA in principle, but the antigen-specific antibody is instead radio-labelled. Hence, the antigen can be quantified and detected through a radioactive signal. The technique is commonly used for determining the concentration of for instance hormones in blood [377].

In order to use immunoassays for quantification of biomarkers, antibodies must be obtained that are selective to the analyte. Today, large libraries of antibodies are available, where an appropriate monoclonal antibody can be selected through phage display. Alternatively, polyclonal antibodies are obtained from animals by inducing an immunological response to the analyte in the animal. Consequently, immunoassays have the potential for developed of new techniques, using the specificity of antibodies to distinguish the target molecule from an infinite number of other molecules present in a mixture. Problems that are often encountered are however cross-reactivity, i.e. the antibodies destined to be selective for the target molecule also bind to other molecules present, giving a false signal which gives either an underestimation or overestimation of the true level of target molecule.

ELISA is a technique commonly used for self-diagnosis tests today, for instance as pregnancy tests etc. Regarding biological monitoring, immunoassays may come to be useful for this application as well for instant exposure assessment.

16 Biological monitoring of other environmental factors

16.1 Organophosphates

16.1.1 Biological relevance

Organophosphates are used as additives to hydraulic- and turbine oils. These compounds are added to oil as a flam retarding agent for the oil to better withstand heat. Due to the occurrence of organophosphates in pesticides, a number of studies have been performed regarding the assessment of occupational exposure to organophosphates for workers who are in contact with pesticides containing organophosphates [261]. Organophosphates belong to a group of very hazardous organic compounds that are a nerve toxic [262, 263]. They are readily absorbed through the skin [264] and exert their neurological effects through inhibition of acetyl cholinesterase which is an enzyme that hydrolyses acetylcholine, a major neurotransmitter [265].

The toxic effect of organophosphate has been associated with the development of multiple sclerosis and Rheumatoid arthritis in individuals exposed to these oils [266]. The formation of biomarkers are very complex depending on the actual organophosphate [267].

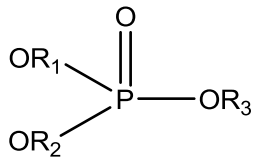


Figure 16.1 General structure of organophosphate. Side chains R_1 and R_2 are generally methyl or ethyl groups [264].

16.1.2 Analytical methods

Metabolites of organophosphates have been used as biomarkers for organophosphates exposure, mainly from pesticides and insecticides. Alkyl phosphate derivatives are biomarkers of a range of different organophosphates and are the result from enzymatic conversion of the parental molecule by cytochrome P450 or esterases, the latter being a group of enzymes that generally hydrolyses esters [265]. Due to the ability of organophosphates to inhibit the cholinesterase and acetyl cholinesterase, the activity of these two enzymes has also been measured by biological effect monitoring as indicator of organophosphate exposure [264, 265]. However, different organophosphates may inhibit the enzyme activity to a varying extent [264, 265]. In another study, the serum cholinesterase activity was determined by a spectrophotometric method where the enzyme activity was used for reduction of potassium hexacyanoferrate resulting in a colour change that can be quantitatively measured [268]. Salivary acetyl cholinesterase have also be used as a biomarker for organophosphate exposure [269]. Decreased activity of these enzymes in blood serum is however a relative insensitive measure of organophosphate uptake and in order to assess that exposure indeed has occurred, the results after potential exposure must be correlated to the baseline activities for the individual. The baseline activity of the enzymes has been proposed to be measurable 60 days after exposure [264].

The low concentrations of organophosphate metabolites in urine found in occupationally exposed workers have not been associated with any significant reductions in blood cholinesterase activity [264].

The general structure of organophosphates, as indicated in Figure 16.1, includes an OR_3 side chain which is more prone to act as a leaving group. While the side chains R_1 and R_2 are generally methyl or ethyl groups, R_3 is more specific to the organophosphate. Measurement of the occurrence of this side chain as a metabolite of organophosphates is hence a more selective method for determination of exposure to certain organophosphates rather than all organophosphates as a group [264]. These metabolites can be measured in urine.

For quantification of organophosphate metabolites in urine, GC-MS with electron impact ionization (EI) have been used. This method was improved in sensitivity by operation in negative ion chemical ionization (NCI) mode. Additionally, isotope dilution gas chromatography-positive ion chemical ionization (PCI) tandem mass spectrometry and GC-MS/MS with isotope dilution have been applied. LC-MS/MS is increasingly in use for analysis of organophosphate metabolites [261]. Organophosphate pesticides are for instance readily analysed using liquid chromatography-mass spectrometry with electrospray ionisation [270], LC-MS/MS with EI and turbo ion spray (TIS) sources and LC-Negative ion electrospray-tandem mass spectrometry (LC-ESI-NI-MS/MS) [261]. For specific organophosphate metabolites (analysis of the compound-specific side chain) LC-negative ion atmospheric pressure chemical ionization-tandem mass spectrometry (LC-APCI-NI-MS/MS) on a triple-quadropole instrument as well as coupled-column LC electrospray tandem mass spectrometry (LC/LC-ESI-MS/MS) has been used, the latter for serum samples [261].

In addition, methods using a portable field detector for air monitoring have been described in literature [271].

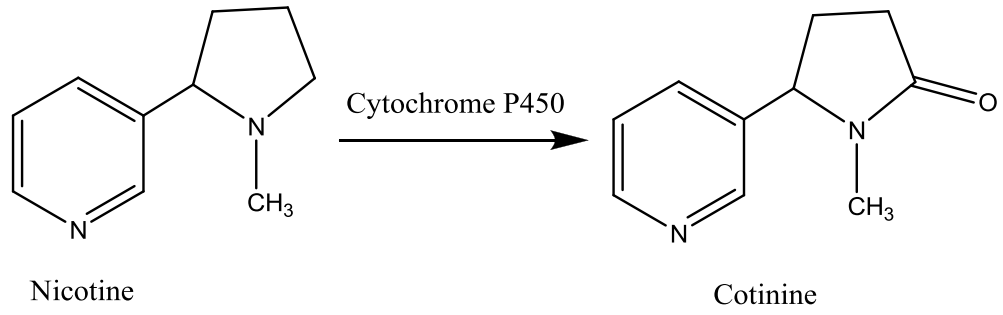
16.1.3 Concluding remarks

Both urinary alkyl phosphate derivatives and blood serum enzyme activity has been used in routine analysis for exposure assessment of organophosphate, based on the occurrence of these substances in pesticides for several years [264]. Both types of these biomarkers provide a measure of the overall uptake of organophosphates.

16.2 Tobacco smoking

16.2.1 Biological relevance

Tobacco smoke contains some thousand different substances and metals, out of which several have been classified as mutagens and/or animal carcinogens, including metals. Nicotine is a CNS stimulant and additive. Nicotine has several metabolites and one of them, cotinine, can be used as a biomarker of nicotine, both for passive and active smoking. Nicotine is converted into cotinine through C-oxidation by the Cytochrome P450 system with a yield of 65-75% [272].



Nicotine Cotinine

Figure 16.2 Nicotine is converted into cotinine, a process catalysed by Cytochrome P450.

Nicotine has also been found in some foods, such as potatoes and tomatoes, however the content is negligible and does not significantly contribute to background levels in test persons. The elimination half-life of cotinine is 18-20 h in adults and children [273, 274]. Even though nicotine is readily converted into cotinine, residual nicotine in lungs and possibly in the gastro intestinal tract after cessation of exposure causes a prolonged absorption phase. The level of cotinine has been associated with risk of asthma in children [274-278]. Cotinine has been found to be a useful biomarker for tobacco smoke in ambient air in workplaces [279], but further studies would be needed in order to list threshold values in ambient air.

16.2.2 Analytical method

The concentration of cotinine is preferably measured in urine and is adjusted to the creatinine content of the sample. Analysis can be performed by capillary gas chromatography with selective ion monitoring with an detection limit of 0.2 µg/L [280]. Trideuterated cotinine was used as internal standard. For the analysis, m/z = 176 and 179 for cotinine and trideuterated cotinine were used respectively. Their molecular ions (M+1) were m/z = 177 and 180 respectively. Samples were injected on the column with split-less technique. Electron impact and chemical ionization was used for the analysis [273]. Cotinine in urine has been used as a biomarker in restaurant personnel [276]. By these means, Statens arbeidsmiljöinstitutt (STAMI) in Norway has also used cotinine as a biomarker for nicotine in bar and restaurant personnel for exposure assessment before and after introduction of smoking permit [281].

16.2.3 Concluding remarks

Cotinine has shown to be a suitable biomarker for tobacco smoke, both in active and passive smokers. Analysis is performed by capillary gas chromatography.

16.3 Cortisol as a biomarker of mental stress

16.3.1 Biological relevance

Cortisol is a hormone secreted from the adrenal cortex, resulting in elevated levels during mental stress. This class of steroid hormones promote gluconeogenesis, i.e. the formation of glucose from non-carbohydrate species and the formation of glycogen, increases the degradation rate of fat and protein while inhibiting the inflammatory response [282]. Stress has even been defined as secretion of cortisol. Hence, cortisol has been implied to be an indicator of mental stress, while this thesis has been contradicted by some studies [283]. Cortisol commonly form conjugates to glucuronic acid prior to excretion in urine. As a consequence, hydrolysis is needed prior to analysis of GC-MS [284].

16.3.2 Analytical method

Cortisol has been analysed in urine samples with LC-MS/MS with deuterium-labelled cortisol as internal standard and electrospray ionization and a C18 column [285]. Prior to this study, atmospheric pressure chemical ionization has been used instead. Isotope dilution gas chromatography mass spectrometry has also been applied [284].

Immunological assays are simple and convenient means to perform routine analyses, but may experience problems associated with cross-reactions, since a number of steroid metabolites are present in blood which may resemble cortisol epitope [284]. For instance can the radio immunoassay (RIA) method be used for determination of cortisol concentration in urine or serum. The RIA method involves mixing of the antigen with a known amount of antigen-specific antibodies in excess. After binding of antigen-specific antibodies and antigen has occurred, radioactively labelled antigens are added, binding to the remaining antigen-specific antibodies. The resulting radioactive signal after precipitation of the sample is hence a quantitative measure of the analyte, which is smaller for a large amount of antigen in the sample than a small amount, since there are less remaining antigen-specific antibodies in the sample to bind the radioactively labelled antigens. This method has however been associated with problems with cross-reactions, i.e. the antibody which is supposed to be specific for cortisol also binds to other proteins present. Such cross reactions may lead to an overestimation of the level of cortisol in urine [286]. Comparison between various immunoassays was however performed in 2006 by Horie et. al, proving an immunoassay method based on RIA being the most selective method due to less cross-reaction. Cross-reaction did however occur with 5α-THF or glucuronide conjugates for other immunoassay kits, while only with 5α-THF for the RIA-based method [284]. Cortisol has also been analysed in saliva by Enzyme-linked immunosorbent assay (ELISA) with subsequent colorimetric analysis at 450 nm [287].

16.3.3 Concluding remarks

Cortisol has been proposed as a possible biomarker for mental stress, which may be a risk factor while working in an offshore environment or during long shifts. Analysis may be performed either by LC-MS/MS or immunoassays.

16.4 Oxidative stress

16.4.1 Biological relevance

There are a number of compounds that are known to induce oxidative stress in cells, but there aren't any relevant biomarkers available. A biomarker of oxidative stress may be used instead to detect any increases in oxidative stress as a result of exposure to, radicals, arsenic [288], Nickel and Cadmium [289], NO, NO_2, SO_2 etc.. Such a biomarker would be unspecific while indicating exposure to any compound or compounds that are able to induce a higher rate of oxidative stress. Of course, oxidative stress occurs naturally at any time, which is the reason to why the body ages, but the rate of oxidative stress can be significantly increased as a result from exposure to specific agents.

The oxidative stress biomarker 8-hydroxy-2'-deoxyguanosine (8-OHdG) has already been associated with occupational exposure to a number of chemicals such as benzene, styrene and asbestos [290].

16.4.2 Analytical method

There exists numerous biomarkers indicating oxidative stress and the literature available is huge. The most commonly used and far most studied biomarker is however 8-hydroxy-2'-deoxyguanosine (8-OHdG). Another biomarker for oxidative stress may be 8-iso-PGF(2α), which is an indicator of lipid oxidation [291]. This biomarker does however not seem to be as extensively used as 8-OHdG in literature.

During oxidative stress, nuclear and mitochondrial DNA is subject to oxidative attack, the guanine base being most prone to oxidation which adds a hydroxyl group to it. Hence, the product 8-hydroxy-2'-deoxyguanosine (8-OHdG) is an indicator of oxidative attack of the DNA and is a commonly used biomarker for oxidative stress [8, 290, 292]. DNA repair products are excreted in urine, and so is also 8-OHdG. As a consequence, the level of 8-OHdG can be measured in urine as a biomarker for oxidative stress [8]. Whether 8-OHdG is a good indicator of increased risk of cancer development is elusive, though a recent study reports about its excretion in urine being correlated to increased incidence of lung cancer in non-smokers [8]. The ability of 8-OHdG to act as an endogenous mutagen once formed [290] does however further imply the significance of this biomarker for cancer development.

Problems associated with the use of 8-OHdG as a biomarker are for instance that the presence of this compound in urine depends on the DNA repair system. Hence, unchanged excretion pattern of 8-OHdG at increased oxidative stress may be a result from impairment of the repair capacity [290]. In addition, smoking (active or passive) also cause oxidative stress, which cause interferences of elevated levels of 8-OHdG as a result. Smokers have been reported to have a 50% higher levels of 8-OHdG as compared to non-smoker.

The level of urinary 8-OHdG has been measured by the use of many different techniques. The most commonly one seems to be HPLC with electrochemical detection (HPLC-EC) [8]. Other common techniques would be GC-MS and ELISA [290]. HPLC-EC includes enzymatic hydrolysis in order to break down the DNA, separation on C18 columns and EC-detection. Constraints in the amount of released 8-OHdG during the enzymatic degradation may cause underestimations [290]. Analysis by GC-MS have resulted in higher measured concentrations

of 8-OHdG as compared to HPLC-EC. Results produced from this method regarding measurement of 8-OHdG has been questioned because of oxidation of guanine prior to analysis [290]. Analysis by ELISA has given results which correlates well with the ones from HPLC-EC. Problems with un-specificity or cross-reactions has however lead to higher measured levels of 8-OHdG when comparing results from ELISA analysis to HPLC-EC [290] quantification. Dose-response relationships regarding occupational exposures have however not been investigated to any high extent [290] and within this area there is still work to be done.

16.4.3 Concluding remarks

On basis of the number of articles published using biomarkers for oxidative stress, 8-OHdG seems to be the most commonly used one by far. Analysis is preferably carried out by HPLC-EC, GC-MS or ELISA. It should be noted that a biomarker for oxidative stress would imply exposure to a number of substances that elevates the level of oxidative stress and not only one single compound.

17 Conclusions

In the petroleum industry, there are safety measures and PPE are widely used. Air levels outside PPE do not contribute to the knowledge regarding the exposure among workers. It is the chemicals that evade the PPE that may result in exposure among workers and this exposure can only be estimated by biomarkers. Still, today the monitoring of biomarkers as compared to air sampling is sparse. The reason for this is unclear, but it may be explained by tradition and lack of sufficient number of reference data or that proper methods for biomarkers are missing. Biomonitoring methods are available for the more common industrial chemicals but additional funding is required to develop (or adapt) methods for new substances.

In several countries there are data bases of high quality regarding e.g., cancer in the population. In order to relate the exposure to disease it would be a great advantage to have access to exposure data from biomarkers. Today, the exposure is often only poorly estimated from a limited number of air measurements. Biomarkers can greatly be of assistance in learning about the relation between the disease and the work place exposure.

Most of the current methods described in the literature are developed at different governmental laboratories and at university laboratories in Europe or the US. The funding is in most cases limited and the transfer of research methods into routine methods has great difficulties. At present, the funding in the world for biomarkers and other methods for the monitoring of pollutants is small and declining. The progress in the area of new and improved methods is unsatisfactory.

In some cases a combination of analyses may be performed e.g., for quantification of the analyte itself and a selected biomarker thereof to assess the total exposure of a substance to human. Furthermore, even if the substance itself isn't toxic to humans, the compound may be transformed into another compound or into metabolites which can affect the health. This overall picture has so far not satisfactory been studied.

By measurement of all possible degradation product including the parent compound and metabolites a more correct picture of the exposure situation should be revealed. In this respect, further development of methods for determination of different protein adducts emerged from chemical exposure is needed, since chemicals may form such adducts together with proteins once they are brought into the body.

Additionally, there is a need to improve or develop analytical methods for biological monitoring of decomposition products of epoxy polymers such as phenolic compounds and Bisphenol A (BPA). In addition new methods for acrylates, aldehydes, NO, NO_2, organophosphates, oxidative stress, protein adducts, nitroso compounds, organo metallic compounds etc. should be developed.

As far as we know, no systematically investigation has been conducted on the exposure of free stable radicals to human. This is of outmost importance since polymers liberate very reactive free radicals when heated or by mechanical stress of the polymeric coating e.g., grinding. Today, there is not any analytical method for assessment of exposure to free radicals, which can be formed during degradation of polymers. Biological monitoring of radicals should be investigated.

Cortisol may be of interest as a biomarker within the petroleum industry, due to a stressful environment and long shift. It needs to be fully evaluated whether cortisol serves as a suitable biomarker for mental stress.

Dose-response relationships regarding occupational exposures to oxidising agents have not been investigated to any high extent [290] and within this area there is still work to be done.

No suitable biomarker for NO_2 exposure exists today. Hydroxyproline which is the most frequently used biomarker, is not applicable to routine analyses due to interferences from the diet. There is a need for a biological method to assess the exposure to NO_2.

In order to improve the biological monitoring of exposure, some analytical methods needs to be further developed. For instance, analytical methods to detect and quantify compounds such as amines, isocyanates as protein conjugates should be developed.

Analytical methods for biological monitoring of decomposition products epoxy compounds (e.g., BPA) should be developed.

Biomarkers of acrylate in urine and/or blood should be investigated. A method for HPLC-MS quantification and detection of TZCA and Me-TZCA should be developed.

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19 Appendix

19.1 Pharmacokinetics

When an individual is exposed to a specific compound, the compound first undergoes an absorption phase, where it is taken up by the lungs, through the skin, or via the gastro intestinal tract (ingestion of dust) and is distributed in the biological fluids of the body. A so-called one-compartment model is used to describe what happens when a chemical compound is brought into the body. Hence, the entire body is referred to as one single compartment that absorbs, distributes and eliminates chemical compounds [8]. In reality, the body can be divided into several compartments, such as various organs, i.e. the compound is initially absorbed and excreted from one compartment into another, the absorption rate of the second compartment being influenced by the excretion rate from the first compartment. Such a model is however rather complicated to describe and the one-compartment model is the one most frequently used. During the absorption phase, the concentration of the chemical compound increases in the blood. The elimination phase then follows, during which the chemical is metabolised in the liver and excreted in the urine. Some compounds may also be distributed in adipose tissue, depending on their lipophilicity.

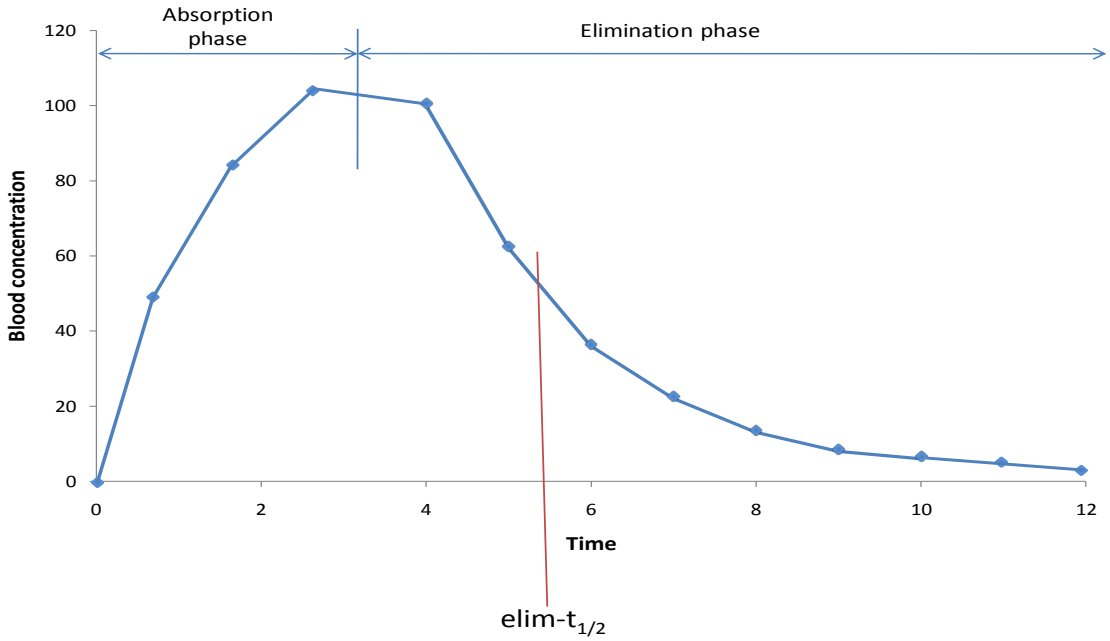


Figure 19.1 The concentration in the blood of a given compound after exposure.

The elimination half-life (elim-$t_{1/2}$) refers to the time required for the concentration in the bloodstream of a given substance to drop by half. The elimination half-life varies greatly between various substances. Information about the elimination half-life of compound should be obtained for biological monitoring in order to estimate the total internal dose of an individual.

Pharmacokinetics describes the rate of elimination of a given compound from the body. The elimination rate is generally described by first-order kinetics, which expresses the elimination rate of a given substance as a function of the concentration in blood (C_t) and the elimination rate constant (k) according to equation 1.

$$\frac{dc}{dt} = -C_t \cdot k \qquad (1)$$

The concentration in blood can hence be calculated from the concentration at the end of exposure (C_0), elimination constant and time (t) [7, 293]:

$$C_t = C_0 \cdot e^{-k \cdot t} \qquad (2)$$

The elimination rate of some compounds can however be expressed by zero-order kinetics. During zero-order kinetics, the elimination rate is independent of the concentration in blood. For example ethanol is metabolised in the body with a constant rate, regardless of the concentration of the ethanol concentration in the bloodstream. The elimination rate and the consequent concentration of such a compound are expressed as follows:

$$\frac{dc}{dt} = -k \qquad (3)$$

$$C_t = C_0 - k \cdot t \qquad (4)$$

Zero-order kinetics is also applicable to describe elimination rate at high exposures due to limitation in the enzymatic systems that metabolises the compound or other compounds being present, taking up all active sites of the metabolic enzymes [6].

For first-order kinetics, the concentration in blood versus time becomes linear with logarithmic scale of the y-axis, which can be seen in Figure 19.2. This provides a graphical means to determine the elimination half-life for a compound.

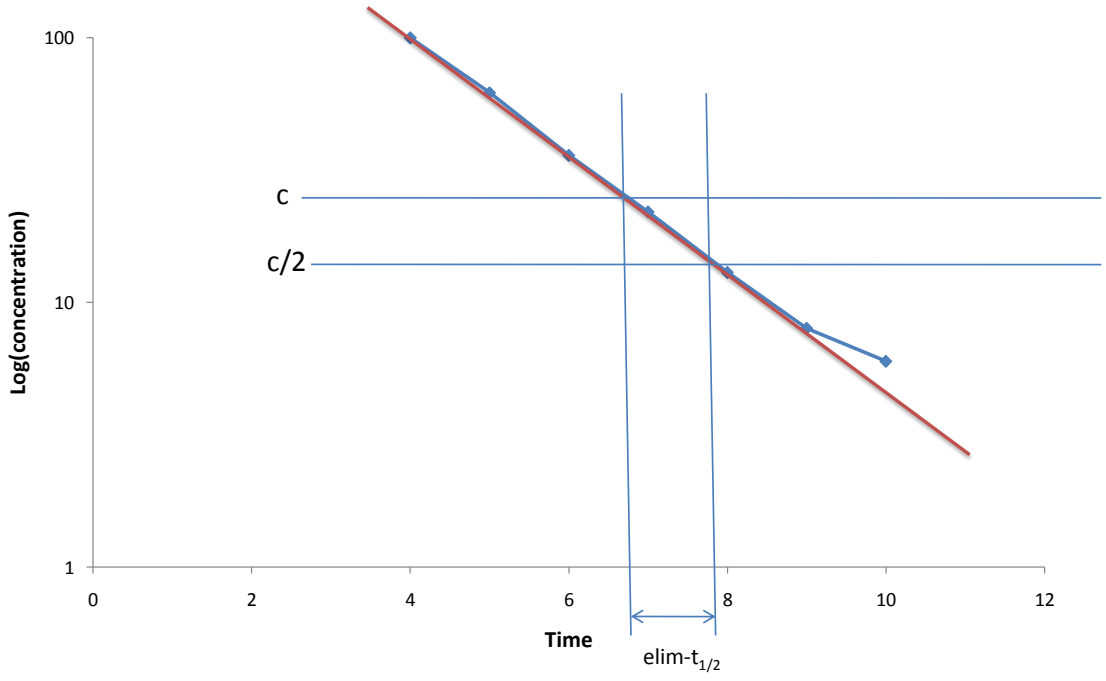


Figure 19.2 Concentration of a compound eliminated by first-order kinetics during the elimination phase with a logarithmic y-axis. The declination in blood concentration becomes linear versus time and the elimination half-life can easily be determined.

Mathematically, the elimination half-life $t_{1/2}$ can be calculated from the elimination rate constant.

$$t_{1/2} = -\frac{\ln 2}{k} \quad (5)$$

For first-order kinetics and

$$t_{1/2} = -\frac{1}{2 \cdot k} \quad (6)$$

For zero-order kinetics.

Generally, the concentration of a compound that a worker is exposed to during the work day is usually reduced before the start of the next day. However, if the elimination half-life of the compound is long, the compound may not be entirely excreted from the bloodstream during the night. As a result, the compound accumulates in the body during the work week as shown in Figure 19.3 [293]. The elimination half-life is of relevance in order to determine when samples for biological monitoring must be taken. For biological monitoring of substances with long elimination half-life, the sampling time is not critical, while for substances with shorter elimination half-lives it needs to be more specified [2]. A biomarker with a long half-life reveals the exposure during the exposure in the past and in some cases during several weeks or longer. A biomarker with a short half-life reveals the recent exposure during the last few hours and exposure to accidents or exposure to recent high exposure peaks will be revealed.

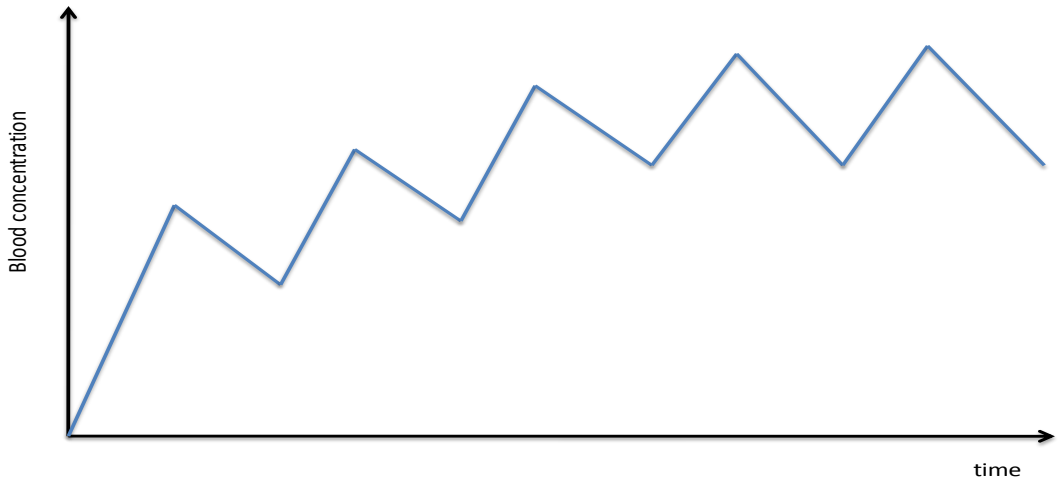


Figure 19.3 Compounds that are slowly excreted through the kidneys may accumulate in the body during the work week.

During a study, urine samples were collected from workers whose job was to manually apply an epoxy resin containing 4,4'-Methylenedianiline (MDA), with brushes. The urine samples were collected during four workdays and one weekend. The analysis showed that MDA was excreted in the urine and that the excretion rate of MDA varied greatly, which can be seen in Figure 19.4.

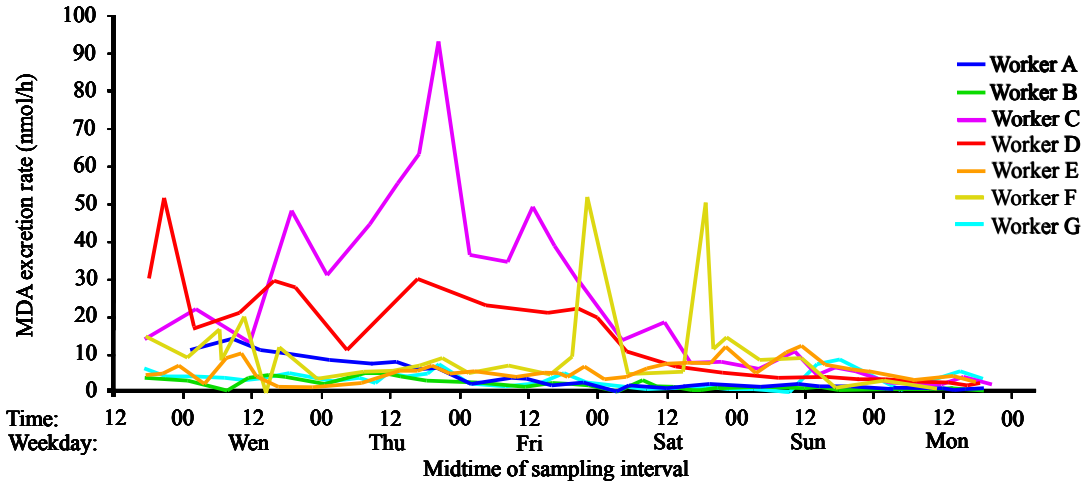


Figure 19.4 Variations in urinary MDA excretion rate in 7 workers with time.

Through comparison between the concentration of total MDA in urine and in blood, a linear relationship could be obtained, indicating that the urinary biomarker served as just as good indicator of exposure as the one in blood. [7-10]

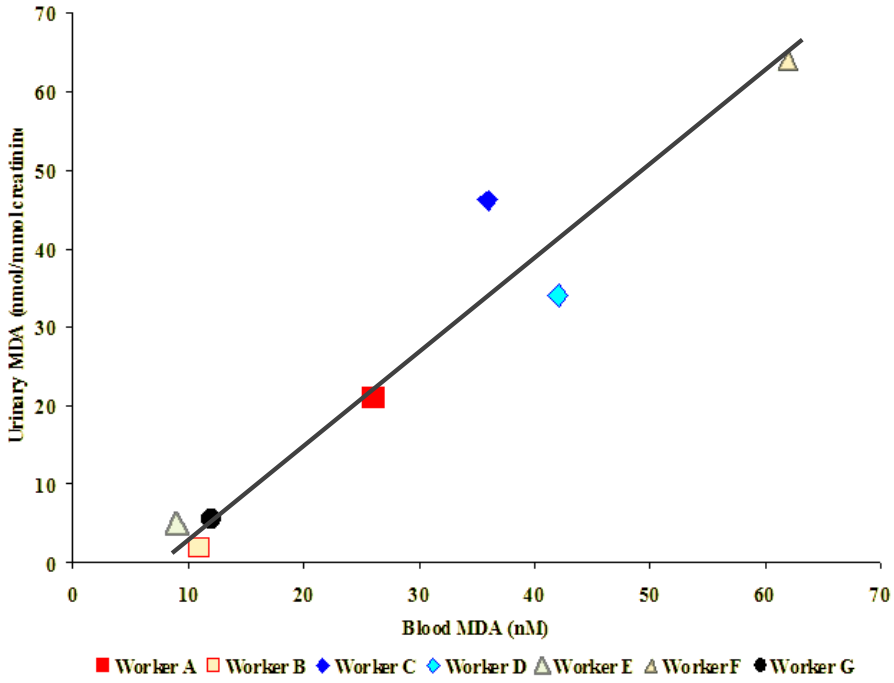


Figure 19.5 Relationship between the urinary concentration of MDA and the concentration of MDA in blood in 7 workers exposed to MDA:

19.1.2 Metabolism

Metabolism is the biotransformation of xenobiotics, i.e. reactions and processes which occur once a substance has been absorbed into the body. The purpose of metabolism is to convert xenobiotic substances into more hydrophilic metabolites that can be more efficiently excreted from the body. Chemical modification of a xenobiotic often leads to alterations in the biological effects of the compound. In many instances, it is the metabolite of the xenobiotic compound that exerts a pharmaco-dynamic effect in the body and not the parental molecule itself. Consequently, chemicals with toxic or tumorigenic effects may become less harmful or lose their toxic properties entirely. In a similar manner, harmless xenobiotic chemicals can undergo biotransformation into toxic or tumorigenic substances.

Some chemicals are able to stimulate the synthesis of enzymes involved in xenobiotic biotransformation. This process is known as enzyme induction. Enzyme induction has been stated as “an adaptive and reversible response to xenobiotic exposure.” [294].

19.1.3 Biotransformation

Xenobiotic biotransformation normally occurs in two steps – phase I and phase II, which takes place in the liver. The enzymes involved in the biotransformation process can be divided between these two groups. The enzymes participating in biotransformation are numerous and include for instance Cytochrome P450, which represents a group of metabolic enzymes that are expressed in most tissues. Phase I biotransformation normally precedes phase II. Molecules may however be transformed during phase II without first entering phase I. Conversion of a bioactive parent compound to less bioactive or inactive metabolites that are efficiently eliminated is termed *metabolic inactivation* or *detoxification* and is generally the purpose with biotransformation.

An illustrating example where biotransformation - instead of converting a chemical to a water-soluble and harmless metabolite - leads to formation of a more harmful substance (e.g., phenoxyl radical) is the biotransformation of benzene.

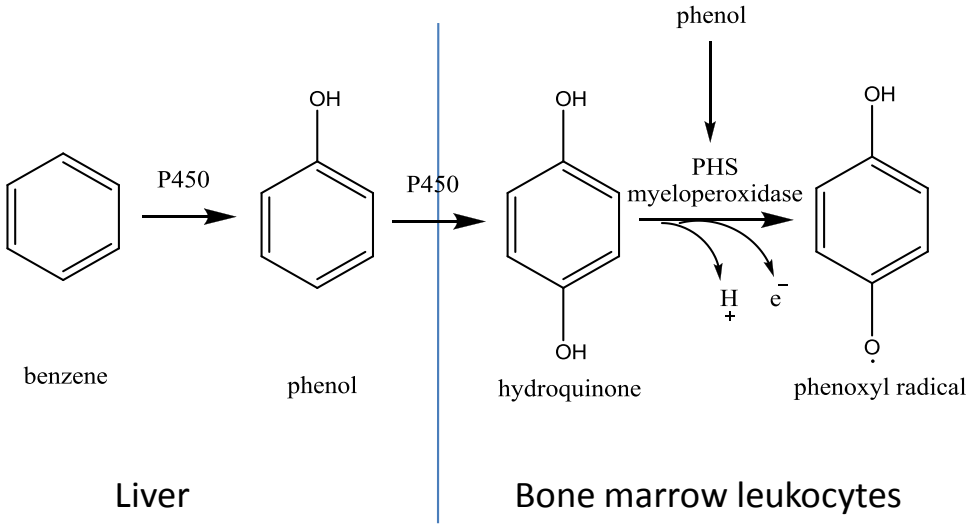


Figure 19.6 Cytochrome P450 (more specifically, CYP2E1) and peroxidase in the activation of benzene to myelotoxic metabolites. The resulting phenoxyl radical is able to bind to proteins and DNA in the bone marrow and eventually cause bone marrow suppression. The reaction of hydroquinone to phenoxyl radical by PHS myelooxidase is stimulated by phenol since it acts as a reducing agent for the enzyme.

19.1.3.2 Phase I biotransformation

Hydrophilic substances are generally excreted in urine, while lipophilic compounds undergo biotransformation in the liver. Phase I reactions involve hydrolysis, reduction and oxidation. These reactions expose or introduce a functional group such as –OH, -NH_2, -SH or –COOH and usually results in only a small increase in hydrophilicity.

Reduction of xenobiotics containing an aldehyde, ketone, disulphide, sulphoxide, quinine, N-oxide, alkene, azo or nitro group often occurs in vivo, although it is sometimes difficult to know whether the reaction proceeds enzymatically or non-enzymatically.

19.1.3.3 Cytochrome P450

The Cytochrome P450 enzymes are the most versatile groups of enzymes participating in detoxification of substances during phase I biotransformation. Other examples of enzymes involved in phase I reactions are alcohol dehydrogenases, monoamine oxidases and esterases.

In two individuals, who have been exposed to equal amounts of a xenobiotic substance, the concentration of a compound in biological fluids may significantly differ. This is due to individual differences in metabolic phenotypes, i.e. the activity of various metabolic enzymes differs between various individuals. Individuals can be grouped into “poor”, “extensive” or “normal” metabolisers with respect to a specific substance. The genotype is the genomic information regarding the expression of a gene. For instance, a gene coding for a metabolic enzyme may be deleted, single or expressed in multiple copies. The phenotype is the resulting expression of the protein and is partly a result from the genotype of the gene coding for the protein. However, the relation between genotype and phenotype can in some instances be

difficult to recognize since the resulting level of a protein in the cells may be influenced by other factors than the genotype, such as post-translational interaction with other proteins, or expression being inhibited on translational level.

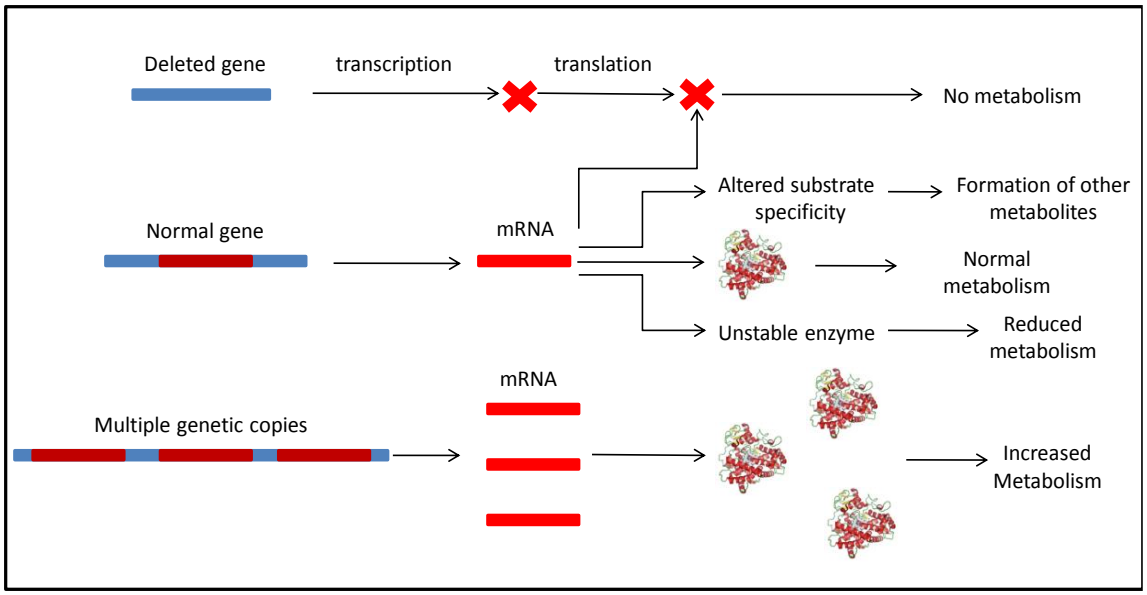


Figure 19.7 Overview of how individual differences in genotype influence the metabolism by affecting the phenotype, i.e. the resulting level of a metabolic enzyme in the cells.

The Cytochrome P450 enzymes include a large group of enzymes, i.e. CYP enzymes. For example may CYP2D6 play a role in the metabolic activation of chemical carcinogens, such as those present in the environment, in the diet, and/or in cigarette smoke. According to this hypothesis, individuals lacking CYP2D6 have a low incidence of cancer due to their inability to activate carcinogenic compounds. CYP2D6 is the enzyme within the Cytochrome P450 family with most phenotypic variability and participates greatly in metabolism of xenobiotics.

Variations occur both within and between different ethnic groups of population in regard of phenotypes [295]. For example, populations in North America and Europe are generally slower acetylators than Asian populations [296]. Table 19.1 provides an overview of differences between various ethnic groups with regards to metabolism.

Table 19.1 Variations in CYP2D6 drug metabolism between different ethnic groups.

Population	Poor metabolisers (%)	Rapid metabolisers (%)
American	7.7	4.3
British	8.9	*
German	7.7	0.8
African American	1.9-6.1	*
Nigerian	0-8.1	*
South African	19	*
Japanese	0	*
Colombian	6.6	1.7
Mexican	3.2	*

*No data is available.

Slow metabolisers are much more prone to be poisoned from a given dose of a specific compound as compared to normal and fast metabolisers. The implication of being slow metabolisers relative a fast metaboliser is when to determine any levels of chemicals or its metabolites in e.g., blood. If individual A is a slow metaboliser and individual B is a fast metaboliser and they are both exposed to the same dose of a substance, the concentration of the substance or its metabolite in the blood of individual A will be higher than for individual B after some time [61, 294].

To determine what group of CYP-specific metaboliser an individual belongs to, a mixture of about 4-6 different compounds with well-known metabolic profiles can be administrated. Examples of such compounds are Caffeine, Debrisquin, Chlorzoxazone and Diclofenac. Each substrate yields a number of metabolites and the resulting concentration measured in blood or urine is used to determine the phenotype of the specific individual [294, 297, 298].

In addition to genetic variations, diet and other chemicals can have an influence of the activity of Cytochrome P450 enzymes. For example do grapefruit juice and certain antibiotics inhibit their activity while tobacco smoke and St John's wort enhances it [296].

19.1.3.4 Phase II biotransformation

In phase I biotransformation the chemical or drug is transformed into a less lipophilic xenobiotic compound. In phase II transformation the xenobiotic is further converted into an even more water-soluble compound that can more efficiently be excreted from the body [294, 296].

Phase II biotransformation of xenobiotics may or may not be preceded by phase I biotransformation. These biotransformation reactions include: glucuronidation, sulphonation, acetylation, methylation, conjugation with glutathione (GSH) and conjugation with amino acids, such as glycine, taurine and glutamic acid. Conjugations reactions catalysed by transferases are the most common reactions during phase II biotransformation [296].

Due to species differences of phase I and II reactions, animal testing does not always reveal how a chemical is metabolised in humans.

19.1.4 Toxicology

Toxicology is the study of adverse effects of chemicals on living organisms. Adverse reaction refers to any unexpected or dangerous reaction to a drug or a chemical. Some toxic effects of chemicals are reversible while others are irreversible, which determines the severity of an injury brought to a tissue. For example, liver cells easily regenerate, while the cells in the

central nerve system (CNS) are highly differentiated and thus cannot multiply. As a consequence, the injuries brought to the liver are commonly reversible, while injuries to the CNS are not. Carcinogenic and teratogenic (ability to disturb the growth and development of an embryo or fetus) effect are also generally irreversible. In order to for a toxic compound to exert its harmful effects, the compound must be distributed to its sites of action in the body for a long enough period of time. As mentioned earlier, it might also be the metabolite of a compound and not the compound itself, which has toxic biochemical or physiological properties. In summation, the resulting response in the body to a toxic compound is not only dependant of the chemical itself but also of its physical properties, the exposure situation, metabolism and the overall susceptibility of the biological system [294]. As a consequence, it is of great importance to locate the metabolites of toxic chemical compounds that workers can be exposed to. Adverse health effects from exposure will be discussed further in chapter 7.

19.1.5 Duration and frequency of exposure

In toxicology, the exposure of experimental animals to chemicals is usually divided into four categories: acute, subacute, subchronic, and chronic. Acute exposure is defined as exposure to a chemical for less than 24 hours and examples of such exposure routes are intraperitoneal, intravenous and subcutaneous injection; oral intubation, and dermal application. While acute exposure usually refers to a single administration, repeated exposures may be given within a 24 hours period for some slightly toxic or practically non-toxic chemicals. Acute exposure by inhalation refers to continuous exposure for less than 24 hours, usually for 4 hours. Repeated exposure is divided into three categories: subacute, subchronic and chronic. *Subacute* exposure refers to repeated exposure to a chemical for 1 month or less, *subchronic* for 1 to 3 months and *chronic* for more than 3 months.

In human exposure situations, the frequency and duration of the exposure are usually not as clearly defined as in controlled animal studies, but many of the same terms are used to describe general exposure situations. Thus workplace or environmental exposures may be described as *acute*, occurring from a single incident or episode, *subchronic*, occurring repeatedly over several weeks or months, or *chronic*, occurring repeatedly for many months or years.

The duration and frequency of exposure has its implications which are illustrated with following the example: For many agents, the toxic effects that follow a single exposure are quite different from those produced by repeated exposure. The primary acute toxic manifestation of benzene is depression of the central nervous system. Repeated exposures to benzene can however result in bone marrow toxicity and an increased risk for leukaemia [294].

19.1.6 Interaction of chemicals

Because of the large number of different chemicals an individual may come in contact with at any given time (work place, drugs, diet, hobbies, etc.), it is necessary to assess the spectrum of responses, in order to consider how various chemicals may interact with each other. Interaction can occur in a variety of ways. Chemical interactions are known to occur by a number of mechanisms, such as alteration in absorption, protein binding, as well as the biotransformation and excretion of one or both of the interacting toxicants. In addition to these modes of interaction, the response of the organism to combinations of toxicants may be increased or decreased because of the toxicological response at the site of action.

The effects of two chemicals given simultaneously produce a response that may simply be additive of their individual responses or it may be greater or less than that what expected from the sum of their individual responses. The study of these interactions often leads to a better understanding of the mechanism of toxicity of the chemicals involved. A number of terms have been used to describe pharmacologic and toxicological interactions:

- *Additive effect* occurs when the combined effect of two chemicals is equal to the sum of the effects of each agent given alone, which means 1+1=2. This effect is most commonly observed when two chemicals are given simultaneously.
- *Synergistic effect* occurs when the combined effects of two chemicals are much greater than the sum of the effects of each agent given alone, meaning 1+1=20. For example, both carbon tetrachloride and ethanol are hepatotoxic compounds (they cause liver damage), but when combined they produce much more liver injury than the mathematical sum of their individual effects on liver at a given dose would suggest. The implication is if you drink alcohol and previously have been in contact with carbon tetrachloride, your liver damage will be severe.
- *Potentiation* occurs when one substance does not have a toxic effect on a certain organ or system, but when addition of another chemical makes it much more toxic, 0+1=10. Isopropanol for example, is not a hepatotoxic compound, but when administered in addition to carbon tetrachloride, it adds to the hepatotoxicity of carbon tetrachloride.
- *Antagonism* occurs when two chemicals administered together interfere with each other's effects, or when one chemical inhibits the actions of another one.

Due to synergistic effects, it is difficult to determine the toxicity response of a person who is submitted to a mixture of chemicals, even though the concentration of each individual substance is relatively low [294].

19.1.7 Undesired effects

The spectrum of undesired effects of chemicals is broad. Some effects are deleterious and others are not. In therapeutics for example, each drug produces a number of effects, but usually only one effect is associated with the primary objective of the therapy, all the other effects are referred to as undesirable or side effects of that drug for that therapeutic indication.

19.1.8 Immediate versus delayed toxicity

Immediate toxic effects occur rapidly after a single administration of a given chemical. Delayed toxic effects occur later on. One example of such an effect is the one of carcinogenic substances. It usually takes 20 to 30 years before tumours are detected in humans after the initial exposure.

19.1.9 Animal testing

Chemicals are generally tested for toxic effects in animals. However, enzyme systems in animals and humans may differ. Despite this fact, it is generally assumed that toxic effects occurring in animals are applicable to humans as well. This premise applies to all of the experimental biology and medicine. In laboratory animals, the toxic effect with regard to dose unit of body surface but not for body weight is within the same range as for humans. On basis of body weight, humans are generally about ten times more vulnerable than animals. From

this information, after animal testing, an appropriate dose of a chemical can be estimated which is safe for humans.

When it comes to carcinogenicity, chemicals that have been found carcinogenic are able to cause cancer in some species of animals, but not in all. Arsenic (As) may however be carcinogenic for all species. It has also been found that chemicals carcinogenic in animals are in all cases are also able to cause cancer in humans. [299-301] Chemicals that are carcinogenic in animals are however mostly considered carcinogenic as well in humans for safety purposes.

In order to assess the hazardous effects of chemicals in humans, laboratory animals must be exposed to it in high doses. It is assumed that the incidence of an effect in a population increases with higher doses of exposure, which is called the principle of quantitative dose-response relationship. Tests with high doses are carried out in order to characterize toxic effects that may arise from a given chemical. Exactly which toxicological tests that must be carried out for a chemical is not stated and partly depends of the intended use of the chemical. Risk assessment is based on toxic effects of the chemical as well as the effects from its structural analogues.

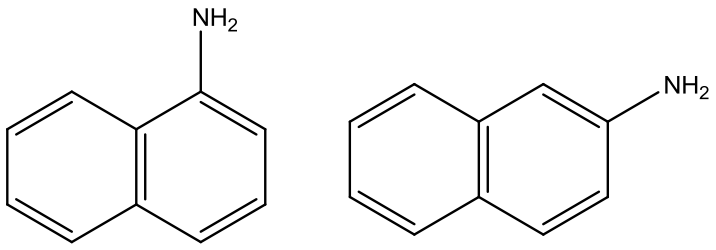


Figure 19.8 Two position isomeric forms of Naphtylamine. Structure A (α-naphtylamine) is "less harmful" but structure B (β-naphtylamine) is highly potent carcinogenic.

19.1.10 Individual differences in response

Exposure of a given chemical does not necessarily produce an equivalent response in all humans, which complicates the assignment of protecting workers against chemical hazards. In addition to variations in response to a chemical between various individuals, there may also be differences in what kind of biological damage that occurs in the tissues.

Individual variations in response to a chemical are due to genetic differences. These variations may also occur within a species. A *genetic polymorphism* is a difference in a specific gene present in more than 1 % of the population. Such polymorphism may cause *chemical idiosyncrasy*, which is termed as abnormal reactivity to a chemical or differences in toxic responses as compared to the general population. [302, 303] An individual who carries this genetic variation may be extremely sensitive to low doses of a given chemical, or extremely insensitive to high doses of it. The response to the chemical is however generally similar to the response in most individuals in other respects. Genetic polymorphism may also have an impact of the susceptibility of cancer. Mutations of tumour suppression genes or tumour-inducing genes increase the risk of development of cancer [294].

19.2 Instrumentation principles for Metal analysis

1. *Atomic absorption spectroscopy: In* short, the electrons of the atoms in the atomizer can be promoted to higher orbitals for an instant by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy (or wavelength) is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity. As the quantity of energy (the power) put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, from Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured. In this concept: 1) a flame is used to atomise the sample [304] 2) a graphite furnace [305] and 3) inductively coupled plasmas [306].

$$C = \frac{1}{\sigma \cdot l} \cdot \ln\left(\frac{I_0}{I}\right)$$

Beer-Lamberts law defines the concentration C as a function of the absorption cross section σ, the path length l and the light intensity of reference (I_0) and the sample (I).

2. *Inductive coupled plasma mass spectrometry* [307] (ICP). This is the most versatile analytical method to be applied on elemental analysis of metals. The instruments are a powerful tool of such analysis including coupled to high performance liquid chromatography (HPLC) including an ability to analyse organic compounds have various element (atoms in the periodic system) There are some principle in the ionisation process and de-clustering of interfering masses. This technique is the most superior analytical technique for determination of metals, the low detection limits are due to the very high degree of atomisation of the metal of interest. The temperature of the plasma by argon ICP is around 6500 K, sufficient to destroy most molecular bonds and ionise most elements. The instrument can either run samples without any separation or together with chromatographic separation, i.e., organometallic compounds with high throughput of samples. More than 90% of the elements in the periodic table can be ionised utilising argon gas.

19.3 Paintings and coatings

19.3.1 Thermal degradation

The constitution of epoxy polymers is the reaction of an organic molecule with an Epoxy group with compounds that have reactive groups e.g., various types amines, alcohols, carboxylic acids, isocyanates etc. When epoxy is thermally degraded at >300 °C several decomposition products are formed that may be harmful [308, 309]. Thermal degradation of Epoxy polymers has been sparingly described in literature [179, 310-312]. It has been concluded that the driving force of the degradation process is the formation of many different low molecular free radicals. What compounds are released during hot work will vary depending upon the cross-linking of the polymer. It has been shown that Bisphenol A is an important emission product during welding and combustion of epoxy polymers. Sensory irritants such as ketones, radicals, nitroso-amines, azo compounds, peroxides, acrolein, formaldehyde, phenols, alkyl benzenes and several different acid anhydrides have also been found during polymer degradation. In addition, diamines (TDA, HDA, IPDA and MDA etc.), isocyanates, flame retardants (organic phosphate esters) and biocides have been found,

depending on the specific product [109, 110, 313]. Some of the ingredients used in the formulation of epoxy polymers are well-known skin sensitizers. These compounds are however not volatile and do therefore not commonly act as respiratory sensitizers.

While heating epoxy plastics, airborne compounds are formed that workers may get exposed to. The epoxy polymers are a diverse group of polymers with great variety in composition. It is not possible to foresee what compounds that will be formed without information about chemical nature of the polymer. Even if the composition of the polymer is known in detail there are analytical methods missing for the most interesting expected groups of compounds, such as radicals and oxidation products. During thermal decomposition of epoxy polymers, the same compounds have been found in the air as the ones used in the formulation of the polymer, but regarding other breakdown products, very little is known. The thermal degradation of epoxy polymers may form several potentially toxic compounds in both gas and particle phase. Some different groups of substances are listed below:

- Amines [Toluene diamines (TDA), Hexamethylene diamine (HDA), Isophorone diamine (IPDA), Methylene dianiline (MDA) and several aliphatic monoamines etc.]
- Aminoisocyanates (of aromatic and aliphatic isocyanates/amines)
- Carbonyl compounds (e.g., formaldehyde and other aldehydes)
- Biocides
- Flame retardants (phosphor based and halogenated compounds etc.)
- Pigments
- Monoisocyanates (ICA, MIC, EIC, PhI)
- Diisocyanates (TDI, HDI, MDI etc.)
- Acid anhydrides (Phtalic anhydride etc.)
- Organic phenol compounds (such as BPA)

We are not aware of any studies revealing details of exposure to thermal decomposition products of epoxy polymers (e.g., Epoxy urethanes) among workers in the petroleum industry.

19.3.2 Formation of radical species from coatings

The chemical formation of various coatings occurs through a radical driving procedure and is sensitive to the reverse effect, e.g., formation of free radicals. Degradation may be induced in a number of ways, such as through *photo-oxidative degradation, thermal degradation, ozone-induced degradation, mechano-chemical degradation, catalytic degradation and biodegradation* [314]. Without stabilizer all coating would break down rapidly. With time, coatings become more susceptible to degradation and thus the formations of free radicals. Radicals are highly reactive species that are formed through degradation of polymers and other organic compounds, through auto-oxidation in living organisms and can be found in exhaust gases and in cigarette smoke where one puff of a cigarette gives as an estimate 10^{15} radicals. Environmental produced radicals in work environments will not be dealt with here.

Practically no papers are seen dealing with this issue. It is further confirmed that in year 2004 a meeting was held in USA with 70 participates to outline this problem and to come up with a strategy [315]. At this meeting the recommendations were: ”*A common theme running through workshop discussions was the need to better characterize and understand the ‘reacting’ indoor environment, with an emphasis on the chemicals that most affect human health—the ‘biologically relevant’ compounds. New methods need to be developed that can detect some of the elusive, short-lived, highly reactive product”.*

19.3.2.1 Photo-oxidative degradation

When a coating material is exposed to UV- and visible light, various processes are initiated, for example extensive chain scission resulting in two free radical species [316]. The formed radical (R^*), forms together with oxygen (O_2), a peroxide radical ($R\text{-}OO^*$) which can undergo further reactions. This kind of degradation of polymers, forming radical species occurs in many instances.

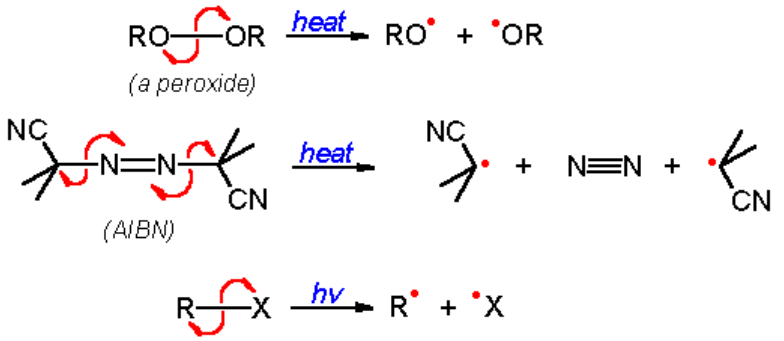


Figure 19.9 Bond scission affording free radicals.

In a polymer such as Polyurethane (PUR), the main process that occurs is scission breakdown of the –N-C- and –C-O bonds in the position α to the carbonyl group by the urethane group [317]. Radicals can react with hydrogen (H) via hydrogen atom abstraction, note that this can never occur with water.

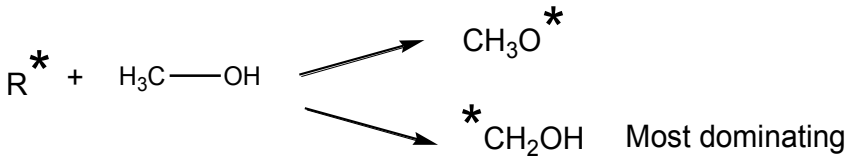


Figure 19.10 Hydrogen abstract of methanol and a free radical whereas another radical is produced.

The total process, described above, is illustrated by the photo-oxidation process for PUR which can be seen in Figure 19.11.

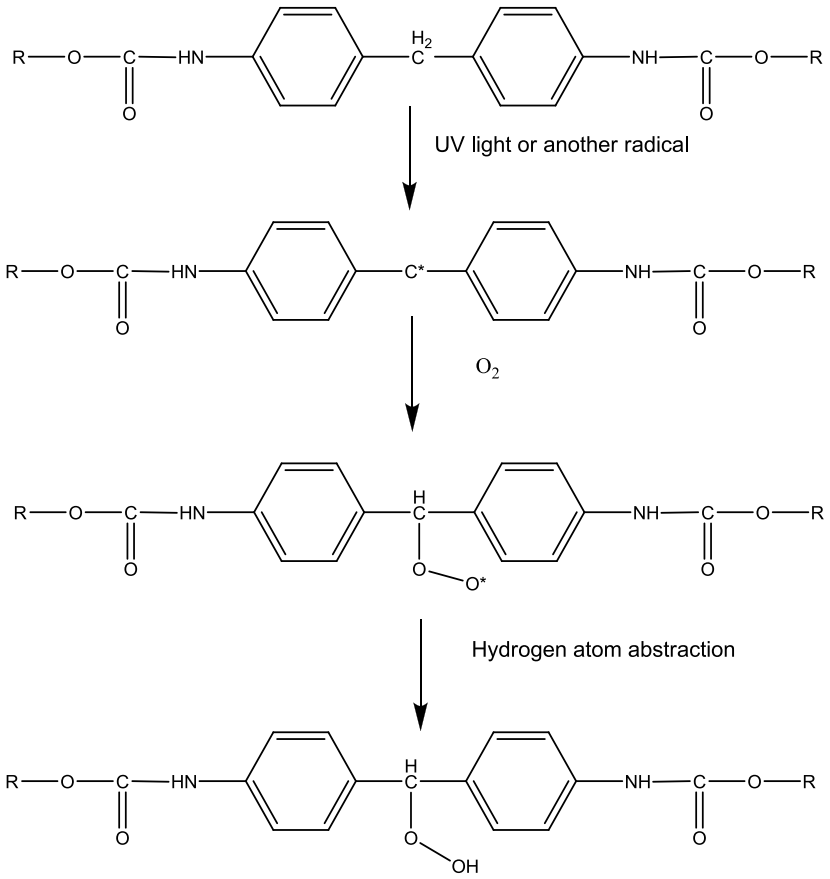


Figure 19.11 Some of the processes involved in Photo-oxidation of PUR.

19.3.3 Photo- and Thermal degradation

Photochemical and thermal degradation are together classified as oxidative degradation. Thus in principle, the degradation process is the same. A major difference between the two, is that photochemical degradation, which is initiated by UV-radiation mainly occur on the surface of the polymer, whereas thermal degradation occurs throughout the entire polymer [318]. The de-polymerization reaction in thermal degradation does not need to be initiated at terminal end of the macromolecule. A large number of polymers de-polymerize at elevated temperatures [319, 320]. For instance, polymethylmethacrylate (PMMA) may be converted almost quantitatively back to the monomer.

19.3.3.1 Ozone-induced degradation

Ozone in the air, even in very low concentrations, markedly accelerate the aging of polymeric materials [321]. The exposure of polymers to ozone results in the rapid and consistent formation of a variety of saturated carbonyl- and unsaturated- carbonyl products based on aliphatic esters, ketones and lactones as well aromatic carbonyl compounds associated from styrene polymers.

19.3.3.2 Biodegradation

Biodegradation is a biochemical transformation of polymers into organic compounds such as carbon dioxide and water [322].

19.3.4 Summary

The formation of free radicals from degradation of polymers can be summarized as follows:

1. *Thermal degradation*: Heat is an important physical factor in oxidation, which involves random breakdown of C-C bond on which carbon-centred radicals $R^{\cdot}$ are formed.
2. *Autoxidation:* The presence of atmospheric oxygen influences thermal degradation of organic material dramatically. This occurs even at very mild conditions. This auto-initiated reaction of organic matter with oxygen is classified as autoxidation. Examples of highly reactive species that are initially formed are $RO_2^{\cdot}$, $HO^{\cdot}$.
3. *Combustion*: Combustion is an extreme oxidation process of organic materials which takes place at temperatures above 400 $^{\circ}$C. The mechanism of high-temperature oxidation differs substantially from the autoxidation process. Hydrogen peroxide is generally formed during degradation reactions at temperatures above 200 °C.
4. *Induction by radiation*: Degradation may be induced by radiation of for example sunlight and other light with high energy.
5. *Mechanical stress*: Linear chain molecules of polymers may be scission and radicals may be formed by mechanochemical processes when exposed to high shear, e.g., during extruding, grinding or calendaring [323].
6. *Atmospheric pollutants*
7. *Biological actions*.

During the conduction of a number of different work activities, workers may be exposed to radical species. Radical species can modify various proteins and DNA and hence promote mutagenicity. In worst case radicals can cause cancer or other serious diseases.

19.4 Polyurethanes

19.4.1 Biological properties of PUR

The metabolic pathway on the formation of nitrosoarene from corresponding amine is described in Figure 19.12.

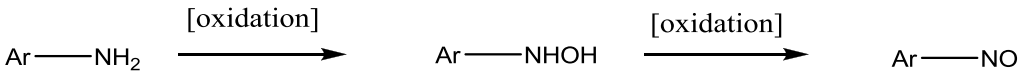


Figure 19.12 The oxidative metabolism of arylamine to N-arylhydroxylamine to Arylnitroso compound.

Figure 19.13 shows schematic reactions of arylisocyanates during biotransformation. When isocyanates are brought into the body, the reactive isocyanate group is immediately hydrolysed to the corresponding amine, (reaction A) or form conjugate with proteins (reaction C). The administrated arylamine or arylamine formed by hydrolysis of the corresponding arylisocyanate is transformed into the corresponding nitroso compound by oxidative processes in the metabolism (reaction A).

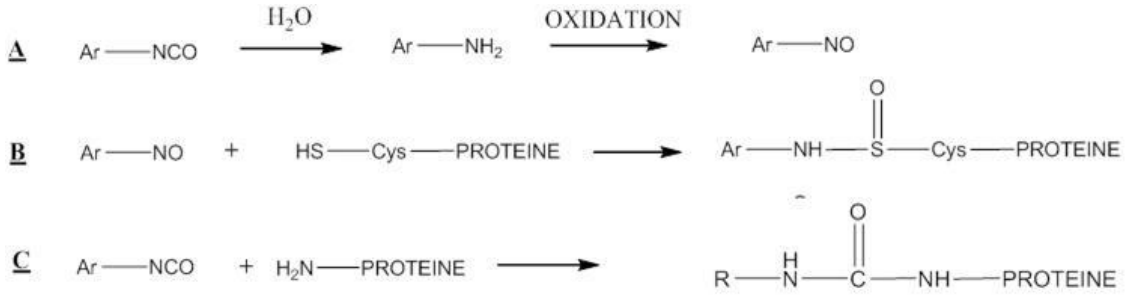


Figure 19.13 Metabolic reaction of arylisocyanate and corresponding arylamine

Arylnitroso compounds are electrophilic and hence very reactive. These compounds react readily with nucleophilic thiols which are found in proteins such as glutathione (GSH) including those found in haemoglobin, as described in reaction B in Figure 19.13. Adduct formation between arylnitroso and haemoglobin can be used for the quantification of human exposure to environmental arylamines and arylisocyanates. Exposure of arylnitroso compound may also be produced by thermal oxidative processes which are included in work related processes. Hitherto, only the arylamino level in urine or blood is determined, which gives the dose of administrated amine and corresponding isocyanate exposure. Determination of exposure of these sole compounds is not sufficient in terms of monitoring essential biomarkers during thermal degradation of PUR. Hence, it would be essential to investigate this further and to develop analytical methods regarding the exposure of arylisocyantes corresponding arylamines and thereof produced arylnitroso compound and corresponding protein conjugates. At present such simple methods do not exist. These methods would enable monitoring of low levels of metabolites of N-arylhydroxylamine and arylnitroso compounds and in addition adducts to haemoglobin emerged from exposure of isocyanates and its corresponding amines [324-326]. Very little is known about their biological effect as protein conjugates and needs to be further investigated.

Isocyanates are able to bind to various proteins in the body, but there are significant differences in which sites of the protein various isocyanates bind to [327]. The N-terminal amino group of the protein or to the side-chain of the amino acids Lysine (primary amine),

Cystein (thiol), Histidine (secondary amine in the imidazole ring), Tyrosine (hydroxyl group) and Serine (hydroxyl group) are all susceptible to isocyanate binding [328]. In vitro experiments showed that for 2,4-toluenediisocyanate (2,4-TDI) and 1,6-hexamethylene-diisocyanate (HDI) the thiol group of the Cystein side chain is the most reactive site of a protein, followed by the N-terminal amino group, Lysine and Tyrosine. Histidine reacts with HDI but not with TDI [327]. In summary, in vivo formation of stable adducts between isocyanates and proteins offer a possibility for biological monitoring of isocyanate exposure in humans. Exposure to isocyanates, such as MDA have also been reported to cause an immunological response, resulting in non-significantly elevated levels of isocyanate-specific IgE-levels [329], thus potentially enabling exposure assessment of immunogenic isocyanates by measurement of isocyanate-specific antibodies.

Due to the reactivity of isocyanates, they have the potential of reacting in a number of different ways once they have been brought into the body. Hence, a number of potential biomarkers for isocyanates exist, as is shown in Figure 19.14.

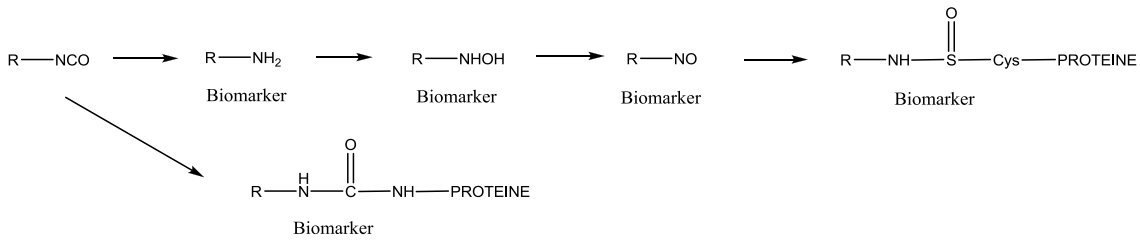


Figure 19.14 General complexity of biomarkers from isocyanates.

The biological monitoring of isocyanates and aromatic amines are frequently occurring as compared to many other biomarkers. For some of the isocyanates there are published controlled studies of volunteers with the relation between the exposure and the levels of biomarkers in urine, erythrocytes and plasma. For MDA there is also published data regarding the dermal uptake among volunteers and the levels in blood together with their urinary excretion. For a few isocyanates there are exposure data from workers and biomarkers essentially confirming the volunteer studies [330-337]. Several papers have been presented regarding the details in the work-up of the samples followed by LC-MS and/or GC-MS analysis [338]. The literature data still suffer from adequate data of exposure related the development of the disease.

In order to know more details regarding the development of the disease it would be an advantage to have data from other possible metabolic pathways in humans, as there are a vast number of potential metabolic routes in the body.

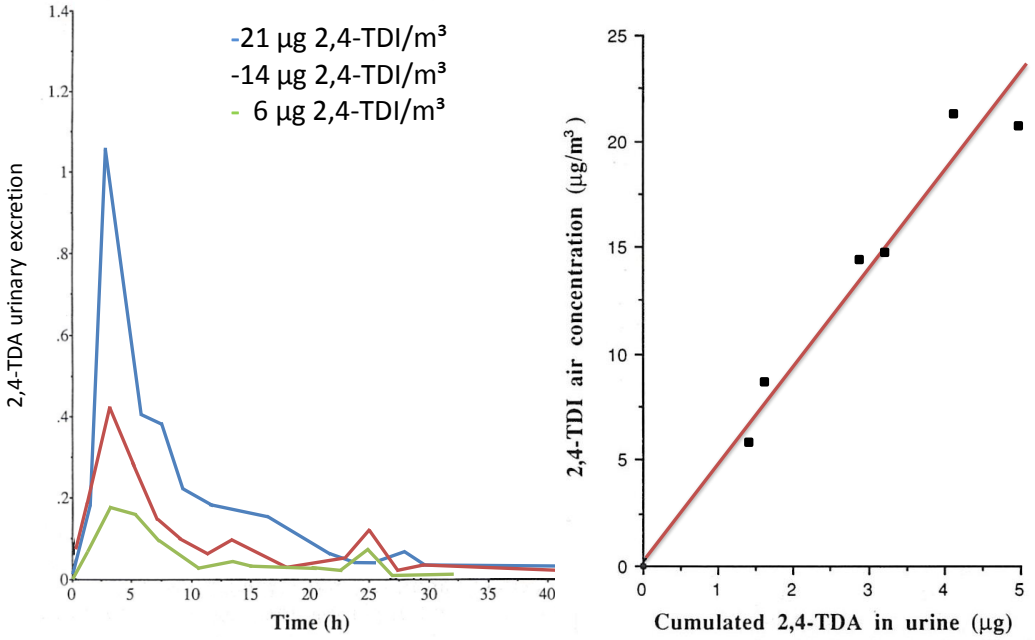


Figure 19.15 (Left) The concentration of 2,4-Toluene diamine as a result to exposure of test subjects to three difference air concentrations of 2,4-Toluene diisocyanate. (Right) The concentration of 2,4-Toluene diisocyanate in air versus the cumulated urinary concentration of 2,4-Toluenediamine, giving a linear relationship [116].

19.5 Epoxy

19.5.1 Biological relevance

Epoxy paints are two-component products [136, 137]. The building blocks are normally diglycidylether bisphenol A (DGBA) with a molecular weight of 340 Da (low molecular weight Epoxy). The DGBA is manufactured by reacting Bisphenol A (BPA) with epichlorhydrine (Figure 19.16). Bisphenol A is listed as a contact allergen and has recently been associated with chronic deceases. In a study performed in the U.S the BPA concentrations was examined together with the health status in the general adult population. The study included 1455 adult test subjects of 18-74 years in ages. High urinary BPA concentrations adjusted to the creatinine content was related to cardiovascular diseases when adjusted to age and sex of the participants. Increased levels of BPA in urine were also associated with diabetes and clinically abnormal concentrations of liver enzymes. Data from the National Health and Nutrition Examination Survey 2003-2004 was used in the study [138-141]. The European Union did however present in the early 2008 a report describing present protective measures related to BPA as sufficient [142].

DGPA is also a potent contact allergen. Attempts are therefore being made to reduce the amount of DGBA by converting it to molecules with longer chains (high molecular weight Epoxy) through pre-polymerization. The amines listed in Table 12.2 are most often used in Epoxy systems for corrosion prevention are polyamines, polyamides or amine adducts such as diethylene triamine (DETA), triethylene tetramine (TETA), tetraethylene pentamine (TEPA), 2,4,6-tris-(dimethylaminomethyl)-phenol, m-xylene α,α diamine (XDA), isophorone diamine

(IPDA), 1,2-cyclohexane diamine, n-aminoethyl piperazine, 3-cyclohexylamine propyl amine, benzyldimethyl amine, 3-dimethylamino propyl amine, n,n-diethyl-1,3-diaminopropane [106, 339]. In many cases, the curing agent may be an isocyanate compound.

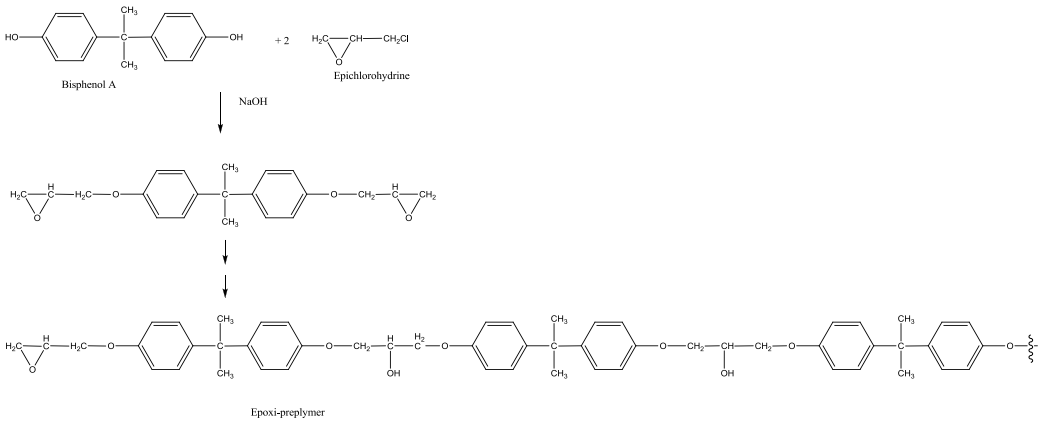


Figure 19.16 Manufacturing of Epoxy prepolymers and some of its constituents which may be harmful in humans.

We are not aware of any studies revealing details of exposure to thermal decomposition products of Epoxy polymers (e.g., Epoxy urethanes) among workers in the petroleum industry. On basis of this information we enforce the development of analytical method(s) for decomposition products. Epoxy consists of a number of chemical substances. Some of them are described in more detail below.

19.6 Bisphenol A (BPA),

19.6.1 Biological relevance

Bisphenol A is a hormonal-related substance [144] with estrogenic characteristics. Only few epidemiological studies have been conducted on its effect on humans. However, a significant relationship between BPA concentration in urine and increased risk of heart diseases and cardiovascular diseases and diabetes has been reported in USA, as discussed earlier. An overview of various BPA analogues, related compounds and their biological effects have been described [145].

Amongst all the possible sub-chemicals in BPA, the *DGEBA* Epoxy resin belong to the most common causes of occupational allergic contact dermatitis [340].

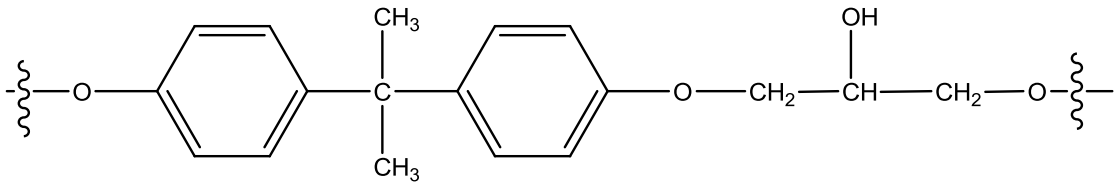


Figure 19.17 Chemical structure of DGEBA Epoxy resin.

Another functional group in BPA to be taken in consideration is the glycidyl ether group, with reactive epoxide functionality which can be seen in Figure 19.18.

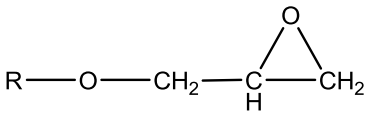


Figure 19.18 The chemical structure of the glycidyl ether group.

The epoxide group is a reactive group which is able to react with several functional groups in living cells. The proposed metabolic pathways for glycidyl ethers are shown in Figure 19.19 [341].

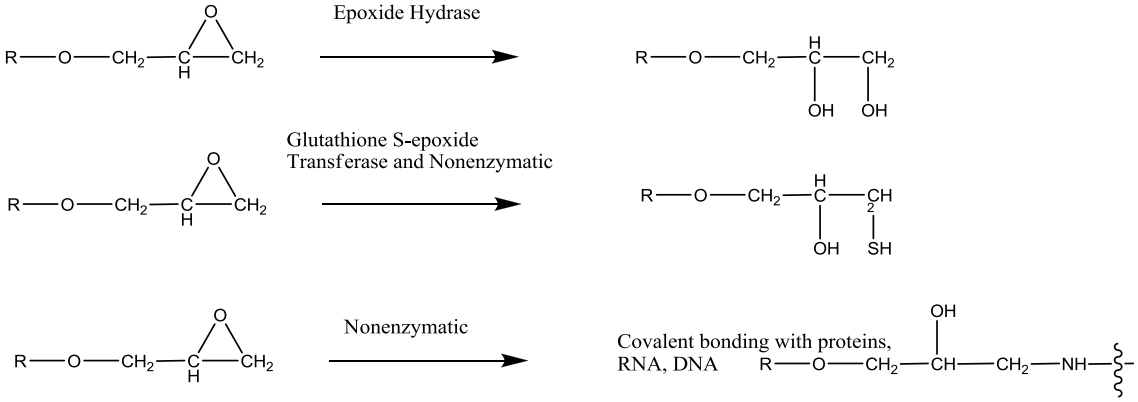


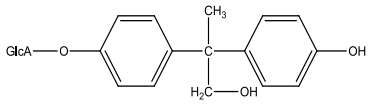
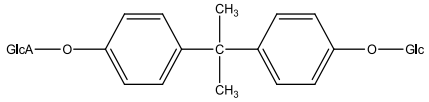
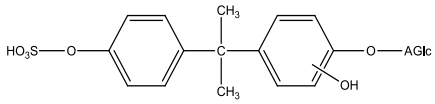
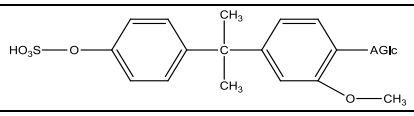
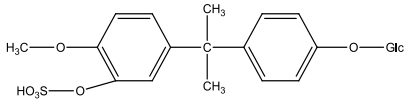
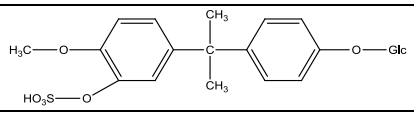
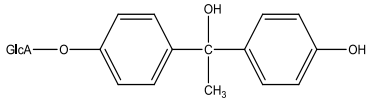
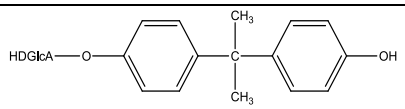
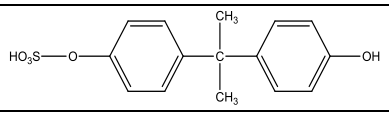
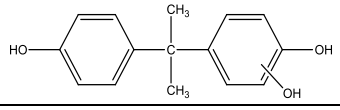
Figure 19.19 The proposed metabolic pathways for glycidyl ethers [341].

19.6.2 Biomarkers

There are a number of primary markers of BPA and amongst them; the liberation of amines may be one important class of biomarkers.

A study describes the biotransformation of bisphenol A in a mammalian model, combined with HPLC-MS/MS [143]. Metabolites were isolated from urine, liver or the digestive tract.

Table 19.2 Metabolites of BPA found in mice [143]. Abbreviations used: Glucuronic acid (GlcA), dehydrated glucuronic acid (DHGlcA) and glucose (Glc).

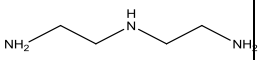
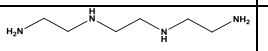
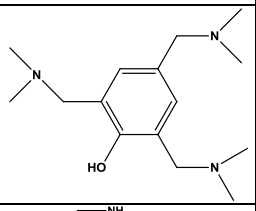
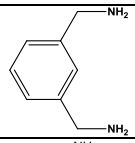
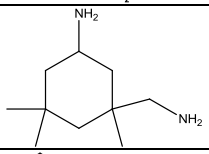
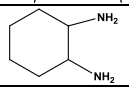
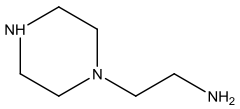
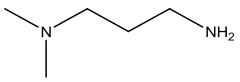
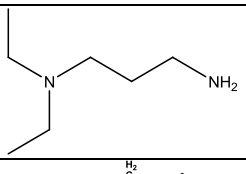
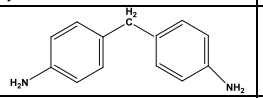
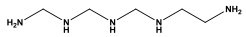
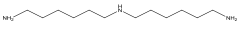
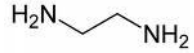
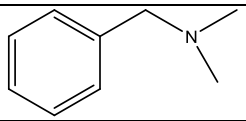
Metabolite	Structure	Metabolite source
GlcA-BPA-OH		Urine
GlcA-BPA-Glc		Urine
HO_3S-BPA(OH)-GlcADH		Digestive tract
HO_3S-BPA(OCH_3)-GlcADH		Digestive tract
HO_3S-BPA(OCH_3)-Glc		Liver, urine
HO_3S-(OH)BPA(OCH_3)-Glc		Liver, urine
GlcA-BPA		Urine, liver, digestive tract
GlcADH-BPA		Digestive tract
HO_3S-BPA		Faeces, urine
HO-BPA		Faeces

It has been concluded, that more extensive studies must be conducted to establish the risk of BPA exposure on humans [145]. However, BPA should be considered as a harmful substance for humans and thus be accounted as harmful for its exposure to human.

19.7 Amines

19.7.1 Some major amines found from hot work

Table 19.3 Amines found from hot metal work [67-69][7]

#	Amines	CAS number	Structure	OEL
1	Diethylene triamine (DETA)	111-40-0		Sensitising substance [7] 1 ppm [61]
2	Triethylenetetramine (TETA)	112-24-3		Sensitising substance [7]
3	2,4,6-Tri(dimethyl amino-methyl)-phenol (TEPA)	90-72-2		-
4	m-Xylene α, α diamine (XAD)	1477-55-0		Sensitising substance STEL*: 0.1 mg/m3 [7]
5	Isophorone diamine (IPDA)	2855-13-2		-
6	1,2-Cyclohexandiamine	694-83-7		-
7	n-Aminoethyl piperazine	140-31-8		-
8	3-Dimethylaminopropylamine (DMPA)	109-55-7		-
9	N,N-Diethyl-1,3-diaminopropane	104-78-9		-
10	4,4-Methylenedianiline	101-77-9		0.1 ppm [61]
11	Tetraethylene pentamine (TEPA)	112-57-2		-
12	Hexamethylene triamine	100-97-0		-
13	Ethylene diamine	107-15-3		10 ppm [61]
14	Benzyldimethyl amine	103-83-3		-

19.7.2 Metabolic pathway of amine

Aromatic amines and aliphatic amines are metabolised differently in the body. There also exist differences in the metabolism of primary-, secondarily- and tertiary amines. One example is the formation of secondary amines into corresponding nitrosamines in vivo, which may be carcinogenic [166].

1,6-Hexamethylenediamine (HDA) is a primary aliphatic amine. A metabolic pathway, described in Figure 19.20, has been proposed for this compound [342-345].

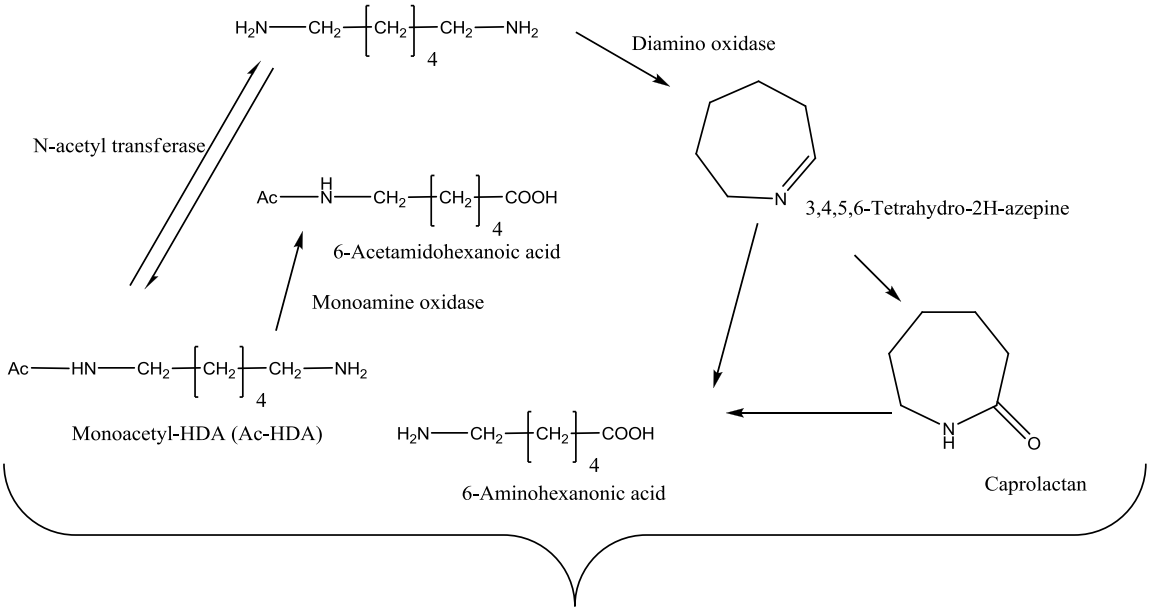


Figure 19.20 Proposed metabolic pathways for HDA in man.

19.8 Acrylates

19.8.1 Thermal degradation

Thermal decomposition of acrylate plastics results in the formation of aerosols containing a mixture of different compounds in gas and particle phase. To reveal the exposure both general methods for different groups of compounds and selective methods for certain compounds are necessary. There are missing information regarding the air exposure to acrylic compounds and especially compounds formed during the thermal decomposition.

Various acrylates have been investigated in terms of thermal breakdown of the polymer. In the thermal process the formation of various small molecules are produced due to random main-chain scission [346], depending on the constitution of chain.

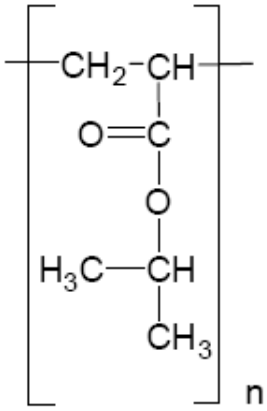


Figure 19.21 Repeating unit (monomer) of one type of acrylate polymer

The most abundant degradation products emerge from the alkyl ester decomposition, see 0. The degradation products may be monomers, dimers, saturated diesters, trimers, olefins, aldehydes, various carboxylic acids or alcohols. Degradation of poly-methyl-methacrylate yields the monomer. In addition, during thermal degradation a number of free radical species are formed. It is clear that what products are formed depends on the nature of the acrylate, e.g., chain length, and the temperature during thermal degradation. In Table 19.4, the distribution of various breakdown products formed during thermal degradation of acrylates versus the monomer chain length. Table 19.6 presents an example of the degradation pattern as a result from various temperatures during thermal degradation.

Table 19.4 Thermal degradation product (%) at 550 °C of poly-methacrylate [346].

Chain length	Olefin	Aldehyde	Alcohol	Methacrylic acid	Monomer
4	8.6	<1.0	<1.0	7.5	82.9
5	20.1	<1.0	<1.0	15.2	62.0
6	17.4	<1.0	<1.0	10.6	69.2
7	24.8	<1.0	<1.0	13.3	58.7
8	19.9	<1.0	<1.0	8.6	68.3
9	25.4	<1.0	<1.0	10.4	59.4

Table 19.5 Thermal degradation product (%) at various temperatures, chain length 6 [346].

Product	400 °C	450 °C	550 °C	650 °C
Olefin	0.9	2.8	17.4	28.9
Aldehyde	<1.0	<1.0	<1.0	<1.0
Methacrylic acid	0.1	1.0	10.6	18.3
Monomer	98.9	95.1	69.2	45.9

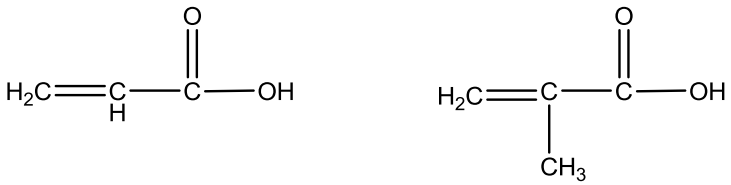
Table 19.6 Thermal degradation product (%) at various temperatures, chain length 6 [346].

Product	400 °C	450 °C	550 °C	650 °C
Olefin	0.9	2.8	17.4	28.9
Aldehyde	<1.0	<1.0	<1.0	<1.0
Methacrylic acid	0.1	1.0	10.6	18.3
Monomer	98.9	95.1	69.2	45.9

19.8.2 Methacrylic acid (MAA) or Methyl methacrylate (MMA)

It is not a simple task to point out specific biomarkers from acrylate degradation, due to the great diversity of acrylates used in industry and the great number of degradation products are formed depends on a number of different factors.

However, one degradation product seems to be feasible to use as biomarker - *Methyl ester of acrylate acid (MEMA)* [347].



Acrylic acid Methacrylic acid

Figure 19.22 Structures of acrylic acid and methacrylic acid.

Methacrylic acid (MAA) is not a harmful substance but is classified as an irritant and as a sensitizer in animals and humans [348]. The irritating effect of the acrylate and methacrylates is derived from the action of acrylic acid or Methacrylic acid formed by ester hydrolysis. The structures of these two acids are shown in Figure 19.22.

Effects of MEMA can be attributed to a reaction with protein-thiol groups of mucous membranes or to Methacrylic acid by ester hydrolysis [349]. Very little data regarding the toxicity of MEMA or its metabolites are available [349]. Urinary methanol has been used as a biomarker for MEMA, for example to investigate the exposure situation of dental laboratory technicians [350, 351].

Table 19.7 Toxicity data of Methacrylic acid and Methacrylic acid alkyl esters. All data are available as data sheets from the corresponding company [104, 188, 352].

Methacrylic acid methyl ester have clastrogenic effects in high doses, i.e. it is able to induce disruptions or breakages of DNA:

Compound	Methacrylic acid	Methacrylic acid methyl ester
CAS number	79-41-4	80-62-6
Irritating effects	Corrosive to eyes and skin.	Slightly irritating to eye and skin. Sensitizing.
Repeated Dose toxicity	Local irritant	Local irritant
In vitro testing of genotoxicity and carcinogenicity	DNA binding screening test positive. Ames test negative.	Contradictory indications of clastrogenic effects in high doses
Reproductive Toxicity		No embryo or fetal toxicity, no developmental effects up to maternally toxic concentrations

Acrylates and methacrylate can both be metabolised through two different pathways in the body. The importance of the metabolic pathways for acrylates and methacrylate do however differ due to higher alkylating properties of acrylic acid esters as compared to methacrylates [353]. The two possible pathways are listed below:

1. By ester hydrolysis (esterase) in various tissues. The toxicokinetic pattern of the hydrolysis depends on the ester alcohol. Esters of branched alcohols very often undergo slower hydrolysis due to the steric hindrance. Generally, hydrolysis is regarded as a mechanism of detoxification, since the intact esters are more lipophilic as compared to the corresponding acids.
2. Acrylates and methacrylates can produce conjugates with glutathione (GSH), which occurs both vitro and in vivo. The conjugation takes place spontaneously and enzymatically by reaction of the double bond in the compound and the thiol group of GSH. This reaction rate is higher for acrylates than for methacrylates due to hindrance of the methyl group. The conjugation reaction does not occur with either acrylic acid or methacrylic acid [353, 354].

Due to the aliphatic double bond in Acrylate and Methacrylate, there's a high possibility that the corresponding epoxides are formed in vivo. Epoxides are often biologically reactive compound, c.i.f., epoxy in section 19.5.

19.9 Polyvinyl chloride (PVC)

19.9.1 Thermal degradation

PVC paints are mainly composed of PVC. The literature shows that in connection with thermal

degradation of PVC, hydrochloric acid gas (HCl) is formed in a process called dehydrochlorination at temperatures above 150 °C [355-357]. This is the primary step during thermal degradation of PVC (Figure 19.23).

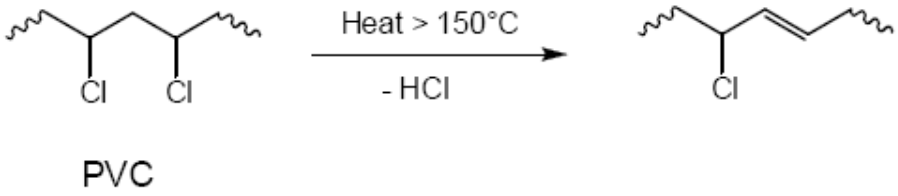


Figure 19.23 Elimination of hydrochloric acid from PVC – a process called dehydrochlorination.

Some sources claim that dehydrochlorination is possible at as low temperatures as 80 °C [136]. The thicker the vinyl coat is – the more HCl will be formed. The dehydrochlorination process is an elimination reaction which leads to the formation of a double bond which favours further vicinal dehydrochlorination. The double bond is also susceptible to the formation of epoxides (Figure 19.24).

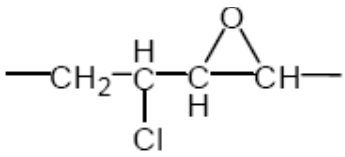


Figure 19.24 Epoxide structural element in PVC.

The biological relevance of epoxides has been discussed in section 2.1.2.1.2. In addition, on the α-position to the chlorine atoms is a position of hyperoxides that further can give free radicals and oxy radicals, which was discussed in section 19.3.2.

19.10 Polyester

19.10.1 Biological relevance

Polyester paint [136, 137] consists of unsaturated polyester adhesive dissolved in styrene or methylstyrene (vinyl-toluene). In the curing process, a reaction between the aromatic vinyl group and the polyester, takes place when accelerators and peroxide are added.

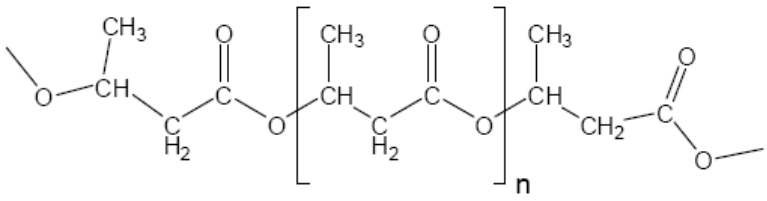


Figure 19.25 Schematic structural formula of polyester polymer

Polyesters often depolymerise and reform the original monomers [187]. This means that in the event of thermal degradation of styrene-based polyester, styrene or methyl styrene (vinyl toluene) will be important components in the low-temperature zone. The metabolic pathways of styrene in man are shown in Figure 19.26, revealing possible biomarkers for styrene [188]. In radical reactions, oxidation products will assumingly form e.g., styrene oxide (see Figure 19.26), a well-known toxicant as well as many other compounds. As a result from styrene exposure, styrene will be present in blood and can hence be used as a biomarker itself, while Mandelic acid and Phenylglyaxylic acid can be detected in urine. Mandelic acid and Phenylglyaxylic acid are the most commonly used biomarkers for styrene exposure[7, 61].

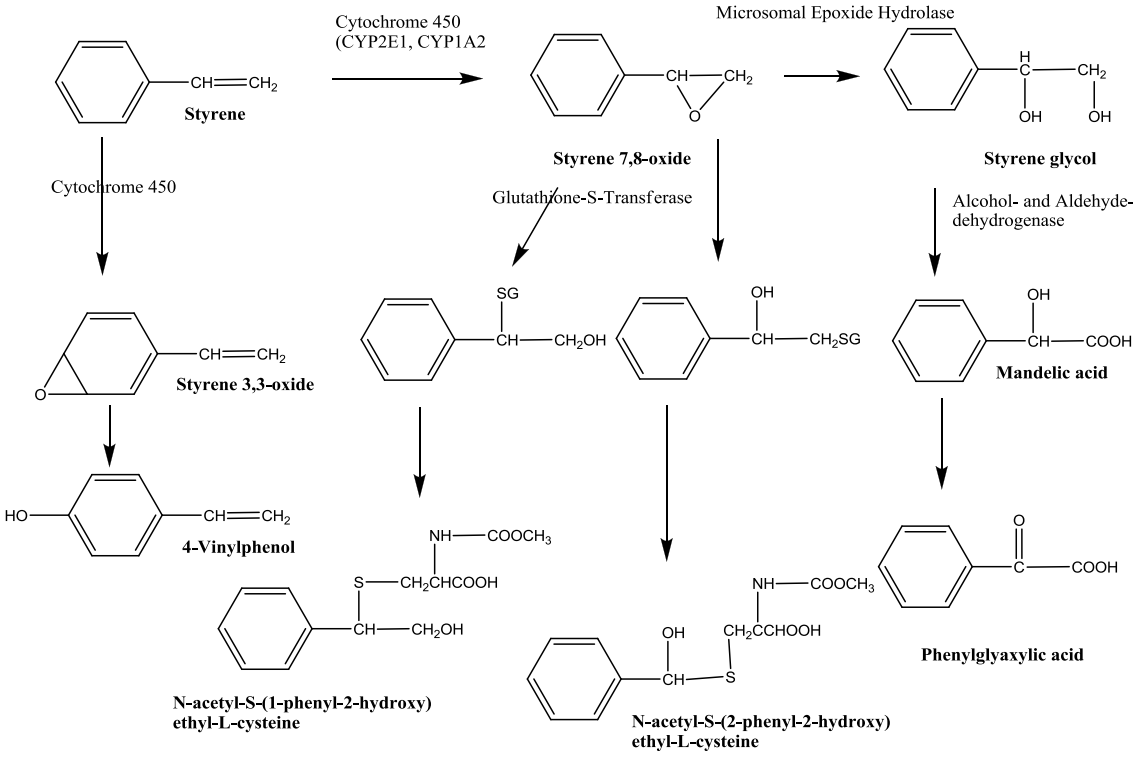


Figure 19.26 Metabolism of styrene in humans [188].

Even more metabolites than those shown in Figure 19.26 have been found for styrene [191], including various conjugates such as sulphate- and glucuronide conjugates, i.e. products from phase II metabolism.

19.11 Solvents

19.11.1 Toxic effects of solvents and vapours

The term solvent refers to a class of liquid organic chemicals of variable lipophilicity and volatility. Non-polar organic solvents of low molecular weight readily diffuse across the membranes in the alveoli, thus entering the blood through inhalation. Non-polar solvents may also be absorbed through the skin due to their lipophilicity. The lipophilicity of solvents enables them to accumulate in fatty rich tissues, such as the central nervous system, which is the primal biological effect of solvents on humans. Various solvents cause different toxic effects, such as carcinogenicity and fatal damages to the liver. The blood brain barrier consists of cells surrounding the capillaries in the CNS thus preventing substances to enter the brain directly from the blood. Lipophilic and uncharged compounds are able to cross the barrier. Due to the ability of solvents to readily cross the blood brain barrier, they are able to directly influence the CNS.

Classification of solvents is made according to molecular structure or functional group. Classes of solvents include aliphatic hydrocarbons, many of which are chlorinated, aromatic hydrocarbons, alcohols, ethers, aldehydes, ketones etc., and complex mixtures that defy classification. Generally, the lipophilicity of a compound increases with increasing molecular weight, while large molecules are less volatile.

The main determinants of solvent's inherent toxicity are: Its number of carbon atoms. Whether it is saturated or has double or triple bounds between adjacent carbon atoms. Its structure i.e., straight-chain, branched-chain or cyclic and the presence of functional groups greatly affects the toxicity.

In some cases, solvents within the same class have similar toxicological properties. For example, amides and amines tend to be potent sensitizers while aldehydes are particularly irritating. Hydrocarbons that are extensively metabolised tend to be cytotoxic and/or mutagenic and many unsaturated short-chain halocarbons are animal carcinogens. The toxicity of solvents within the same class may however vary dramatically.

Exposure of solvents often includes exposure to a number of different compounds, rather than one in particular. It is therefore important to have information of the toxicology of solvents when in a mixture. As mentioned earlier, during exposure of mixtures, the resulting toxic effect may be additive of the effect of the individual solvents. Alternatively, they can interact synergistically or even antagonistically. In many cases, reliable risk assessments cannot be made due to too little data available regarding mixtures of solvents. Even though the hazard varies between one solvent to another, all solvents can cause toxic effects, such as narcosis and irritation of skin and mucous membranes as long as the dose is sufficient. Several solvents are classified as animal carcinogens and a handful as human carcinogens. Health effects from solvents are influenced by the toxicity of the solvent, the exposure route, the amount or rate of exposure, the duration of exposure, individual susceptibility and interaction with other chemicals. These factors also influence the effect not only from solvents, but from all other chemicals. It needs to be mentioned, for e.g., dermal uptake, that solvents can act as carriers for other compounds that have difficulties to be absorbed through the skin.

One example of a non-additive effect is when the activity of Cytochrome P450 is increased in an individual due to high alcohol intake. Hence, other solvents are more readily converted into cytotoxic metabolites. However, simultaneous ethanol consumption may occupy most Cytochrome P450 enzymes and therefore prevent metabolism of these other solvents. For example, these enzymes have a much higher affinity for ethanol than for methanol. As a consequence, methanol poisoning is treated through administration of ethanol in order to prevent methanol being converted into formaldehyde and then further on into formic acid, causing blindness and acidosis, the latter symptom being fatal.

Another example is the simultaneous exposure to toluene and benzene. Benzene is less efficiently metabolised than toluene and genotoxic and erythropoietic toxicity that is related to benzene exposure is reduced as compared to exposure of only benzene [358]. Organic solvents are classified as presented in Table 19.8 and Table 19.9 [359].

Table 19.8 Chemical classes of solvents -1

Solvent	Example
Aliphatic hydrocarbons	Gasoline
Cyclic hydrocarbons	Cyclohexane
Aromatic hydrocarbons	Benzene, toluene
Ketones	Acetone
Aldehydes	Acetaldehyde
Alcohols	Ethanol
Esters	Ethyl acetate

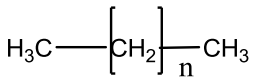
Table 19.9 Chemical classes of solvents -2

Solvent	Example
Nitro-hydrocarbons	Ethyl-nitrate
Halogenated alkanes	1,1,1-Trichloroethane, chloroform
Halogenated alkenes	Trichloroethylene
Mixture	Kerosene

Due to the diversity of the great number of different solvents some general biological relevance is described here. Some specific solvents will be described separately later on [359]. Even though there are a large number of solvents, there are toxicological effects that are common for most solvents. For instance, solvents cause local dermal effects and act suppressive on the central nervous system. Solvents are generally neurotoxic, cause hepatotoxic effects (liver damage) and increase the risk of cancer to a variable extent.

Regarding solvents of the chemical class -1, n-Hexane and methyl-n-butyl ketone are known to cause peripheral neurotoxicity, i.e. effects on the peripheral nervous system. Styrene and perchloroethylene are suspected to exert these effects as well.

Aliphatic solvents, such as gasoline exhibit some common characteristic when it comes to toxicity, e.g., glomerulonephritis and aspiration pneumonitis. The chemical structure of aliphatic solvents is shown in Figure 19.27. Aliphatic solvents typically contain both straight and branched chain isomers.



n= 1.....24

Figure 19.27 General chemical structure of straight chain aliphatic solvent(s).

Halogenated aliphatic solvents have some characteristic toxicological effects. For instance, per-chloromethane (CCl_4) and tri-chloromethane (chloroform, $CHCl_3$) are known to cause severe hepatotoxic effects. Halogenated aliphatic solvents are also known to cause nephrotoxicity and cardiac arrhythmias (irregularities in heart rate). Halogenated alkanes containing more than one chloro atom (polychlorides) and monochloric alkenes are also known to be carcinogenic.

19.11.2 Metabolism of solvents

Especially for solvents, the biotransformation is crucial for the toxicity. Through bio-transformation, solvents are converted into more hydrophilic derivatives that are excreted in urine. Toluene is for example metabolically inactivated or detoxified through conversion into hydroxyl and carbonyl metabolites that are too polar to diffuse into bio membranes. Unmetabolised, toluene accumulates in neuronal membranes and inhibits their function as the membranes become saturated and less permeable for other compounds. In other cases, solvents are converted into cytotoxic and/or mutagenic metabolites through metabolic activation. One example of metabolic activation of a solvent is benzene, which has been shown in Figure 19.29.

19.12 Chlorinated solvents

19.12.1 Biological relevance

The biotransformation of chlorinated solvents is quite complicated due to the diversity of such solvents. Chlorinated aliphatic solvents have been associated with numerous health effects on basis of the toxicology of these solvents, animal metabolism and studies which have been conducted regarding occupational exposure [237]. These effects are exerted on the central nervous system (CNS), the liver and kidneys. In addition, some chlorinated solvents are carcinogenic. In Table 19.10, some common chlorinated solvents and their adverse effect is listed.

Table 19.10 Some aliphatic chlorinated solvents and their biological effect [237].

Compound	Formula	CAS Number	Target organ and biological effect
Methyl chloride	CH_3Cl	74-87-3	Possible CNS
Methylene chloride	CH_2Cl_2	75-09-2	Possible CNS, liver, carcinogen
Chloroform	$CHCl_3$	67-66-3	Possible CNS, liver, kidney, carcinogen
Carbon tetrachloride	CCl_4	56-23-5	Possible CNS, liver, kidney, carcinogen
1-chloroethane	C_2H_5Cl	75-00-3	Possible CNS
1,1-dichloroethane	$C_2H_4Cl_2$	75-34-3	Possible CNS, liver, kidney
1,2-dichloroethane	$C_2H_4Cl_2$	107-66-2	Possible CNS, liver, kidney, probably carcinogen
1,1,1-trichloroethane	$C_2H_3Cl_3$	71-55-6	Possible cardiac, CNS, liver, kidney
1,1,2-trichloroethane	$C_2H_3Cl_3$	79-00-5	Possible CNS, liver, kidney
1,1,1,2-tetrachloroethane	$C_2H_2Cl_4$	630-20-6	Possible CNS
1,1,2,2-tetrachloroethane	$C_2H_2Cl_4$	79-34-5	Possible CNS, liver, kidney
Pentachloroethane	C_2HCl_5	76-01-7	Possible CNS
Hexachloroethane	C_2Cl_6	67-72-1	Possible CNS, liver, kidney
Vinyl chloride	C_2H_3Cl	75-01-4	Possible circulatory system, liver, spleen, known carcinogen
1,1-dichloroethylene	$C_2H_2Cl_2$	75-35-4	Possible liver, kidney
1,2-dichloroethylene	$C_2H_2Cl_2$	540-59-0	Possible liver,
1,1,2-Trichloroethylene	C_2HCl_3	79-01-6	Possible CNS, liver, kidney, carcinogen
Perchloroethylene	C_2Cl_4	127-18-4	Possible CNS, liver, kidney, carcinogen

Suitable biomarkers of exposure to chlorinated solvents would be metabolites of the solvents, since these compounds are metabolised in the liver after exposure. For instance, perchloroethylene is metabolised by Cytochrome P450 and GSH s- Transferases into small organic compounds such as dichloro-ethenethione, acetic acid chloride and acetic acid.

However, some of these products are very reactive and form adducts with proteins as indicated in ref. [238].

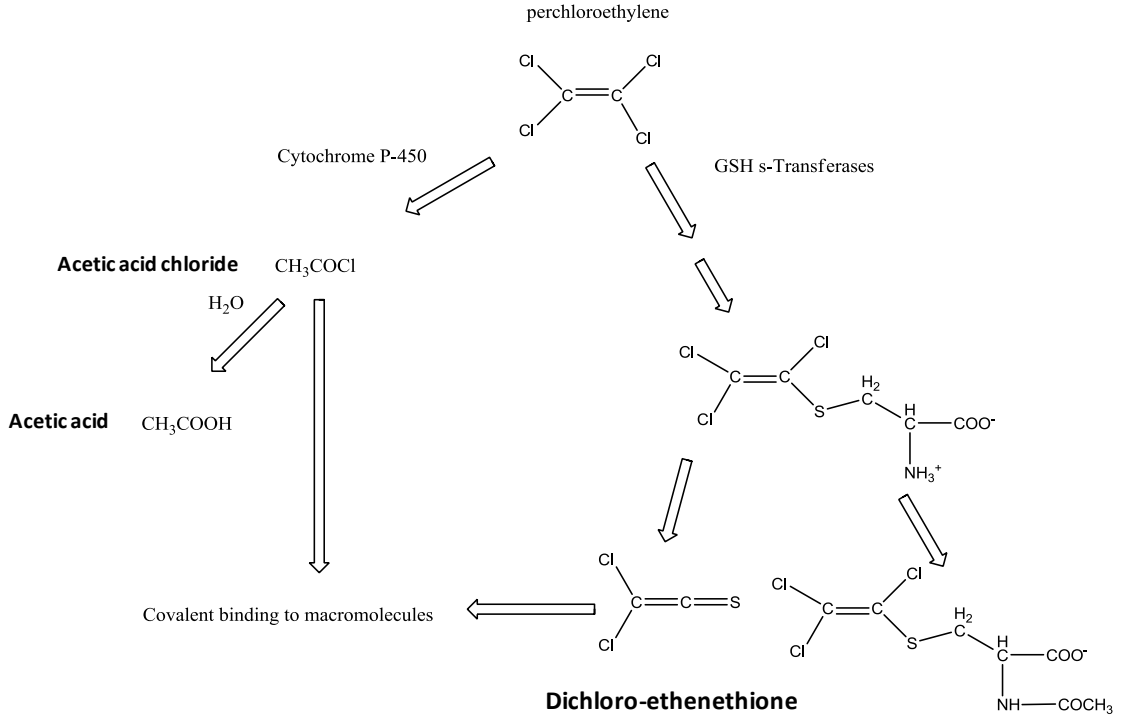


Figure 19.28 Metabolic pathway of perchloro ethylene

Chlorinated solvents are metabolised in the liver and the metabolites of these compounds can be used as biomarkers. Analysis can generally be performed with for instance GC-MS. However it would be impossible do give detailed description of analytical methods for all the hundreds of chlorinated solvents and their metabolites.

19.13 Benzene

19.13.1 Biological relevance

Benzene is a toxic solvent and is classified from the International Agency for Research on Cancer (IARC) as a human carcinogen (group 1) [240]. The toxicity of benzene is also related to individual genotype and personal exposure [241]. A review article on biomarkers of benzene has recently been published [242].

The metabolism of benzene is complex, producing a number of metabolites. Three metabolites, t,t-muconic acid [243, 244], S-phenylmercapturic acid and phenols [241] in urine are used as biomarkers for benzene exposure.

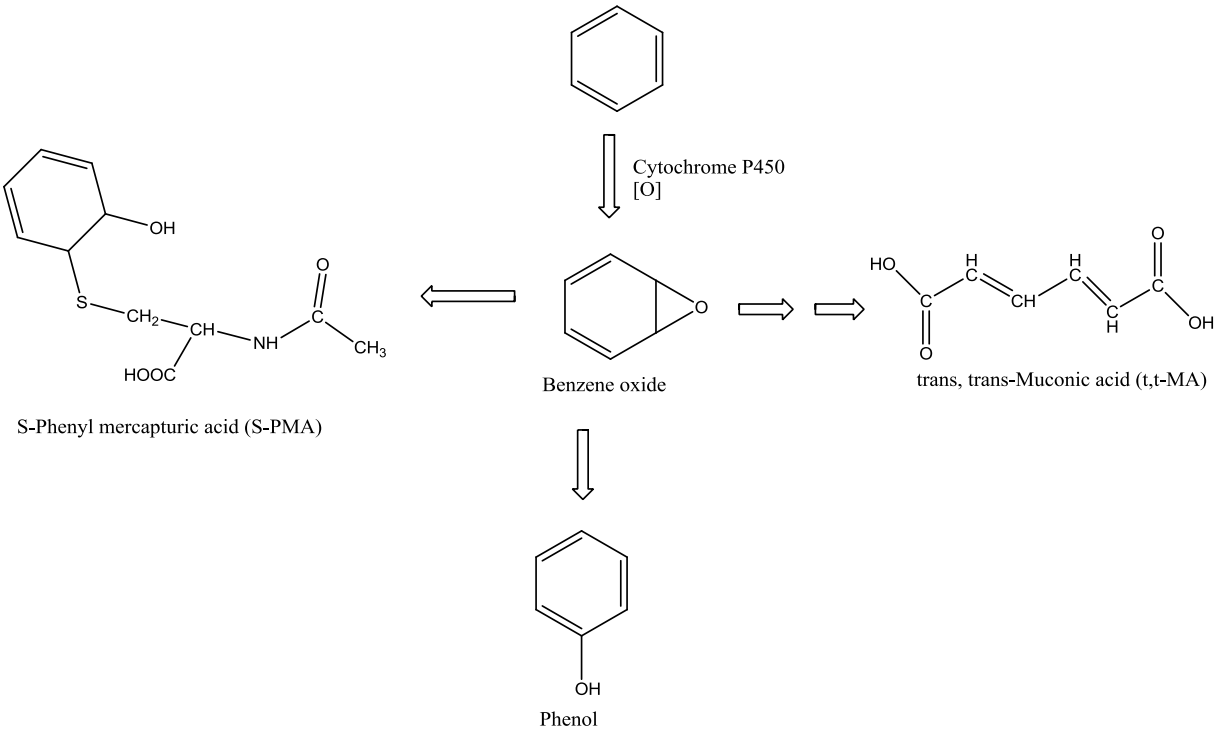


Figure 19.29 Benzene metabolism and its three biomarkers.

Benzene in exhaled air can be analysed as such with a reliable field method utilising GC-MS [245]. Estimation of unmetabolised benzene is used for the evaluation of benzene exposure in human in urine [246] and blood [247].

A number of GC-MS and HPLC-UV methods have been described for benzene metabolites [248]. This includes a sensitive and specific HPLC-MS/MS assay for the determination of benzene metabolites such as t,t- muconic acid, S- phenylmercapturic acid, hydroquinone and catechol in urine [249]. In addition, a LC-MS/MS method for the determination phenylmercapturic acid, benzylmercapturic acid and *o*-methylbenzyl mercapturic as biomarkers of benzene, toluene and xylene has been validated [250]. Also GC-MS has been applied [251, 252].

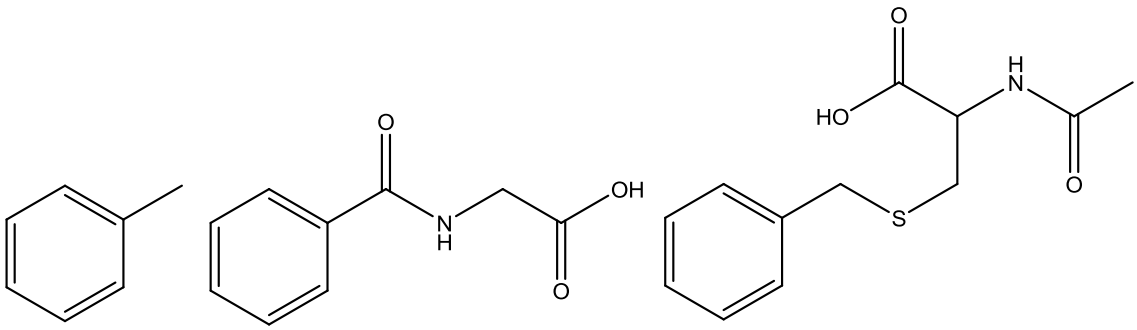
Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed in urine using GC-MS [252] [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

19.14 Toluene

19.14.1 Biological relevance

Toluene can cause brain dysfunction as a result from long term exposure [253]. Currently, no evidence exists which suggests carcinogenic properties of toluene. Nevertheless, exposure to high concentrations of toluene can induce changes in the central nervous system and other neurotoxic effects [254].

One main biomarker of toluene that was commonly used is Hippuric Acid. However, due to the high levels of hippuric acid in urine (from dietary benzoic acid) this is no longer recommended. Another biomarker is of benzene exposure is benzylmercapturic acid (BMA) [250] although there is some doubt about its application to biomonitoring of toluene exposure. Ortho cresol is another metabolite useful for assessing exposure to toluene around the current exposure limits. Toluene itself has also been measured in breath, blood and urine as an indicator of exposure.



Toluene Hippuric acid Benzylmercapture acid

Figure 19.30 The chemical structure of Toluene and its metabolites Hippuric Acid and Benzylmercapturic acid (BMA).

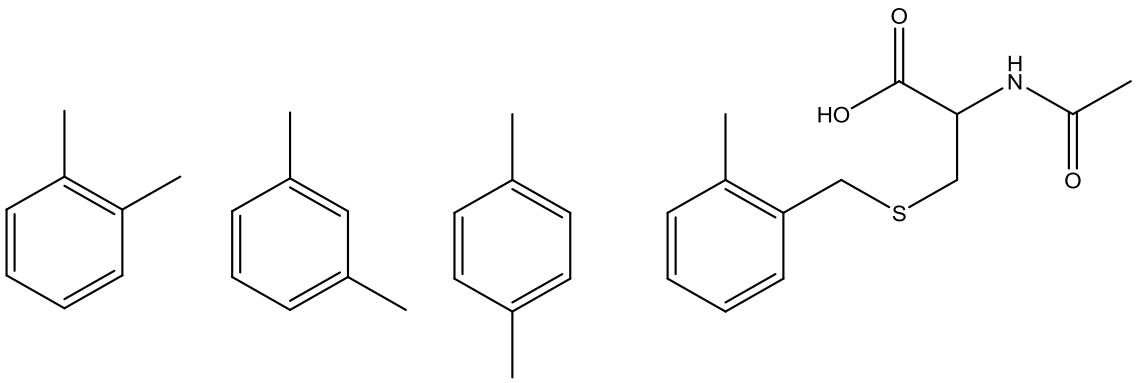
Saliva can be used as specimen for sampling instead of urine or blood samples for biological monitoring in the workplace utilising GC-MS [255]. The analysis of biomarkers from toluene the method described [249] has been used. A validated LC-MS method validated according to U.S. Food and Drug Administration (FDA) guidance [250], Also GC-MS can be applied on a biomarker from toluene [251, 252].

Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with a validated GC-MS from urine [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256]

19.15 Xylene

19.15.1 Biological relevance

In principle as described for toluene, O-Methylbenzyl mercapturic acid (MBMA) is proposed to be a reliable biomarker of the exposure of xylene [250]. A more common and widely used method detects methyl hippuric acid in urine. The ACGIH and DFG have a BEI and BAT for methyl hippuric acid



ortho-xylene meta-xylene para-xylene O-Methyl mercapture acid

Figure 19.31 All xylene isomers and the metabolite O- Methyl Benzylmercapturic acid.

Methyl hippuric acid can be detected in urine by a simple HPLC-UV method. A validated LC-MS method validated according to U.S. Food and Drug Administration guidance has been reported [250]. Also GC-MS can be applied on a biomarker from xylene [251, 252].

Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with a validated GC-MS from urine [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

19.16 Ethylbenzene

19.16.1 Biological relevance

Ethylbenzene is a solvent and its main metabolite is mandelic acid and phenylglyaxylic acid are used as biomarkers in urine for ethylbenzene exposure [251, 252]. Mandelic acid and phenylglyaxylic acid are also biomarkers for styrene exposure.

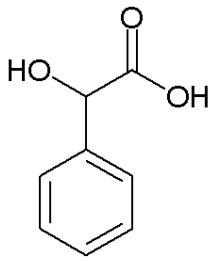


Figure 19.32 Chemical structure of mandelic acid

Mandelic acid in urine as a biomarker of ethylbenzene can be analysed with a validated GC-MS method [251, 252].

Biomarker(s) of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with a validated GC-MS from urine [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

19.17 Styrene

19.17.1 Biological relevance

Exposure from styrene can be assessed from measurement of styrene in blood or its metabolites Mandelic acid and Phenylglyaxylic acid, which are the same as for Ethylbenzene. Biological relevance and analytical methods was described in earlier chapters (section 12.8) regarding styrene and will not be further discussed here.

Biomarker(s) in urine of benzene, toluene, xylene, ethylbenzene and styrene can be analysed with validated GC-MS methods [256]. This also include several other methyl substituted benzene derivatives e.g., cresols [256].

19.18 Polycyclic aromatic hydrocarbon (PAHs)

19.18.1 Biological relevance

Polycyclic aromatic hydrocarbons (PAHs) are emitted from oil, coal and tar deposits and are formed during heating of oil products. In addition to polyaromatic hydrocarbons, there are polyaromatic heterocyclic compounds, polyaromatic nitro and amines formed during the heating of oil products.

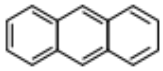
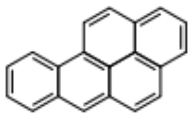
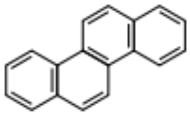
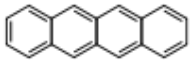
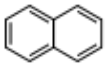
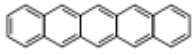
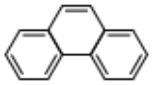
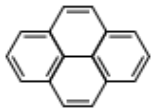
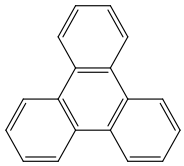
The toxicity of PAHs is very structurally dependent, with isomers (PAHs with the same formula and number of rings) varying from being non-toxic to being extremely toxic. Thus, highly carcinogenic PAHs may be small or large. One PAH compound, benzo[a]pyrene, is notable for being the first chemical carcinogen to be discovered (and is one of many carcinogens found in cigarette smoke). The EPA (US Environmental Protection Agency) has

classified seven PAH compounds as probable human carcinogens: benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]anthracene, chrysene, and indeno[1,2,3-cd]pyrene [257].

Figure 19.33 Emission of PAHs occurs generally during combustion of fossil fuels (Crown Copyright 2009).

PAHs known for their carcinogenic, mutagenic and teratogenic properties are benz[a]-anthracene and chrysene benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, coronene, dibenz[a,h]anthracene (C20H14), indeno[1,2,3-cd]pyrene (C22H12) and ovalene [360]. The chemical structures of these compounds are presented in Table 19.11.

Table 19.11 Some important PAHs

Chemical compound	Structure	Chemical compound	Structure
Anthracene		Benzo[a]pyrene	
Chrysene		Coronene	
Corannulene		Naphthacene	
Naphthalene		Pentacene	
Phenanthrene		Pyrene	
Triphenylene		Ovalene	

PAHs are extensively described according to their biomarkers [129, 361], carcinogenicity [361] and their determination in urine [362]. PAHs occur as a mixture of compounds. Since pyrene is almost always found in this mixture, pyrene and its metabolite 1-hydroxypyrene (1-OHP) has been considered as an appropriate biomarker for PAH exposure [362]. Also the 3-hydroxybenzo[α]pyrene (3-OHPBaP) metabolite is found in human urine after exposure of PAHs [258].

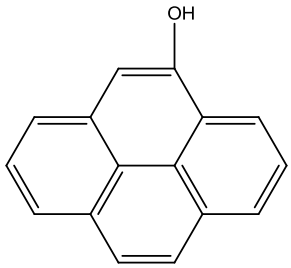


Figure 19.34 Chemical structure of 1-hydroxypyrene (1-OHP).

19.19 Analytical methods and their criteria

19.19.1 Introduction

The quantification and determination of biomarkers in biological fluids such as blood and urine samples requires a lot of attention. Not only the correct handling of samples and/or in which way the analyses are performed is an important matter. The choice if appropriate instrumentation is of great importance, there may be difference between various instrument manufacturers, and factors such as the skilfulness of the laboratory and certain knowhow of the personnel. In most biological samples the concentration of the analyte is very often extremely low and very sensitive instrumentation is necessary. The matrix has also a great influence for e.g., the analysis of urinary carboxylic acids, where the limitation factor to achieve highest sensitivity is the high content of creatinine that interferes with the derivatisation process [363].

Biological samples contain a lot of endogenous compounds present together with water and salts. The sample pre-preparation is of utmost importance. In all biological samples the analytes of interest need to be separated from interfering compounds present. This requires hyphenated (a combination of analytical instrumentation) analytical techniques such as ICP-MS for elemental analysis or either gas chromatography (GC) or high performance liquid chromatography (HPLC) in combination with mass spectrometry (MS) for organic compounds.

Clearly electrospray ionisation (ESP) have revolutionised MS and today ESP which is a robust technology used worldwide at scientific and routine laboratories. There are several other available MS techniques that have a potential for being useful tools for the analysis of biomarkers but most of these do not enable quantitative determinations. Matrix assisted laser desorption ionisation (MALDI) enables the identification and characterisation of macromolecules. An analytical technique called Direct analysis in real time (DART) has been developed by JEOL, a Japanese mass spectrometry company [364, 365]. This method enables ionisation directly on the sample surface or of solid samples, such as on various materials, clothing etc. In addition, direct analysis of biological fluids, such as saliva, urine and blood, can be conducted without prior purification. DART also enable analysis of samples that normally cannot be analysed with electrospray or atmospheric pressure chemical ionisation. Liquid samples can simply be analysed by dipping a glass road in the sample and then insert the glass road into the ion source. Gaseous samples can be analysed by direction of the gas stream into the ion source.

Depending on the nature of the analyte and the sample matrix, different methods are required. In addition, several other steps in the analytical chain are crucial for the result, such as: sampling, sample preparation, sample handling, derivatisation, and recovery from the biological matrix, stability (towards heat and/or oxidation), separation and ionisation in the mass spectrometry.

Some basic general knowledge regarding methods for biomarkers is necessary [366]:

- Accuracy
- Precision
- Specificity
- Limit of detection
- Limit of quantification
- Linearity and range
- Robustness
- Uncertainty

The measurement of the concentration of biomarkers in biological samples has associated with it an uncertainty that may be expressed as overall uncertainty or expanded uncertainty (at the 95 % confidence level). Estimation of the contributions of the different uncertainties for analyte mass, correction of analyte mass and between laboratory variations has to be performed [367]. The uncertainty depends also on the concentration (uncertainty is higher at low levels). When essential data is known and/or estimated the uncertainty for a given method can be calculated.

Table 19.12 An example of performance characteristics for determination of a biomarker in a biological sample

Uncertainty contribution	Uncertainty (%)	Comments
Analyte mass	6	
Analyte stability during storage	0.1	
Reaction/ Extraction efficiency	4	
Mass of analyte in calibration standards	1	
Calibration lack-of-fit	3	
Response drift between calibrations	3	
Analytical precision	1	
Selectivity	negligible	
Blank level	5	
Correction of analyte mass	4	In urine samples: correction of analyte mass with creatinine or comparison with total sample volume. In blood samples: correction of analyte mass with haemoglobin.
Between-laboratory variations	7	

When combining the uncertainties specified for the performance characteristics in Table 19.12 a worst-case situation will result. The resulting combined relative uncertainty will be about 10 % and the expanded uncertainty (at the 95 % confidence level) will be 20 %.

The Food and Drug Administration (FDA) has also published a guidance for the validation of bio analytical methods [105].

The experiments for validation of a method should be carried out by an experienced analyst to avoid errors due to inexperience. The analyst should be well trained in the technique and operation of the instrument. Before an instrument is used to validate a method, its performance specifications should be verified using generic chemical standards. Satisfactory results for a method can be obtained only with equipment that is performing well. Special attention should be paid to those equipment characteristics that are critical for the method. For example, if detection limit is critical for a specific method, the instrument's specification for baseline noise and, for certain detectors, the response to specified compounds should be verified.

Inter-laboratory calibrations are essential in order to ensure the quality of the analyses. For within and between-laboratory quality assurance schemes to ensure the consistency of results – e.g. schemes operated by the Finnish institute of occupational health (FIOH) and University of Erlangen in Germany are valuable.

All chemicals used for the analysis, such as reagents and reference standards, should be:

1. Available in sufficient quantities
2. Accurately identified
3. Sufficiently stable
4. Checked for exact composition and purity.

All other materials and consumables, for example, chromatographic columns, should be new and qualified to meet the column's performance criteria. This ensures that one set of consumables can be used for most experiments and avoids unpleasant surprises during method validation.

Operators should be sufficiently familiar with the technique and equipment. This will allow them to identify and diagnose unforeseen problems more easily and to run the entire process more efficiently.

Numerous papers are published regarding analysis of a great number of compounds, however many of these publications are on a limited set of compounds in a mixture and may not be adapted to "real sample conditions". Therefore it is of great importance that the selected method of choice is carefully evaluated for the biological sample to be analysed.

In all methods either for air samples or for biomarkers there is a need for analytical standards. These are essential not only for the quantification but also for the qualitative analysis. In many cases it is not only necessary to have the analyte (the compound to be analysed) in a pure form but also to have access to reference samples containing the analyte (e.g., cotinine in urine). It is a well-known fact that the matrices often greatly influence the quality of the analysis. For many interesting compounds there are difficulties to get the reference compounds and reference samples necessary for the analysis. A limited number of standards are certified at e.g., NIST and a few are commercially available at a high cost. Some laboratories make a lot of effort, at a high cost, to produce standards. In many cases the compounds of interest are not stable and needs to be prepared shortly before the analysis. For organic compounds the access of standards of corresponding metabolites may be very troublesome to get. In fact, one of the greatest difficulties for biomarkers is access to relevant standards and is one of the most limited factors.

For some compounds and their biomarkers there are proven routine methods. Basically, the biomarkers that are in routine analyses in many countries have a reasonable good knowledge base. To estimate how good a biomarker is depends of course on how many details regarding the analysis that are known but also to what extent it is in use. How good a biomarker is, also depends on the purpose of the sampling and whether the biomarker reflects to the most relevant compound present at the workplace. There may be unknown compounds present and other compounds present with greater importance for the health.

19.19.2 Organic mass spectrometry (MS)

MS is the first choice to use as a detector for biomarkers due to its high selectivity (MS/MS) and sensitivity. HPLC-MS/MS is the method of choice. The method is normally robust and enables high throughput of samples and in addition can accommodate nearly all kind of solvents including water.

GC-MS is the choice of technique when analysing complex samples which require high degree of separation combined with high sensitivity. A limitation is that the analyte needs to be volatile. The best sensitivity in GC-MS often requires derivatisation of the analyte. However, for biological samples this can be troublesome since water and salts may be present which are not compatible with GC-MS. The highest sensitivity in GC-MS is obtained in negative ionisation mode. The ability to run negative GC-MS is highly dependent on the quality and the vendor of the MS instrumentation.

For the quantification using MS internal standards are necessary. The internal standard should be similar to the analytes due to different response of various compounds. The best choice is carbon 13-labelled analytes and the second choice deuterium labelled analytes as internal standards. Deuterium labelled standards often has different HPLC separation characteristics and is more lipophilic and the response may differ compared to the response of the analyte. Quantification of analytes requires standards in order to create standard curves from which the concentration of the analyte can be calculated. Standard curves are obtained by using standard concentration of the analyte of interest together with internal standards. In many cases, the analyte is not commercially available and therefore must be synthesised if that is possible. In such cases if the molecule contains certain atoms e.g., nitrogen, a nitrogen specific detector can be used. Based on the properties with the nitrogen sensitive detectors and the standards obtained by MS, response factors can be calculated for the MS. Another way is to use ICP-MS for quantification, which can be used for a number of elements such P, S, Br etc. [368]. This method is further described below.

19.19.3 Inorganic mass spectrometry (ICP-MS)

Inorganic mass spectrometry, e.g., ICP-MS (Inductively coupled plasma mass spectrometry) is a very important method in elemental analyses. It has outstanding features to determine elemental concentrations at ultra-trace concentration levels [61, 369]. Inorganic mass spectrometry has created an increasing interest over the last decade due to the dramatic improvements in this technique in combination of the successful commercialisation of improved instrumentation and its ability to solve increasingly difficult analytical problems. Furthermore, the coupling of HPLC on-line with ICP-MS makes separations possible of the analyte of interest in complex mixtures. Also, the use of time-of flight (TOF) mass spectrometry for accurate mass determination and isotopic ratio determinations make this technique versatile.

However, ICP-MS requires special equipment and handling of the instrument. In addition, it requires a lot of argon gas and problems have been experienced with overlapping and interfering ions depending on the configuration of the instrument used [370]. The oxide of one element can interfere with another element. One example amongst numerous others is Cd and MoO since these species have the same mass.