Aerosols of Isocyanates, Amines and Anhydrides

Sampling and Analysis

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Abstract

This thesis presents methods for air sampling and determination of isocyanates, amines, aminoisocyanates and anhydrides. These organic compounds are generated during production or thermal degradation of polymers such as polyurethane (PUR) or epoxy. Isocyanates, amines and anhydrides are airway irritants known to cause occupational asthma. Some of the isocyanates and diamines are listed as human carcinogens.

Isocyanates and anhydrides are reactive and needs to be immediately derivatized during sampling. Methods have been developed for determination of airborne isocyanates and aminoisocyanates using di-n-butylamine (DBA) as the reagent to form stable urea derivatives. Anhydrides were derivatized with DBA to stable amide derivatives. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) enabled instrumental detection limits as low as 10 attomoles. Methods for preparation and characterization of reference solutions of technical grade isocyanates, aminoisocyanates and anhydrides are presented. A nitrogen-selective LC-detector enabled the quantification of the DBA-derivatives.

A novel sampler is presented. The sampler consists of a denuder in series with a three-stage cascade impactor and an end filter. The sampler made it possible to reveal the distribution of isocyanates between gas and different particle size fractions. During thermal degradation of PUR, isocyanates were associated to particle size fractions (<1 µm) that may penetrate to the lower airways. The distribution during 8 minutes changes noticeably. Gas phase toluene diisocyanates (TDI) and methylene diphenyl diisocyanate (MDI) become associated to small particles (<1 µm). Many workers are exposed to this kind of aerosol. As a reference method air-sampling was performed using an impinger filled with di-n-butylamine (DBA) in toluene, connected in series with a glass fiber filter. There was a good agreement between the denuder impactor sampler and the reference method.
List of Papers

The thesis is based on the papers listed below. References to the papers in the text are assigned with roman numerals.

I  Determination of technical grade isocyanates used in the production of polyurethane plastics.
    Marand, Å., Dahlin, J., Karlsson, D., Skarping, G., Dalene, M.
    *Journal of Environmental Monitoring*, **2004**, 6, 606-614

II Determination of Airborne Isocyanates as di-n-butylamine derivatives using liquid chromatography and tandem mass spectrometry.
    Karlsson, D., Dahlin, J., Marand, Å., Skarping, G., Dalene, M.
    *Analytica Chimica Acta*, **2005**, 534(2), 263-269

    Dahlin, J., Spanne, M., Karlsson, D., Dalene, M., Skarping, G.
    *Manuscript*

IV Size-separated sampling and analysis of isocyanates in workplace aerosols. Part II. Ageing aerosols from thermal degradation of polyurethane.
    Dahlin, J., Spanne, M., Karlsson, D., Dalene, M., Skarping, G.
    *Manuscript*

V Determination of isocyanates, aminoisocyanates and amines formed during thermal degradation of polyurethane.
    Karlsson, D., Dahlin, J., Skarping, G., Dalene, M.
    *Journal of Environmental Monitoring*, **2002**, 4, 216-222

VI Determination of airborne anhydrides using LC-MS monitoring negative ions of di-n-butylamine derivatives.
    Dahlin, J., Karlsson, D., Skarping, G., Dalene, M.
    *Journal of Environmental Monitoring*, **2004**, 6, 624-629
In paper I, II and V the respondent contributed to the experimental work, field measurements and the writing of the papers. In paper III the respondent was responsible for the majority of the experimental work and the writing of the paper. In paper IV and VI the respondent performed all the experimental work and wrote the papers.

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2PP</td>
<td>1-(2-pyridyl)-piperazine</td>
</tr>
<tr>
<td>2-MP</td>
<td>1-(2-methoxyphenyl)-piperazine</td>
</tr>
<tr>
<td>AA</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>APCI</td>
<td>Atmospheric pressure chemical ionization</td>
</tr>
<tr>
<td>CLND</td>
<td>Chemiluminescent nitrogen detection</td>
</tr>
<tr>
<td>DBA</td>
<td>Di-(n)-butylamine</td>
</tr>
<tr>
<td>DEA</td>
<td>Di-(n)-ethylamine</td>
</tr>
<tr>
<td>DPA</td>
<td>Di-(n)-propylamine</td>
</tr>
<tr>
<td>EC</td>
<td>Electrochemical</td>
</tr>
<tr>
<td>ECD</td>
<td>Electron capture detector</td>
</tr>
<tr>
<td>ES</td>
<td>Electrospray</td>
</tr>
<tr>
<td>ET</td>
<td>Ethyl chloroformate</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionization detector</td>
</tr>
<tr>
<td>FL</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>HA</td>
<td>(cis)-Hexahydrophthalic anhydride</td>
</tr>
<tr>
<td>HAI</td>
<td>Hexamethylene aminoisocyanate</td>
</tr>
<tr>
<td>HDA</td>
<td>Hexamethylene diamine</td>
</tr>
<tr>
<td>HDI</td>
<td>Hexamethylene diisocyanate</td>
</tr>
<tr>
<td>HFBA</td>
<td>Heptafluorobutyric anhydride</td>
</tr>
<tr>
<td>HMDI</td>
<td>Dicyclohexyl methane diisocyanate</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>HSE</td>
<td>Health and safety executive</td>
</tr>
<tr>
<td>IARC</td>
<td>International agency on research of cancer</td>
</tr>
<tr>
<td>ICA</td>
<td>Isocyanic acid</td>
</tr>
<tr>
<td>ICRP</td>
<td>International commission on radiological protection</td>
</tr>
<tr>
<td>IPDI</td>
<td>Isophoronediisocyanate</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>MA</td>
<td>Maleic anhydride</td>
</tr>
<tr>
<td>MAI</td>
<td>Methylene diphenyl aminoisocyanate</td>
</tr>
<tr>
<td>MAMA</td>
<td>9-(N-methylaminomethyl)-anthracene</td>
</tr>
<tr>
<td>MAP</td>
<td>1-(9-anthracenylmethyl)-piperazine</td>
</tr>
<tr>
<td>MCP</td>
<td>Multi channel plate</td>
</tr>
<tr>
<td>MDA</td>
<td>Methylene diphenyl diamine</td>
</tr>
<tr>
<td>MDI</td>
<td>Methylene diphenyl diisocyanate</td>
</tr>
</tbody>
</table>
MIC  Methylisocyanate
MMNTP  4-methoxy-6-(4-methoxy-1-naphtyl)-1,3,5-triazine-2-(1-piperazine)
MRM  Multiple reaction monitoring
MS  Mass spectrometry
NBDPZ  4-nitro-7-piperazino-2,1,3-benzoxadiazole
NDI  Naphtalene diisocyanate
NIOSH  National institute of occupational safety and health
Nitro  N-4-nitrobenzyl-N-n-propylamine
NPD  Nitrogen-phosphorous detector
OEL  Occupational exposure limit
OSHA  Occupational safety and health administration
PA  Phthalic anhydride
PAC  9-anthracenylmethyl-1-piperazine carboxylate
PFBBBr  Pentafluoro benzylbromide
PFPA  Pentafluoro propionic anhydride
PFU  Phenyl-formaldehyde-urea
PhI  Phenyl isocyanate
pMDI  Polymeric MDI
PUR  Polyurethane
PVC  Polyvinylchloride
RH  Relative humidity
SIR  Selective ion recording
TA  Tetrahydorphthalic anhydride
TAI  Toluene aminoisocyanate
TDA  Toluene diamine
TDI  Toluene diisocyanate
TLC  Thin layer chromatography
TMA  Trimellitic anhydride
TOF  Time of flight
TRIG  Total reactive isocyanate group
TSD  Thermionic specific detector
TWA  Time weighted average
UV  Ultraviolet
1 Introduction

Exposure to airborne chemicals is very common in several different industrial branches. To investigate exposure in different work environments it is necessary to select proper sampling and analytical methods. Authorities in most countries have established limit values for the exposure to different chemicals that results from measurements are compared with.

There are different ways to obtain information about exposure to airborne compounds. For a practicing industrial hygienist and for authorities a total level of some compound with an occupational exposure limit (OEL) value is often sufficient information. If the value obtained is well below the limit, the situation is regarded as acceptable and no measures are needed to improve the situation. On the other hand, if the value obtained is above the OEL, measures must be taken to lower the exposure levels for the workers. However, if not only the OEL is taken into account there are many more aspects to study. In and industrial environment the air is usually not very clean. Dust, particles, different gases and moisture are almost always present. It has been shown that depending on size, particles can reach different parts of the human airways. Some particle size fractions are able to penetrate all the way down to the alveolar region, where the residence time is longer and particle clearance is less efficient. Hazardous compounds may be transported to this region if adsorption of the compounds onto particle surfaces occurs. To obtain a more detailed view of an aerosol it is necessary to use methods which not only obtain a total concentration of a certain compound, but also has the ability to separate relevant particle size fractions. If the air level of a compound is below the OEL, it may be viewed as acceptable. However, if the compound is associated to small particles that can penetrate deep into the airways, the levels may be too high. This is a very good reason for the development of samplers that provides results that better reflects the risks. For this purpose there is a need for methods that can show how toxicants are distributed in an aerosol.

The fate of chemicals in the body is an important issue. Some are not very reactive e.g. solvents and may be exhaled after inhalation and only a small amount is absorbed and metabolized. Isocyanates and anhydrides are examples of extremely reactive compounds. If inhaled, there are many compounds in the airways that are highly reactive towards isocyanates and anhydrides. Adducts may be formed with compounds containing e.g. amine groups
(-NH₂) and hydroxyl groups (-OH). The formation of biological adducts may damage e.g. a protein in an irreversible way.

Isocyanates are chemical compounds used in the manufacture of polyurethane (PUR) plastics. PUR is a versatile polymeric material. The properties of PUR range from hard and rigid to soft and flexible. In the modern society PUR is used in great amounts for production of paints, soft and rigid foam, elastomers, glues, clothing, shoe soles and several other products. But since the introduction of PUR there have also been published reports about health effects among workers. The health effects arise from exposure to isocyanates. Isocyanates are highly reactive and in most air-sampling methods used today, amine reagents are used. The amine reagent protects the isocyanate group, to avoid side reactions with other compounds. Isocyanate sampling is a complex field. There are numerous types of isocyanates with different properties. Small monoisocyanates are volatile, while diisocyanates and isocyanate adducts are heavier and have lower volatility. Exposure is seldom restricted to just one type of isocyanate. Instead an aerosol consisting of gaseous isocyanates and isocyanates associated to particles is present. Air sampling must be done with methods capable of sampling both gases and particles, without discrimination.

Exposure to reactive monomers may occur during production of polymers, but there are also other kinds of exposure. In fact, exposure to thermal degradation products of polymers is more common and hazardous compounds may be generated. For PUR, isocyanates are reformed, but diamines and aminoisocyanates that are closely related to diisocyanates, are also generated. Organic acid anhydrides are another type of reactive compounds that can be generated during thermal degradation of epoxy or alkyd resins.

This thesis covers sampling and analysis of reactive compounds that are closely associated to polymers, either used in production or formed during thermal degradation. Sampling methods which are able to separate an aerosol in different fractions have been developed. Methods that are able to screen for several compounds of different types in the same sample have been developed, to simplify exposure assessment. For sample analysis the instrumental techniques have been improved to obtain lower detection limits and greater selectivity. New methods to bring forward appropriate analytical standards have been developed.
2 Aims of the Thesis

• To develop air sampling and analytical methods for complex isocyanates commonly used in the industry.

• To investigate the possibility to use tandem mass spectrometry for analysis of isocyanate-DBA derivatives.

• To develop air sampling methods for the assessment of the distribution of isocyanates in an aerosol and how the aerosol varies with time.

• To study reactive aerosols formed during thermal degradation of polyurethane regarding amine and aminoisocyanate formation.

• To develop methods for the determination of airborne anhydrides simultaneously with isocyanates.
3 Isocyanates

3.1 Introduction

The common feature for all isocyanates is the isocyanate group –NCO. Isocyanates are strong electrophils and react with compounds containing active hydrogens, such as amines, alcohols, thiols or water. Aromatic isocyanates are usually more reactive than aliphatic isocyanates, due to the electron-withdrawing properties of aromatic rings. The synthesis of isocyanates was discovered in 1848 by Wurtz. The most important industrial application for isocyanates, production of polyurethane (PUR) from diisocyanate monomers, was invented by Otto Bayer in 1937.

3.1.1 Monoisocyanates

In comparison with diisocyanates the volumes of monoisocyanates used in industrial applications are small. Monoisocyanates are used in the synthesis of pharmaceutical products, for production of pesticides and modification of polymers. In the Bhopal accident in 1984 it was methyl isocyanate (MIC) used in the production of an insecticide, carbaryl, that was released and killed at least 3800 people. Monoisocyanates have also been identified as thermal degradation products of polyurethane and phenyl-formaldehyde-urea (PFU) resins. Monoisocyanates studied in this thesis are listed in Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Vapour pressure (Pa)</th>
<th>CAS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>H-N≡C-N</td>
<td>13300 (-19°C)</td>
<td>75-13-8</td>
</tr>
<tr>
<td>MIC</td>
<td>N≡C-N</td>
<td>46400 (20°C)</td>
<td>624-83-9</td>
</tr>
<tr>
<td>EIC</td>
<td>N≡C-N</td>
<td>14700 (20°C)</td>
<td>109-90-0</td>
</tr>
<tr>
<td>PIC</td>
<td>N≡C-N</td>
<td>6900 (20°C)</td>
<td>110-78-1</td>
</tr>
<tr>
<td>PHI</td>
<td></td>
<td>250 (20°C)</td>
<td>103-71-9</td>
</tr>
</tbody>
</table>
3.1.2 Diisocyanates

The majority of isocyanates used industrially are diisocyanates, containing two isocyanate groups. The global market of isocyanates in the year 2000 was about 4.4 million tons. For production of polyurethane a diisocyanate is mixed with a polyol, and the bond formed is called a urethane bond (figure 2).

Several diisocyanates are used in the industry, to achieve different properties of the polyurethane. For production of soft foam, toluene diisocyanate (TDI) is used, but TDI can also be used in lacquers. Rigid foam is produced from methylene diphenyl diisocyanate (MDI). MDI and TDI are also be used in the production of elastomers. MDI-based binder is used for building sand moulds for iron casting. There are also isocyanate glues based on MDI. Coatings are produced from aliphatic isocyanates like hexamethylene diisocyanate (HDI), isophorone diisocyanate (IPDI) or dicyclohexylmethane diisocyanate (HMDI). Coatings need to be UV-resistant, so the aromatic isocyanates are not appropriate in this application. Naphthalene diisocyanate (NDI) is another aromatic diisocyanate that is used in the production of hard elastomers used for example for production hard wheels for trucks or roller skates. Polyurethane is one of the most versatile plastic materials known, and the texture of PUR ranges from soft and flexible to hard and rigid.\(^5\) Diisocyanates included in this thesis are summarized in table 2.

Diisocyanates are manufactured by a number of processes, involving different starting materials, but the common step for almost all isocyanate production is a phosgenation reaction, where an amine is reacted with highly toxic phosgene. During the First World War phosgene was used for chemical warfare.\(^6\) A lot of research about isocyanate production without phosgene has been performed, but so far no viable processes that completely can replace phosgenation has been found.\(^7\)
Table 2. *Diisocyanates*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Vapor pressure (Pa)</th>
<th>CAS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,6-HDI</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>7 (20°C)</td>
<td>822-06-0</td>
</tr>
<tr>
<td>2,6-TDI</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>2 (20°C)</td>
<td>91-08-7</td>
</tr>
<tr>
<td>2,4-TDI</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>3 (20°C)</td>
<td>584-84-9</td>
</tr>
<tr>
<td>IPDI</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>0.04 (20°C)</td>
<td>4098-71-9</td>
</tr>
<tr>
<td>4,4’-MDI</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>6.65*10⁻⁴ (20°C)</td>
<td>101-68-8</td>
</tr>
</tbody>
</table>

Some processes for production of polyurethane involves pure diisocyanate monomers, for example production of soft foam where a mixture of 2,4- and 2,6-TDI in the proportions 80:20 is used for the foaming process. Other polyurethane manufacturing involves isocyanate adducts, prepolymeric isocyanates or oligomers, containing 3 or more isocyanate groups.

### 3.1.3 Isocyanate adducts and Oligomers.

To obtain different properties for the isocyanate mixture or to lower the volatility, isocyanate adducts or oligomers are often used in the industry. For example is HDI often further reacted during the production, to form biuret, alophanate or isocyanurate adducts. This reduces the volatility of the HDI, and is utilized in the production of polyurethane coatings. During the production of MDI, a crude mixture of different MDI-isomers (2,2’-, 2,4’- and 4,4’-MDI) and oligomers with 3 or more aromatic rings and isocyanate groups are obtained. For the production of for example rigid foam it is not necessary to separate the isomers, instead the crude mixture of oligomeric isocyanates is used directly. This mixture of MDI, isomers and oligomers are usually referred to as polymeric MDI (pMDI). Structures for some isocyanates found in technical mixtures are summarized in table 3. Isocyanate mixtures are also sometimes prepolymerized, that is mixed with a small amount of polyol, to change volatility, reactivity, storage stability or some other property.
Table 3. *Isocyanate oligomers and adducts.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>CAS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isocyanurate</td>
<td><img src="image" alt="Isocyanurate structure" /></td>
<td>3779-63-3</td>
</tr>
<tr>
<td>Biuret</td>
<td><img src="image" alt="Biuret structure" /></td>
<td>4035-89-6</td>
</tr>
<tr>
<td>pMDI</td>
<td><img src="image" alt="pMDI structure" /></td>
<td>9016-87-9</td>
</tr>
</tbody>
</table>

### 3.2 Health Effects

A few years after polyurethane production was started industrially, reports were published about respiratory disorders in workers handling the isocyanates.⁸ Today, isocyanates are viewed as one of the chemical agents causing the most cases of occupational asthma.⁹ Between 5-15 % of workers handling isocyanates are estimated to develop occupational asthma.¹⁰,¹¹ Isocyanate asthma has been reported for HDI¹²,¹³, and HDI adducts¹⁴, TDI¹⁵,¹⁶, TDI prepolymer¹⁷ and MDI.¹⁸,¹⁹ In some cases asthma attacks due to isocyanates has caused death.²⁰,²¹ The mechanisms for isocyanate asthma and sensitization have not been completely understood. IgE or IgG antibodies to isocyanates are sometimes found in sensitized patients,²² but antibodies are not always present in symptomatic patients, suggesting nonantibody-mediated mechanisms.²³ After exposure to isocyanates biomarkers for isocyanates can be found in blood and urine.²⁴

Besides from asthma, isocyanates also causes other airways disorders like hypersensitivity pneumonitis,²⁵,²⁶ rhinitis,²⁷,²⁸ and chronic obstructive airway disease.²⁹,³⁰ Isocyanates are also skin irritants, and may cause eczema and contact dermatitis.³¹,³²

Some diisocyanates are suspected carcinogens, and has been studied by the International Agency for Research on Cancer (IARC). 2,4-TDI is classi-
fied as a group 2B agent (possibly carcinogenic to humans), while NDI, MDI and pMDI are classified in group 3 (not classifiable as to carcinogenicity to humans).33

Since the volumes of monoisocyanates used in the industry are much smaller than for diisocyanates, the number of reports about their health effects is limited. However, much information is available regarding MIC, where victims of the tragic Bhopal disaster have been studied. Several reviews have been published, where the acute symptoms of methyl isocyanate exposure are presented. These include pulmonary edemas, eye and throat injuries, vomiting and nausea, neurological disorders and unconsciousness.34,35 Animal studies confirm the acute toxic effects of MIC, but long-term effects showing decreased lung function in exposed workers have been difficult to establish.36 Phenyl isocyanate (PhI), which sometimes has been present as a by-product in polymeric MDI mixtures, has been showed to have sensitizing properties38, and produce asthma-like symptoms in rats.39

Due to all the negative health effects ascribed to isocyanates, their occupational exposure limits (OEL) are very low. In Sweden, the time weighted average (TWA) value, that is the maximum permissible concentration of isocyanates in the air during an eight hour work shift, is 10 ppb for monoisocyanates. For diisocyanates the value is 2 ppb.40

3.3 Exposure

Exposure to airborne TDI and MDI has been reported during production of soft TDI-based PUR-foam41,42,43 and rigid MDI-foam.44 Spray painting of polyurethane coating may give rise to high levels of isocyanates, typically HDI-adducts like HDI biuret or isocyanurate that are common components in coatings. Several studies concerning spray painting has been published.45,46,47,48 Spraying of MDI is also sometimes performed, for example for insulation purposes49 or in more specialized processes like production of “bed liner”, a protective and softening coating on pick-up trucks.50 The isocyanate mixture used here, polymeric MDI, is non-volatile but spraying makes airborne exposure possible.

Isocyanate exposure occurs in several industrial branches, not just during manufacture of polyurethane or spray painting. In recent years the exposure associated with thermal degradation of PUR has been thoroughly studied. During flame lamination of TDI-foam with textile the foam is degraded, and TDI is released to the surrounding air.15,51 Other situations where polyurethane is thermally degraded are for example during welding, cutting or grinding in PUR-coated metal sheets.52,53 Car workshops has been studied to assess this exposure situation.54 MDI glues, or plaster casts used at hospitals has been shown to cause occupational asthma.19,55 Isocyanate exposure can also occur during thermal degradation of other polymers, for example has
exposure to isocyanic acid (ICA) and methyl isocyanate (MIC) been observed during thermal degradation of phenol-formaldehyde-urea resins.\textsuperscript{56,57}

Another route of exposure to isocyanates is dermal exposure. Reports on contact dermatitis caused by dermal isocyanate exposure have been published. Glues\textsuperscript{58}, lacquers\textsuperscript{59}, paint or binders\textsuperscript{60} are example of different products that has been showed to cause allergic skin reactions in exposed workers.

To study possible exposure situations and evaluate air sampling methods for this thesis a number of different workplace studies have been performed. In paper I spray painting using HDI-based coatings, spray foaming using MDI and casting of PUR plastic details was studied. In paper V different situations where thermal degradation of PUR takes place were studied. Measurements were made during welding in coated metal sheets and welding in PUR-insulated heating pipes. The primary goal for the field measurements was not exposure assessment. Instead, field samples were collected to evaluate air sampling methods for sampling of different PUR-related compounds.

### 3.4 Air Sampling

Due to the early reports about the isocyanates ability to cause airway disorder, there were soon developed methods for determination of air levels. In 1957 Marcali presented a procedure for isocyanate determination involving air sampling using a bubbler containing acidic medium for hydrolysis of TDI to toluene diamine (TDA). The formed amines were then diazotized and the air levels were determined photometrically.\textsuperscript{61} A drawback of the Marcali method is that it not only measures isocyanates, if TDA is present in the air it will also be included in the result.

#### 3.4.1 Reagents

The reactivity of isocyanates makes it necessary to protect them already during air sampling. Otherwise, they may be lost in reactions with other compounds present. A number of different derivatizing reagents for isocyanates have been developed, most of them using the reaction between the isocyanate and an amine to form a urea-derivative. Lower occupational exposure limits has been one reason that new reagents are still developed. Reagents often have some special analytical property highlighted, to make the derivatives suitable for analysis by a certain detector. New reagents have also been developed to obtain faster reaction rates for the derivatization, or increased stability for reagents and derivatives.

The first amine reagent developed was the “nitro” (N-4-nitrobenzyl-N-n-propylamine)-reagent.\textsuperscript{62} The reagent was not very stable, it had to be stored as the hydrochloride salt, but the formed derivatives were reported stable
during analysis. Another amine reagent, 1-(2-pyridyl)piperazine (1-2PP) was introduced in 1979, as having several advantages, for example better stability and higher molar absorptivity, compared with the nitro reagent.\textsuperscript{65}

To take advantage of fluorescence (FL) detection, a detection method with low detection limits, urea derivatives were formed by reaction of isocyanates with 9-(N-methylaminomethyl)-anthracene (MAMA).\textsuperscript{64} When it was introduced it was compared with the nitro-reagent, and a ten to twenty times lower detection limit was claimed with the use of MAMA.

A reagent that still is frequently used is 1-(2-methoxyphenyl)piperazine (2-MP).\textsuperscript{65} When it was introduced it offered higher selectivity in isocyanate analysis, since it was detected by two detectors, UV and electrochemical. It also had higher collection efficiency for isocyanates when compared with the nitro-reagent and 1-2PP. Another reagent developed for double detection techniques is 3-(2-aminoethyl)indole, also known as tryptamine. Fluorescence and amperometric detection was used in series for analysis of isocyanate derivatives.\textsuperscript{66} In later studies tryptamine has been compared with 2-MP, 1-2PP, and the nitro-reagent. It was concluded that tryptamine and 2-MP had about the same relative reaction rates to isocyanates, while 1-2PP and the nitro reagent reacted much slower with isocyanates.\textsuperscript{57}

1-(9-Anthracenylmethyl)-piperazine (MAP) is a reagent that structurally resembles MAMA as they both contain an anthracene group suitable for UV or FL detection. In the first publication\textsuperscript{68} presenting the MAP reagent, reaction rates were compared with 2-MP, MAMA and tryptamine. The reaction rate for MAP was comparable with 2-MP, and detection properties were in most cases more attractive than for the other reagents in the comparison. MAP has also been used for determination of total reactive isocyanate groups (TRIG). Determination of TRIG is performed by using a reagent that gives equal response for all isocyanate groups, regardless of the structure of the isocyanate. The idea of TRIG measurements is that the concentrations of isocyanate groups can be determined without standards for all analyzed compounds. 2-MP\textsuperscript{69}, MAMA\textsuperscript{70,71} and tryptamine\textsuperscript{72} have also been evaluated for measurements of TRIG.

Di-n-butylamine (DBA), the reagent used for air sampling of isocyanates in paper I-VI, was initially used for determination of isocyanate content in technical mixtures used for polyurethane production. A known amount of DBA is added to an isocyanate mixture, and the isocyanate groups are then derivatized and DBA is consumed. The excess DBA is titrated with hydrochloric acid. The concentration of isocyanate groups in the isocyanate mixture can then be calculated. This method was used to determine isocyanate content in technical mixtures in paper I. Air sampling of isocyanates using DBA as a reagent has been used for about a decade.\textsuperscript{73} A drawback with DBA is that it is an aliphatic molecule, and UV-response for the derivatives is obtained only if it is reacted with aromatic isocyanates. However, mass spectrometric detection makes it possible to analyze aliphatic isocyanaytes deri-
vatized with DBA as well. The DBA reagent has other advantages in that it is stable, as are the derivatives formed, due to a high solubility it can be used in high concentrations in toluene for impinger sampling and it has been showed to have high reaction rates when compared with other reagents. Excess reagent can easily be removed by evaporation or extraction.

The reagents described so far have been the most studied, but in recent years a few new reagents have been presented, that also should be mentioned.

A method for determination of TRIG in air was developed using 9-anthracenylmethyl-1-piperazinecarboxylate (PAC). Isocyanate groups on all types of isocyanates were reacted to a single analyte. In this way the isocyanate concentration could be determined without knowledge of the structure or a special standard. However, the method had problems in obtaining blank samples. 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ) is a new reagent that has been tested with several isocyanates. Reaction rates have been compared with MAMA, and found to be higher. However, NBDPZ is not very stable in some solvents. Two other new reagents are ferrocenoyl piperazide (Fc-PZ) and 4-methoxy-6-(4-methoxy-1-naphtyl)-1,3,5-triazine-2-(1-piperazine) (MMNTP), but since the introduction no new reports has been published. For reaction of MMNTP with aliphatic isocyanates a 2 hour “incubation-period” is recommended that seems to make this reagent unsuitable for air monitoring of isocyanates, where fast reactions are necessary. Structures of different isocyanate reagents are presented in table 4.

Besides from the amine reagents, reports have also been published where isocyanates form urethanes by reaction with ethanol in an impinger or bubbler. To accelerate the reaction the ethanol was made alkaline by addition of potassium hydroxide.
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Structure</th>
<th>Reference</th>
<th>Reagent</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitro</td>
<td><img src="image" alt="Nitro Structure" /></td>
<td>62</td>
<td>1-2PP</td>
<td><img src="image" alt="1-2PP Structure" /></td>
<td>63</td>
</tr>
<tr>
<td>MAMA</td>
<td><img src="image" alt="MAMA Structure" /></td>
<td>64</td>
<td>2-MP</td>
<td><img src="image" alt="2-MP Structure" /></td>
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</tr>
<tr>
<td>Tryptamine</td>
<td><img src="image" alt="Tryptamine Structure" /></td>
<td>66</td>
<td>MAP</td>
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<td>68</td>
</tr>
<tr>
<td>DBA</td>
<td><img src="image" alt="DBA Structure" /></td>
<td>73</td>
<td>PAC</td>
<td><img src="image" alt="PAC Structure" /></td>
<td>76</td>
</tr>
<tr>
<td>NBDPZ</td>
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<td>77</td>
<td>Fc-PZ</td>
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</tr>
<tr>
<td>MMNTP</td>
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<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.4.2 Sampling Techniques

Sampling of airborne isocyanates has been performed using both wet (impingers or bubblers containing a reagent in some solvent) and dry (filters or different solid sorbents impregnated with reagent) sampling techniques. Dry samplers are generally preferred when personal measurements are performed, while impingers have advantages for example regarding the ability to provide reagent to the collected isocyanates. A difficulty is that isocyanates are present both in the gas phase and associated to particles, which puts extra demands on the sampler. Since PUR often is manufactured by spraying, it is possible to obtain airborne exposure even for non-volatile isocyanates.

3.4.2.1 Impinger sampling

In the first method for airborne isocyanates, the Marcali method, air sampling was performed using a bubbler and traditionally wet sampling has been the method of choice when new reagents have been introduced. Sampling using the nitro reagent, MAMA, 1-2PP, 2-MP, tryptamine, DBA and MAP were all initially presented using impingers. Impingers have also been used for sampling of isocyanates in acid or alkaline ethanol. Impingers are known to have poor sampling efficiency for particles in the sizes between 0.01 and 1.5 µm, but this problem can be solved by placing a filter after the impinger. For 2-MP a reagent impregnated filter has been used after the impinger. The DBA method uses an unimpregnated filter after the impinger. The high concentration of DBA in the impinger solution and the volatility of the reagent provide efficient derivatization on the filter as well. Impinger-filter sampling using DBA in toluene was used in paper I for samples where the composition of technical isocyanate solutions was compared with the isocyanate composition in air samples. It was also used as a reference method for comparison of total air levels in paper III and IV.

3.4.2.2 Filter and solid sorbent sampling

Due to the advantages with dry sampling techniques for personal measurements a lot of research has been done to find dry methods that perform as well as impingers. For several reagents impregnated glass fiber filters has been used, but other types of dry samplers, like different solid sorbents, have also been evaluated.

The nitro-reagent has been used in samplers with coated glass powder, coated glass wool and glass fiber filters. When comparing with wet sampling methods, air levels were equal or higher for the dry methods. MAMA has been used adsorbed on XAD-2. When compared with a bubbler method there was good agreement in air levels for sampling of
HDI.\textsuperscript{95} A special kind of sampler consisting of a MAMA-coated denuder in combination with a MAMA-impregnated filter has also been developed for sampling of HDI and oligomers.\textsuperscript{96} Impregnated glass fiber filters have been used for dry sampling using 1-2PP in several studies.\textsuperscript{97,98,99} A method with sampling tubes containing 1-2PP impregnated sorbent has also been presented for sampling of TDI.\textsuperscript{100} Comparison with an impinger method has been done for glass fiber filters.\textsuperscript{97} At low humidity the samplers showed comparable results, but when the humidity was almost 100 \%, the dry sampler failed and much lower levels were obtained for the dry sampler as compared to the impinger sampling.

In recent years several studies has been presented using 2-MP impregnated glass fiber filters.\textsuperscript{56,101,102,103,104,105,106} One of the methods use dual filters, to minimize breakthrough.\textsuperscript{56} In comparisons with impinger methods very different results were obtained. In some studies filters gives the highest air levels\textsuperscript{101} while equal results for impingers and filters are reported in other studies.\textsuperscript{102,104,106} There are also examples when impinger sampling has been superior.\textsuperscript{56,103,105} Besides from the filter methods 2-MP has also been used on extraction columns\textsuperscript{107}, sintered glass\textsuperscript{108} and a sampler where a PUR sponge is coated with the reagent.\textsuperscript{109}

Tryptamine has also been evaluated for dry sampling using sampling tubes filled with coated XAD-2, but so far the best results for this reagent has been obtained using impinger sampling.\textsuperscript{110,111} The fairly new reagent MAP has also been tested for dry sampling of HDI and adducts like isocyanurate and biuret. It was reported that the MAP-impregnated filter and impinger showed equally good performance.\textsuperscript{112}

Because of the high volatility of DBA it is troublesome to use it in dry samplers alone. The reagent evaporates quickly when air is drawn through the sampler and something that prevents the DBA from evaporating must be present in the sampler. A dry sampler containing DBA in polydimethylsiloxane for sampling of gaseous TDI was presented a few years ago.\textsuperscript{113} Polydimethylsiloxane is also used as a stationary phase for gas chromatography. The sampler consisted of a denuder and a filter in series. The amounts of isocyanates in air were lower than for impinger sampling, when the sampling methods were compared. In later studies the denuder was evaluated for sampling of HDI and IPDI\textsuperscript{114} and MDI\textsuperscript{115} and reasonable agreement with the impinger method was obtained. Another way to reduce the volatility of DBA is to let it form an ion pair with acetic acid. This principle was used for impregnation of a glass fiber-coated denuder in series with a filter.\textsuperscript{116} This sampler has been thoroughly evaluated for several different types of exposure, both laboratory-generated atmospheres and sampling in the field. Comparisons were made with impinger sampling, and the sampler was found to be a convenient alternative to impinger sampling. The impregnation technique with DBA and acetic acid on glass fiber filters has been used in Paper III and IV for impregnation of the fractionating sampler.
3.4.2.3 Passive sampling
All air sampling methods presented so far are active sampling methods, i.e. a pump is used to drive the air flow through the sampler. For sampling of gaseous isocyanates passive sampling methods has also been presented, but the research has been limited, because situations were only gaseous exposure exists are rare. Solid phase micro extraction using DBA as a reagent has been used for sampling of gaseous TDI.\(^{117,118}\) For sampling of MIC diffusive samplers using 2-MP\(^{119}\) and NBDPZ\(^{120}\) has been used for derivatization of isocyanates.

3.4.2.4 Fractionated Sampling
The exposure situation for isocyanates is often complex, and isocyanates may, depending on volatility, molecular weight or how they are emitted, be associated with particles or be present as gases. Because of this it is important to use sampling methods that are able to collect the aerosol in a representative way. The distribution between gas and particles in the aerosol is also important to consider when lung deposition and the effects on the human airways are studied. Different models for deposition of gas and particles in the human airways have been presented. In one of these models is presented by the International Commission on Radiological Protection (ICRP).\(^{121,122}\) In the ICRP deposition model it can be seen that large particles (>2.5 µm) are mainly deposited in the upper airways due to inertial impact, while smaller particles are able to reach deeper parts (tracheobroncial and alveolar region) of the airways.\(^{123}\) Gaseous substances are deposited in the head and nose region as well, due to diffusion. To better understand where the isocyanates are deposited and obtain a connection between exposure types and disease, samplers with the ability to fractionate the aerosol are necessary.

There are a few examples of samplers for isocyanates that can separate gas and particles during sampling. The IsoCheck method uses two filters in series for separation. The first filter is an unimpregnated Teflon-filter that collects particles containing isocyanates. The second filter is a MAMA-impregnated glass fiber filter, that collects gas-phase isocyanate.\(^{124}\) After sampling the front filter is put in a solution containing 2-MP to derivatize the isocyanates associated to particles. However, the use of an unimpregnated Teflon filter probably causes some loss of isocyanates, since the isocyanate groups are not derivatized and therefore unprotected after collection. A variant of the IsoCheck sampler is a triple filter system, introduced in 2003.\(^{125}\) This sampler contains 2 unimpregnated Teflon-filters before a 1-2PP impregnated glass fiber filter. The second Teflon filter is used to estimate the gaseous isocyanates collected on the first filter.

A denuder in series with a filter has also been presented as a sampler with ability to separate gas phase from particles. For collection of MDI-aerosols
the nitro-reagent was used for coating of denuder and filter.\textsuperscript{126} For collection of HDI and adducts a denuder-filter system with MAMA-coated parts was used.\textsuperscript{96} To prevent non respirable particles to enter the samplers, a preseparator (a cyclone or an impactor) were used in front of the denuder parts.

Impingers may also be used for fractionated sampling. If the impinger is used with a back-up filter, and the filter is analyzed separately, a fractionation is obtained. Small particles between 0.01 and 1.5 µm are collected on the filter. Large particles and gas phase isocyanates are collected in the impinger flask.\textsuperscript{53} This principle has been used in Paper V.

To obtain a more detailed view of the isocyanate distribution in aerosols a fractionating sampler consisting of a denuder in series with a cascade impactor and a glass fiber filter is presented in Paper III. The denuder-impactor separates the aerosol in five different fractions. The sampler was designed for a total flow rate of 5 l min\textsuperscript{-1}. The sampler has been used for sampling of different types of isocyanate aerosols, generated by thermal degradation of polyurethane, evaporation of isocyanates (gas-phase isocyanates) or spraying of polyurethane coating compounds. As a reference sampler for comparison of total air levels, the impinger-filter sampler with DBA in toluene was used.\textsuperscript{53} The differences in total air levels of isocyanates between the denuder/impactor and the reference sampler were small, despite the big differences in the two samplers regarding design and sampling flow. The denuder-impactor sampler is presented in more detail below

3.4.2.4.1 Denuder

The first part of the sampler introduced in paper III is a channel-plate denuder for collection of gaseous isocyanates (figure 2). The denuder consists of eight parallel glass fiber plates, mounted together in a plastic holder made from polypropylene plastic, and held together with stainless steel bolts. The glass fiber plates mounted in the holder are initially 4 cm wide and 7.2 cm long, but the holder covers about 0.7 cm on each side of a plate, so the effective width facing the air stream is 2.6 cm. The eight denuder plates faces the air stream on both sides, so the total filter surface facing the air stream is 2.6*7.2*8*2 = 299.5 cm\textsuperscript{2}. A thin, but rigid filter material without binder (type MGC, Munktell, Falun, Sweden) was chosen for the denuder plates. Each plate was impregnated with 1.4 M DBA-acetic acid in methanol before the plates were mounted in the denuder.

The efficiency for a denuder can be calculated by using the Gormley-Kennedy equation. The original equations were developed for a cylindrical denuder.
An expression for rectangular denuders have been given by Dasgupta et al.\textsuperscript{127} as:

\[ f = 1 - 0.91e^{-\left(\frac{7.54wDL}{Qs}\right)} \]  \hspace{1cm} (1)

where \( f \) is the efficiency, \( w \) is the channel width, \( D \) is the diffusion coefficient for the studied compound, \( L \) is the length of the channel, \( Q \) is the volumetric flow and \( s \) is the channel height. Values of the diffusion coefficients for TDI, HDI and IPDI were adopted from Nordqvist.\textsuperscript{128} The equation is only valid if the flow through the channel is laminar. The flow is considered laminar if the Reynolds’ number is below 2000. The Reynolds’ number for a square channel is calculated as

\[ \text{Re} = \frac{\nu \times d \times \rho}{\eta} \]  \hspace{1cm} (2)

where \( \nu \) is the linear flow rate, \( d \) is the hydraulic diameter \( \rho \) is the density of the air and \( \eta \) is the viscosity of the air. For a square channel the hydraulic diameter is calculated as \( 4 \times \text{channel area/circumference} \).\textsuperscript{129} Design parameters for the denuder are presented in table 5.
Table 5. Design parameters for the denuder

<table>
<thead>
<tr>
<th>Channel plate denuder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plates</td>
</tr>
<tr>
<td>Total effective area (cm²)</td>
</tr>
<tr>
<td>Calculated efficiency (%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Reynolds’ number&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated according to equation 1, for IPDI.
<sup>b</sup> Calculated according to equation 2

To extract the isocyanate-DBA derivatives from the denuder plates after sampling the plates were cut out and placed in test tubes. The plates were extracted by shaking with 1 mM sulfuric acid, methanol and toluene. For efficient extraction it was found that the toluene extraction should be repeated twice. The toluene was separated and evaporated. The residue was dissolved in acetonitrile for LC-MS analysis.

In paper III the sampling efficiency for gas phase isocyanates was tested by sampling in a test chamber where gaseous isocyanates were generated by evaporation. 2,4- and 2,6-TDI, HDI and IPDI were the isocyanates included in the tests. The breakthrough of gaseous isocyanates was small, and did not exceed 5% of the total amount collected. This agrees reasonably with the theoretical calculations for the denuder. The calculated efficiency for IPDI (diffusion coefficient: $5.06 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ at 25°C) was about 98%, using equation 1.

3.4.2.4.2 Impactor

A cascade impactor (figure 3) with three impaction stages was connected in series after the denuder part. Cascade impactors are used for fractionation of airborne particles. It is an inertial classifier in the sense that the collection principle is based on the inertia of a particle. An air stream is directed towards an impaction plate. Close to the surface of the impaction plate the air stream deviates and flows around the plate. Particles with a high inertia are unable to follow the deviating air stream and are deposited on the plate. In the following impaction stage the diameter of the jet directing the air stream towards the plate is decreased, which increases the velocity of the air passing through the jet. In this way some of the small particles that were able to follow the air stream in the first stage now have an inertia that is too big, so these particles are deposited on the following stage instead.
Figure 3. Cross-cut view of impactor stages and collection principle. Particle-sizes are not to scale.

The design of an impactor is based on numerical solutions of the Navier-Stokes equations which were presented in 1974 by Marple and Liu. Impaction stages are usually described with the cut-off diameter, $d_{50}$, which is the aerodynamic diameter of particles that are collected by the stage with an efficiency of 50%. By calculation of the dimensionless Stokes’ number, the cut off diameter for an impaction stage can be obtained. For the type of impactor used in this work, that is with a single orifice and a circular jet $d_{50}$ can be calculated using the formula

$$d_{50} = \frac{9 \eta W}{\rho_p C_c U \sqrt{Stk_{50}}}$$

In this equation $\eta$ is the viscosity of the medium surrounding the particle, $W$ is the nozzle width, $\rho_p$ is the particle density and $U$ is the velocity through the nozzle. $C_c$ is the Cunningham slip correction factor that becomes more important with decreasing particle size. When the particle diameter comes close to the mean free path of the individual molecules, the surrounding gas no longer acts as a continuous media, but as individual gas molecules exerting forces on the particle. The Cunningham slip correction factor compen-
sates for this. For particles larger than 1 µm the $C_c$ has a value close to 1. Stokes’ number is a dimensionless parameter and represents the ratio between a particle’s stopping distance to a characteristic diameter. $\text{Stk}_{50}$ is the Stokes’ number for 50% collection of the particles with the specified diameter $d_{50}$. For a circular jet impactor the $\text{Stk}_{50}$ is 0.22.

To obtain a sharp cut-off curve for an impactor, the Reynolds’ number should be between 500 and 3000 calculated as:

$$\text{Re} = \frac{v \cdot D \cdot \rho}{\eta}$$  \hfill (4)

In equation 4 the linear air velocity is represented by $v$, $D$ is the diameter of the circular jet, $\rho$ is the density of the air and $\eta$ is the viscosity of the air.

![Graph](image)

**Figure 4.** Collection efficiency for the two top impaction stages. The efficiency was determined by measuring the penetration of a polydisperse glass aerosol.

The three impaction stages used for the measurements had cut-off values ($d_{50}$) of 2.5, 1.0 and 0.5 µm. Design parameters for the impactor stages are presented in table 6. The collection efficiency for the top impactor stages was checked by measuring the penetration of a glass aerosol by connecting the stages to an aerodynamic particle sizer (figure 4). For stage 1 and 2, the cut-off diameters were close to the calculated values *paper III*.

Due to the reactive nature of the isocyanates the derivatizing reagent must be applied to the impaction plates where the particles are deposited. As substrates for the impactors thin DBA-acetic acid impregnated glass fiber plates
were used in paper III and IV. To avoid filtration and retention of small particles on the top plates, a concentrated and highly viscous solution of DBA and acetic acid was used.

Table 6. Design parameters for the impactor stages.

<table>
<thead>
<tr>
<th>Impactor Stage</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nozzle width (mm)</td>
<td>2.7</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Jet to plate distance (mm)</td>
<td>3.55</td>
<td>2.35</td>
<td>1.90</td>
</tr>
<tr>
<td>S/W&lt;sub&gt;a&lt;/sub&gt;</td>
<td>1.32</td>
<td>1.57</td>
<td>1.85</td>
</tr>
<tr>
<td>Reynolds’ number&lt;sub&gt;b&lt;/sub&gt;</td>
<td>2585</td>
<td>4653</td>
<td>6980</td>
</tr>
<tr>
<td>Linear velocity (m s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>14.6</td>
<td>47</td>
<td>106</td>
</tr>
<tr>
<td>Calculated d&lt;sub&gt;50&lt;/sub&gt; (µm)&lt;sub&gt;c&lt;/sub&gt;</td>
<td>2.5</td>
<td>1.08</td>
<td>0.58</td>
</tr>
</tbody>
</table>

<sup>a</sup> S is the distance between the nozzle exit and the substrate, W is the nozzle width.

<sup>b</sup> Calculated according to equation 4

<sup>c</sup> Calculated according to equation 3

For collection of the smallest particles that passes the last impaction stage a 25 mm glass fibre filter was mounted after the last impaction stage. The end filter was impregnated with a solution of DBA-acetic acid in methanol.

After sampling, the impactor substrates and the end filter were extracted in test tubes containing 1 mM sulfuric acid and toluene. The samples were shaken and placed in an ultrasonic bath before the toluene phase was separated and evaporated. After evaporation the samples were dissolved in acetonitrile before the LC-MS analysis.

3.4.2.4.3 Results from laboratory evaluation

In paper III measurements with the denuder-impactor sampler are presented. Besides from sampling of gas phase isocyanates, thermal degradation products of polyurethane and spraying of polyurethane coating compounds were studied. Sample generation was performed in a 0.3 m<sup>3</sup> sampling chamber, built from stainless steel and glass. The humidity in the chamber was controlled by mixing dry and humidified air. To avoid isocyanate aerosol to escape outside the chamber, the pressure in the chamber was below the ambient pressure (controlled by the exhaust ventilation of the chamber).

The results from the measurements with the denuder-impactor showed that different isocyanate distributions were obtained depending on how the isocyanates were generated. When comparing the total air-levels measured with the denuder-impactor with air-levels measured simultaneously using impinger-filter, good agreement between the samplers was obtained.
The isocyanate distribution for samples collected during thermal degradation of PUR showed that gas-phase isocyanates and isocyanates associated to small particles dominated in the aerosol (figure 5).

Figure 5. Relative distribution of isocyanates in the denuder-impactor. Sample collected in the sampling chamber during thermal degradation of PUR.

Figure 6. Relative distribution of isocyanates in the denuder-impactor sampler. Sample collected in the sampling chamber during spraying of PUR-coating.
Non-volatile MDI was exclusively found on the impaction stages, probably due to fast condensation.

Samples collected during spraying of isocyanate coating showed that isocyanates for this type of generation were associated with the larger particles, and the top stage with a \(d_{50}\) of 2.5 µm collected the majority of the isocyanates (figure 6).

In paper IV, ageing isocyanate aerosols were studied. After isocyanate generation, three consecutive denuder-impactor measurements were made. The analysis of these samples revealed that the isocyanate distribution in the aerosol was time dependent. Aromatic isocyanates were initially associated with the gas phase or small particles, but after a few minutes the distribution of isocyanate was changed, and the aromatic isocyanates became associated with particles. The monoisocyanates and HDI remained in the gas phase for the whole sampling period.

The results obtained with the denuder-impactor have shown that this sampler has great potential for investigation of aerosols containing isocyanate. However, some points should be highlighted regarding the evaluation. For example, this type of sampler has not been designed for personal measurements. Instead the sampler was constructed as a tool with ability to study how different isocyanate aerosol behaves. So far, the denuder-impactor has only been evaluated in chamber measurements. To fully characterize the sampler, field measurements are also necessary. The sampler should also be evaluated for other types of aerosol associated with different work operations like casting of PUR or spray foaming.

### 3.5 Sample Analysis

#### 3.5.1 Colorimetric methods

The low occupational exposure limits for isocyanates and complex composition in air samples has contributed to the development of sensitive and selective analytical methods. The first air sampling methods for isocyanates relied on spectrophotometric detection of isocyanates. Before analysis the isocyanates are hydrolyzed to amines, that in turn are diazotized, and the colored complexes are analyzed.\(^{61}\) This way of analysis also analyses the corresponding amines and aminoisocyanates if they are present in the air, so a sum of these compounds is obtained. To measure the isocyanates alone, an improvement of the Marcali-method was introduced in 1970. Sampling was performed using two different sampling solutions, one containing hydrochloric acid for hydrolysis of isocyanates. The other solution contained an amine, for reaction with the isocyanates. After sampling both solutions were diazo-
tized, and the difference in response between the samples represented the response for the isocyanates.132

3.5.2 Chromatography

When derivatizing agents for isocyanates were introduced, it became necessary to develop methods that could separate the formed derivatives from the excess reagent. Separation of the derivatives also provides a higher selectivity, which is necessary if several isocyanates are present in the air sample. For the first derivatizing reagent, the nitro reagent, thin layer chromatography (TLC) was used for separation.62 TLC has also been used for analysis of isocyanates derivatized using 1-2PP.84

The dominating technique for separation of isocyanate derivatives is liquid chromatography. A LC method for analysis of the nitro reagent was developed in 1976133, and for methods using other reagents as 1-2PP63, MA-MAMA64, 2-MP65, tryptamine66, MAP68 and DBA73, HPLC separation was used for sample analysis when the reagents were introduced.

Chromatographic analysis in paper I-VI has been performed using micro-LC, that is with columns with small internal diameters and low flow-rates between 70-100 µl min⁻¹. Micro-LC has also been used for determination of MAMA-derivatives of isocyanates.134 Compared with conventional LC there is several advantages, as for example a low consumption of mobile phases and less maintenance of the instrument. Low flow rates also allow small column particles to be used without getting a high column back pressure. This increases the resolution and selectivity of the chromatography. All columns used have been 50 mm long reversed-phase columns with octadecylsilica (C₁₈) particles in sizes between 2.5-3.5 µm.

To improve the chromatography, sample injection has been performed using on-column concentration in paper I-VI. This means that a small sample plug, usually about 2.5-5 µl, surrounded on both sides with a weak eluent (usually 95/5 water/acetonitrile) is injected to the column. When this package reaches the column the analytes in the sample are retarded on top of the column, and focused in a narrow band. The analytes are released when the gradient starts, but the peaks eluted are much sharper than for conventional injection methods, where the sample is injected directly into the mobile phase. This is illustrated in figure 7 where the same amount of isocyanate-DBA derivatives has been injected.
Figure 7. The effect of on column concentration. Chromatogram A: sample dissolved in acetonitrile, injection without focusing. Chromatogram B: sample dissolved in mobile phase. Chromatogram C: concentrated sample dissolved in acetonitrile, sample surrounded by 95/5 water/acetonitrile in the injection loop.

The sample was dissolved in 20 µl acetonitrile, 20 µl mobile phase (60/40 water/acetonitrile) and 2.5 µl acetonitrile surrounded by 17.5 µl 95/5 water acetonitrile. Pure acetonitrile is a good solvent for the derivatives, but the peaks are severely broadened and distorted. For the sample dissolved in mobile phase it is only the early eluting peaks that are broadened, but there could be solubility problems for some derivatives when the concentration is increased. The concentrated sample surrounded by weak mobile phase shows excellent peak-shape for all the derivatives, and there are no problems with the solubility of the derivatives. If the sample was dissolved in water the effect would have been the same as for on column focusing, but due to the low solubility of the derivatives in water this is not appropriate.
3.5.3 Detection Methods

For the early chromatographic methods for determination of urea derivatives of isocyanates ultraviolet (UV) detection has been one of the most popular detection techniques. Analysis of monomeric isocyanates and prepolymer or adducts using LC-UV has been presented for both the nitro reagent\(^\text{46,133,135,136}\) and 1-2PP.\(^\text{63,137,138}\) UV detection has also been used for MAMA\(^\text{64}\), 2-MP\(^\text{65}\), MAP\(^\text{68}\) and DBA.\(^\text{73}\) All the reagents except for DBA contain aromatic rings, so absorbance in the UV-region is obtained for aromatic as well as aliphatic isocyanates. Analysis of isocyanates using UV and DBA can only be performed for aromatic isocyanates.

To obtain lower detection limits and higher selectivity for isocyanates in complex air samples, several other detection methods has been used for analysis. Sometimes dual detection systems are used to confirm analytical peaks as isocyanates.

Fluorescence (FL) detection has been used for MAMA\(^\text{64}\), tryptamine\(^\text{66}\) and MAP.\(^\text{68}\) MAMA and MAP both contain an anthracene group, suitable for fluorescence detection. Tryptamine may also be detected using fluorescence, but the response is lower than for MAP and MAMA.\(^\text{68}\) Compared with UV detection, lower detection limits are obtained using FL-detection.

The advantage of using two detectors in series is increased selectivity since the ratios between the detector responses must be the same for samples and standards. This makes it possible to exclude peaks that are not isocyanates. TRIG-measurements are usually performed using dual detectors. When 2-MP was introduced as a reagent, UV detection in series with electrochemical (EC) detection\(^\text{65}\) was used for sample analysis. The detection technique has later been adapted for sampling of prepolymer and adducts.\(^\text{69,139}\) Prepolymer identification was originally based on the response ratio between the EC and UV detector, and quantification was done by comparing the EC-response to the response of a monomer. This way of isocyanate identification and quantification makes standards containing anything else but monomers unnecessary, which simplifies the analysis. Later research has showed that some isocyanates may be quantified wrong\(^\text{140}\) or not identified as isocyanates\(^\text{141}\) using the response ratio method for 2-MP.

MAP was introduced as a better alternative to determination of unknown isocyanates using a monomer for determination of isocyanate concentration.\(^\text{68}\) UV detection in combination with EC has been used, as well as UV in combination with FL-detection.\(^\text{68,85,142}\) The FL-detector was introduced as a more robust alternative than an EC-detector.\(^\text{142}\)

For determination of TRIG, MAMA has also been evaluated using dual detection with UV and FL. However, the ratio between the different detectors was not used for identification, instead the ratio between absorption at two different wavelengths was used for identification, since this ratio was found to be fairly constant for different isocyanates.\(^\text{70,71}\) Tryptamine has also
been used for determination of TRIG, by using a combination of a FL and an amperometric detector.\textsuperscript{72}

### 3.5.3.1 Mass Spectrometry

Mass spectrometry (MS) is a sensitive and selective detection technique and very low detection limits can be obtained. This makes MS a very attractive technique for analysis of isocyanates in air, where OEL values are low. However it is only in the last ten years that MS has been used as a routine detection technique for isocyanates. Partly this depends on that it initially was an expensive technique, not affordable for many laboratories. Old mass spectrometers were also difficult to use, the response could be unstable, and the interfaces that were possible to use in combination with LC were poorly developed. Nowadays, it is possible to build inexpensive and more robust mass spectrometers, and with the electrospray (ES) interface coupling to LC is straightforward. DBA, which has been used as a reagent for isocyanates in paper I-VI, was initially analyzed using LC-UV\textsuperscript{73}, but LC-MS methods for determination of aromatic\textsuperscript{143,144} and aliphatic\textsuperscript{4,74} isocyanates were soon introduced. In paper I LC-MS was used for determination of isocyanates used in technical grade qualities and for analysis of air samples.

In a mass spectrometer ions are separated by their mass to charge ratio (m/z). Mass spectrometric detectors using two different types of MS instruments, quadropole and time of flight (TOF), has been used in this thesis.

For routine analysis of air samples different types of quadropole instruments have been used. In the single quadropole used in paper I, V and VI charged ions from the electrospray ion source are focused by different electronic lenses and introduced between four rods. The rods work as electrodes, and dc and ac voltages are applied to the rods. The mass spectrum is scanned by increasing the ac and dc voltages, while the ratio between the voltages is kept constant. Depending on the voltage applied, ions with a unique value of m/z can pass through the quadropole in stable trajectories. The other ions are lost by collision with the quadropole rods. Scanning of a spectrum is obtained by increasing the voltage gradually. Selective ion recording (SIR) can be performed by selecting a few number of voltage settings, that only allows ions with a specified m/z to pass through the quadropole. By selecting only a few ions for analysis lower detection limits are obtained, because the analysis time for these ions is increased.

A triple quadropole instrument, which was used in paper II-IV, is constructed in the same way as a single quadropole, but three mass analyzers are combined in series. The second quadropole is used as a collision cell for fragmentation of ions from the first quadropole. The ions are collided with a collision gas, usually argon, and the fragments obtained are analyzed by the third quadropole. For routine analysis multiple reaction monitoring (MRM) was used. In MRM ions with one m/z are selected by the first quadropole, these ions are collided with argon, and a second fragment ion with another
m/z is selected by the third quadropole. The selectivity in determination is improved compared with a single quadropole used in the SIR-mode. A triple quadropole can be used in several different modes, for example in daughter scanning mode ions with one m/z are selected by the first quadropole, the ions are collided, and third quadropole is scanned to obtain a spectra of the collision fragments. In parent-scanning mode the first quadropole is scanned and the third is held static. In this way all ions releasing a selected fragment in the collision cell are detected. For detection of ions in quadropole instruments electron multipliers are used.

In paper I a time of flight (TOF) MS was used for identification of isocyanate derivatives. In the TOF-MS all ions entering the mass analyzer are accelerated with an electrical pulse of several kilovolts. The ions are accelerated into a field free drift tube, all with the same kinetic energy. Depending on the mass of the ions they will travel through the tube with different velocity. Light ions pass faster through the tube than heavy ones. At the end of the tube the ions are detected by a multi channel plate (MCP) detector. The MCP detector can register the response from several ions at the same time, in contrast to the electron multiplier, that only can detect one ion at the time. Depending on the time it takes for an ion to travel through the drift tube, the m/z of the ion can be calculated. Exact masses of ions at the ppm level can be obtained with TOF-MS, but this type of instrument needs to be calibrated more often than quadropoles. Because they are less robust than quadropoles, they are usually not suited for routine measurements of several hundred samples.

All the MS-instruments used in paper I-VI were equipped with electrospray (ES) interfaces. In this type of interface the effluent from the column is sprayed through a capillary, by assistance of a coaxial nitrogen gas-flow. Between the capillary tip and the MS inlet a voltage of several kilovolts is applied. The sprayed droplets become charged, and the solvent is evaporated from the droplets by assistance of a heated nitrogen gas flow and the electric field. There are different theories about how the charged ions escape from the droplets. In one theory ions are released from the small droplets when the concentration of charges becomes too high on the droplet surface. In the other theory solvent evaporation continues until all solvent is evaporated and only the ions are left. The ions enter the MS by assistance of electrical fields, and a negative pressure gradient. Electrospray is a “soft” ionization method, and little fragmentation of the ions is obtained. By varying voltage settings in the entrance to the mass analyzer, for example sample cone voltage, the degree of fragmentation can be increased. By using this method it is possible to perform some fragment analysis in TOF and single quadropole instruments as well. The ES interface can operate both in the positive and negative ionization mode.

The fragmentation pattern for isocyanate-DBA derivatives usually contains a few typical ions when positive ionization is used. The molecular ion
[M+H]$^+$ is usually abundant, as well as the adduct ions with sodium [M+Na]$^+$. Sometimes the potassium [M+K]$^+$ adduct is present as well. Ions where one DBA molecule has been lost are present in the spectra as [M+H-129]$^+$. Ions from the reagent are also present as m/z = 130, [DBA+H]$^+$ and m/z = 156, [DBACO]$^+$.

When the single quadropole was used for isocyanate analysis in paper I and V the instrumental detection limits for the monoisocyanates was between 20 and 70 fmol. For the diisocyanates the same values were about 10 times lower, between 3 and 5 fmol. In paper II-IV a triple quadropole instrument was used instead. This lead to improved detection limits, between 0.5-5 fmol for monoisocyanates, and 0.01-0.06 fmol for the diisocyanates. The lowest detection limits were obtained using MRM, with analysis of the reaction [M+H]$^+ \rightarrow [DBA+H]^+$. 

Except for isocyanate-DBA derivatives, LC-MS has also been used for determination of isocyanates derivatised with 2-MP. In the first publication about MS determination of 2-MP derivatives particle beam ionization was used for the MS analysis, but in later methods electrospray ionization has been used. MS/MS analysis using either a triple quadropole or ion trap MS has also been presented. To lower detection limits a method for 2-MP using coordination ion spray (adduct formation with lithium ions) has been presented. In paper II this was tested for DBA-derivatives and lithium acetate was added to the LC mobile phases for adduct formation (figure 8). The molecular ion disappeared from the spectra, and was replaced by [M+Li]$^+$, [M+H-129]$^+$ was replaced by [M+Li-129]$^+$ and [DBA+H]$^+$ was replaced by [DBA+Li]$^+$. When MS/MS analysis was performed, and the [M+Li]$^+$ ion was chosen in the first quadropole for fragmentation, the product spectra was dominated by [M+Li-129]$^+$. If the same procedure is carried out without lithium in the mobile phase, [DBA+H]$^+$ is the dominating ion in the product spectra. The fragmentation pattern was clearly different for the lithium adducts compared with the fragmentation of protonated molecular ions, but when the detection levels were compared, analysis of protonated molecular ions was still superior compared with the analysis of lithium adducts.
Figure 8. Addition of lithium to the mobile phase alters the fragmentation pattern for the isocyanate-DBA derivatives (spectra A), compared to regular reversed phase chromatography (spectra B). All ions formed for 2,4-TDI-DBA were lithium adducts, when lithium acetate was added to the mobile phase.

For DBA-derivatives, GC-MS methods have also been used for determination of monoisocyanates. Mass spectrometric detection has also been used for isocyanates derivatized with the NBDPZ-reagent. Atmospheric pressure chemical ionization in combination with MS/MS analysis works well for methyl isocyanate and different mono- and diisocyanate derivatives. Another recently presented isocyanate reagent, ferrocenoyl piperazide has also been analysed using LC-MS/MS with atmospheric pressure chemical ionization (APCI).

3.5.3.2 Standard preparation
A demand for all analytical methods including mass spectrometry is standards for all compounds that shall be analyzed. Response factors for MS are very different even for molecules with similar structures. For some isocyanate monomers it is possible to obtain pure substances that can be used as standards after derivatization, but for the different compounds present in e.g. polymeric isocyanate mixtures and thermal decomposition products of PUR are not available as purified chemicals.

A method for preparation of reference solutions of different isocyanates derivatives has been used in papers I, II and V. By using characterized reference solutions, the problem with synthesis and isolation of separate compounds present in technical mixtures is circumvented. For preparation of
reference solutions, a nitrogen selective LC-detector, chemiluminescent nitrogen detector (CLND) has been used. The CLND has an equimolar response for nitrogen-containing organic compounds, which makes it possible to use any nitrogen containing compound as external standard. In the detector the effluent from the LC-column is mixed with oxygen and argon and nebulized into a pyrolytic tube placed in an oven heated to 1050°C. Organic compounds are oxidized to carbon dioxide, water and different other oxides. Nitrogen bonded to organic molecules is converted to nitrogen oxide (NO). The pyrolysis products from the oven are splitted and one part is passed to a membrane dryer, where water is removed. The other part goes to the exhaust ventilation. The dried oxides are lead to a chamber where they are reacted with ozone. The NO reacts with ozone to nitrogen dioxide in the excited state (NO$_2^*$). The NO$_2^*$ relaxes to its ground state by emission of a photon.

![Figure 9. LC-CLND chromatogram (A) and total ion current chromatogram (B) for a technical isocyanate mixture, Desmodur N 3300 derivatized with DBA. Chromatograms C-G represents extracted mass chromatograms for HDI-DBA, isocyanurate-DBA, diisocyanurate-DBA, triisocyanurate-DBA and tetraisocyanurate-DBA.](image-url)
The photons emitted by NO\textsubscript{2}* are detected by a photomultiplier tube. Response for an organic compound is thus obtained only if the compound contains nitrogen. Mobile phases used for CLND-analysis must be free from nitrogen, which makes it impossible to use acetonitrile. Instead methanol and water are used for preparation of mobile phases. As a standard for determination of the nitrogen content in the analytical peaks, caffeine dissolved in water was used. Caffeine standards are easily prepared by weighing. The standard solutions are stable for long time, and caffeine is an inexpensive chemical. Caffeine is also rather easy to handle as it has a high melting point and it is not very hygroscopic.

For the preparation of standards from technical mixtures, small amounts of different technical mixtures were dissolved in toluene containing DBA in excess. The solutions were stirred and agitated in an ultrasonic bath. For completion of the reaction between DBA and the isocyanate groups the solutions were left standing for a few hours, before they were evaporated to dryness in a vacuum centrifuge. The residues were dissolved in methanol, and the samples were analyzed by LC-CLND (figure 9).

No structural information is obtained from CLND-measurements. This makes it necessary to use mass spectrometry for identification of the different compounds. To simplify the mass spectrometric analysis and identification, the same chromatographic parameters were used as for the CLND-analysis. For identification a time of flight mass spectrometer was used. Deuterium-labeled DBA was also used for the identification of different isocyanate-DBA derivatives. By comparing spectra from isocyanates derivatized with the deuterium-labeled DBA and derivatives with non-labeled DBA the number of isocyanate groups for different ions can be calculated. These methods have been used in paper I for identification of prepolymeric isocyanates and isocyanate adducts.

When the concentrations of all isocyanate species in a reference solution were determined, the solution was diluted in methanol to a concentration that is easy to use for preparation of standards for the quantitative MS analysis. The standard concentration used for most of the reference solutions was usually set to 1 µg ml\textsuperscript{-1} for the most abundant compound in the solution.

3.5.3.3 Internal standard for MS

For determination of isocyanates with LC-MS it is necessary to use internal standards. An ideal internal standard for mass spectrometry has a similar structure as the analyte and a retention time in the LC separation that is close to the analyte. Isotope-labeled analogues of the analytes are often used as internal standards. In paper V deuterium-labeled isocyanate-DBA derivatives, prepared from deuterated amines were used as internal standards. The internal standards were prepared by the thermal decomposition of deuterium-labeled urethane derivatives of deuterium labeled amines. When these types of derivatives are heated they are thermally degraded to deuterium labeled
isocyanates. The isocyanates were collected in an impinger containing toluene-DBA, and deuterium-labeled isocyanate-DBA derivatives were obtained. The synthesis has been described by Karlsson et al. Internal standards with deuterium were available for MIC, HDI, 2,6- and 2,4-TDI and MDI. These internal standards were also used for quantification of other isocyanate-DBA derivatives, even though they are not ideal internal standards. In papers I and II deuterium-labeled isocyanate-DBA derivatives prepared by reaction of isocyanates with deuterium labeled DBA were used instead. The advantage of using the later internal standards is that internal standards can be easily prepared for all the isocyanates analyzed. These internal standards are prepared in the same way as described for standards obtained from technical isocyanate mixtures by dissolution of the appropriate isocyanate in a solution containing deuterium-labeled DBA. For preparation of the DBA-derivatives of isocyanic acid (ICA) and MIC a somewhat different method was used. Isocyanic acid is not commercially available. It is possible to purchase MIC, but due to its high volatility and its toxic effects it is not desirable to handle this compound in the laboratory. Instead ICA and MIC were generated by thermal degradation of urea for ICA and 1,3-dimethylurea for MIC. The isocyanates are emitted during the thermal degradation, and these are collected in toluene containing deuterium-labeled DBA. This method was also used for synthesis of standards for of ICA and MIC.

Two different types of deuterium-labeled DBA were evaluated for synthesis of internal standards, one containing nine deuterium atoms and one with eighteen deuterium atoms. The more deuterium that is incorporated in the molecule, the more different it becomes compared with the non-labeled derivative. The different types of DBA and internal standards synthesized from amines were compared in paper II. The retention time differences are increased with the number of deuterium in the molecules. A diisocyanate derivative with two DBA containing 18 deuterium atoms each elutes almost 20 seconds earlier than the analyte. This affects the analytical precision and the spread of the residuals are much greater for this calibration than for isocyanate-DBA derivatives containing nine deuterium atoms. The lowest spread of the residuals is obtained for the DBA-derivatives obtained from DBA-derivatives of the deuterium-labeled amines. These derivatives also had retention times almost the same as the non-labeled derivatives. However, regarding the possibility to have internal standards for all analyzed compounds, and the easier way of preparation of internal standard derivatives, the nona-deuterium labeled DBA was chosen for analysis. This type of internal standard was also used for determination of air samples in paper III and IV.
4 Amines and Aminoisocyanates

There are some diamines that are closely related to the diisocyanates discussed in chapter 3, the diamines used for production of diisocyanates, hexamethylenediamine (HDA), toluenediamine (TDA) and methylenediphenyldiamine (MDA). Exposure to diamines and also aminoisocyanates has been demonstrated during thermal degradation of PUR.\textsuperscript{81,150} An aminoisocyanate is a diisocyanate where one of the isocyanate groups has been converted to an amine group. Aminoisocyanates can not be synthesized as pure substances because they would polymerize with themselves, but they exist as airborne intermediates in the smoke during thermal degradation of polyurethane plastics.

4.1 Diamines - properties and uses

Except for being used in the production of isocyanates, where diamines are phosgenated in the last reaction step, diamines are used in the production of other type of polymers.

Table 7. Diamines

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Vapor pressure (Pa)</th>
<th>CAS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,6-HDA</td>
<td><img src="image1" alt="Structure1" /></td>
<td>25 (20°C)</td>
<td>124-09-4</td>
</tr>
<tr>
<td>2,6-TDA</td>
<td><img src="image2" alt="Structure2" /></td>
<td>2130 (150°C)</td>
<td>823-40-5</td>
</tr>
<tr>
<td>2,4-TDA</td>
<td><img src="image3" alt="Structure3" /></td>
<td>100 (106°C)</td>
<td>95-80-7</td>
</tr>
<tr>
<td>4,4'-MDA</td>
<td><img src="image4" alt="Structure4" /></td>
<td>$2.7 \times 10^{-5}$ (25°C)</td>
<td>101-77-9</td>
</tr>
</tbody>
</table>

Hexamethylenediamine (HDA) is used in the production of nylon, used for production of textile fibers. Nylon polymers are polyamides, formed by reac-
tion of diamines with diacids. For production of Nylon 6/6, HDA is reacted with adipic acid.\textsuperscript{151}

Methylenediphenyl diamine (MDA) is used as a curing agent in some types of epoxy plastics. In contrast to anhydride cured epoxy, it is not necessary to heat diamine-cured epoxy for the curing reaction to take place.\textsuperscript{151,152}

No examples for the use of toluenediamines (2,4- and 2,6-TDA) outside the isocyanate and polyurethanes industry have been found for this review. However, in the production of soft foam from TDI, the reaction of TDI with water, where TDI is converted to TDA, is often used. TDI and TDA react and form polyurea. Carbon dioxide is released during the conversion of isocyanates, and used as a blowing agent.\textsuperscript{153} Another isomer, 2,5-TDA, is used in some types of hair-dye.\textsuperscript{154}

Aminoisocyanates only exists as reactive intermediates during thermal degradation of PUR. The structures for the aminoisocyanates studied are presented in table 6.

\textbf{Table 8. \textit{Aminoisocyanates}}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Vapor pressure (Pa)</th>
<th>CAS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAI</td>
<td></td>
<td>-</td>
<td>95566-41-9</td>
</tr>
<tr>
<td>2,6-TAI</td>
<td></td>
<td>-</td>
<td>22683-71-2</td>
</tr>
<tr>
<td>2,4-TAI (1)</td>
<td></td>
<td>-</td>
<td>99626-87-6</td>
</tr>
<tr>
<td>2,4-TAI (2)</td>
<td></td>
<td>-</td>
<td>99626-88-7</td>
</tr>
<tr>
<td>MAI</td>
<td></td>
<td>-</td>
<td>148913-78-4</td>
</tr>
</tbody>
</table>

\textbf{4.2 Exposure and Health Effects}

Except for MDA, there are rather few reports on occupational exposure to diamines used as pure chemicals. MDA is used for epoxy curing, and both long term exposure\textsuperscript{155} and acute toxic effects\textsuperscript{156} have been studied.

Exposure to diamines also occurs in the PUR-industry. During thermal degradation of PUR, diamines are formed, as well as aminoisocyanates. For example, flame lamination of soft foam with textile generates 2,4-, and 2,6-

45
TDA as well as the corresponding tolueneaminoisocyanates (TAI). During welding in PUR-coated metal HDA can be detected, as well as hexamethylene aminoisocyanate (HAI). In paper V it was shown that MDA and methylenediphenyl aminoisocyanate (MAI) was formed during welding in PUR-insulated district heating pipes. The pipes were insulated with rigid PUR foam based on MDI. Exposure to amines and aminoisocyanates was also demonstrated during welding in coated metal.

Human exposure not related to industrial activity has also been considered for MDA and TDA. MDA has been shown to be released from medical products made from MDI-based polyurethane. Several studies have investigated exposure to TDA from PUR-covered breast implants. The PUR material surrounding the implants has been shown to be non stable and easily degraded, with the formation of TDA.

Two of the diamines studied, 2,4-TDA and MDA, have been classified by IARC as group 2B agents (possibly carcinogenic to humans). Reports about acute toxicity for MDA have described liver injuries and jaundice for the exposed individuals. The reports concerns both suspected dermal exposure as well as accidental ingestion causing these symptoms. For HDA, the knowledge about effect on humans is limited, but several studies on animals have shown that HDA is an irritant to the upper respiratory tract as well as the eyes and the skin. Similar effects have been found on exposed humans due to airway exposure. The situation about known effects on humans for TDA is somewhat similar as for HDA, in that most of the information comes from animal studies. In a criteria document, the toluenediamines are described as irritants to eyes, skin and the upper respiratory tract. The 2,4-, 2,5- 2,6- isomers are all mutagenic, but only 2,4-TDA is carcinogenic in animals. Some investigations have found TDA as a reproductive toxin affecting spermatogenesis, but the available data was limited.

In Sweden no OEL values are given for the diamines discussed here. However, 2,4-TDA and MDA may only be handled by authorized laboratories, due to the fact that these diamines are suspected carcinogens.

### 4.3 Air Sampling

Air sampling methods devoted only for sampling of aromatic amines have been developed, but there are also methods that are developed for simultaneous sampling of amines and isocyanates, as they often occur together in the industry.

For collection of airborne amines several methods using acid-impregnated glass fiber filters have been presented. Usually the filters are treated with sulfuric acid, and during sampling the amines are stabilized as sulfates. For desorption from the filters after sampling, alkaline solutions are used. It is possible to analyze free amines by chromatography, but derivatization before
the analysis is common. Amine derivatization will be described in more de-
tail later in the text.

The American organizations National Institute of Occupational Safety and
Health (NIOSH) and Occupational Safety and Health Administration
(OSHA) have presented filter methods for MDA. In the OSHA method the
amines are derivatized before analysis, while the NIOSH method analyses
free amines. The OSHA method have been developed and adapted for analy-
sis of toluenediamines as well. Another MDA method using acidic glass
fiber filters, where the amines were derivatized before analysis has also been
published.

Methods using acidic filters are not suitable for determination of amines
in the presence of isocyanates, since isocyanates will be converted to amines
in contact with acids. In methods that can measure amines and isocyanates in
the same sample the isocyanates are derivatized upon sampling, and the
amines are either derivatized after sampling or analysed as such. In the early
methods where both types of compounds can be determined, alkaline ethanol
in impinger bottles was often used for sampling. Isocyanates react with the
ethanol forming urethanes, and are in this way protected from hydrolysis to
corresponding amines or further reaction with the sampled amines. This
technique has been used for sampling of PUR degradation products from
coatings and soft foam during flame lamination. Derivatisation was per-
formed before the analysis. A method where the amines are left underivat-
ized has also been used for sampling during flame lamination, and for
sampling of MDA in the presence of MDI.

Isocyanates react faster with amines than with ethanol and most isocy-
anate derivatization reagents used today contain an amine group for protec-
tion of the isocyanates. In wet sampling methods the amine reagent is dis-
solved in some organic solvent, for example toluene. Amines and aminoiso-
cyanates present in the sampled air are also collected in the reagent solution.
This collection principle has been used in the NIOSH 2-MP method. After
sampling the impinger solution is analyzed for the presence of both iso-
cyanates and amines.

Collection of amines and aminoisocyanates in the isocyanate sampling so-
lution has also been used in paper V. DBA dissolved in toluene was used for
protection of the isocyanate groups and by derivatisation after sampling it
was possible to analyze airborne amines and aminoisocyanates as well. Air
sampling was performed using an impinger connected in series with a 13
mm glass fiber filter, with a pore-size of 0.3 µm.

4.4 Amine derivatization

For analysis of amines with chromatographic methods it is common to deri-
vatize the amines before the analysis. Amine groups are active, and amines
may if they are not derivatized become adsorbed by hydrogen bonding to different surfaces in the analytical instrument. Derivatization protects the amine group from oxidation. Derivatization has also often been used to obtain lower detection levels and better selectivity in the analytical procedure.

4.4.1 Derivatization with anhydrides

Acid anhydrides react with amines, and different anhydrides have been used for derivatization of amines. In the NIOSH air sampling method for sampling of amines in the presence of isocyanates, acetic anhydride is added for derivatization of amines. The acetic anhydride also consumes the excess isocyanate reagent, 2-MP, which facilitates the chromatographic analysis. The acetic anhydride reacts slowly with toluene diamines present in the sample, and heating is necessary for completion of the reaction. Acetic anhydride derivatization of MDA (and m-phenylenediamine) has also been used in a filter method. Filters were extracted with an aqueous base (phosphate buffer) mixed with acetonitrile, and acetic anhydride was then added to this solution.\(^{169}\)

Fluorinated acid anhydrides are very common for derivatization of amines, not just for air sampling, but also for analysis of amines in biological samples\(^{171}\) and in PUR-foam.\(^{172}\) The fluorinated acid anhydrides react fast with amines in organic solvent, and high recoveries are obtained for aromatic amines.\(^{173}\) The acid formed by the reagent is extracted from the organic phase with a buffer prior to analysis.

Heptafluorobutyric anhydride (HFBA) is used in two NIOSH methods for filter sampling of toluenediamines\(^{168}\) and MDA.\(^{166}\) The filters are shaken with alkaline solution and toluene. The amines are extracted to the toluene. The toluene is separated and the HFBA reagent is added to for derivatization of the amines.

HFBA have also been used for amine-derivatization when amines and isocyanates have been collected in alkaline ethanol. After sampling the sampling solution was evaporated to dryness. Phosphate buffer and toluene was added, together with HFBA.\(^{81}\) HDA and HAI were determined in the toluene phase. A similar derivatization method has also been used for derivatization of toluenediamines, but instead of HFBA pentafluoropropionic anhydride (PFPA) was used.\(^{150}\)

4.4.2 Derivatization with chloroformates

Another reagent, ethyl chloroformate (ET), have been used for amine derivatization in the DBA method for isocyanates.\(^{143}\) The amines are reacted to urethanes in this type of derivatization. The reaction of amines with ET takes place in a two-phase reaction, with an aqueous and an organic phase. The water solution should be alkaline to force the amines into the organic solvent
where they react with the chloroformate. After the reaction the organic layer containing amine-ET and isocyanate-DBA derivatives is separated and evaporated. The residue is dissolved in a solvent appropriate for the following analysis. Chloroformate reagents have been widely used for derivatization of amines preceding GC-analysis of amines. The reaction is quickly completed without heating, and by selecting an appropriate buffer the selectivity of the reaction can be very high. In paper V ET was used for derivatization of amines and aminoisocyanates before analysis.

4.5 Determination of amines and aminoisocyanates

4.5.1 Preparation of standards

Standards for diamine analysis are easily prepared as the diamines are commercially available. Aminoisocyanate standards for analysis are not commercially available, but different methods have been used for synthesis of derivatives. In two previous methods where aminoisocyanates were analyzed, diamines were used as starting material for synthesis of derivatives. In the first method, where aminoisocyanates were analyzed with underivatized amine groups, a deficit of ethyl chloroformate was used for derivatization of diamines. The second method also included the reaction with ethyl chloroformate in deficit, and the unreacted amine-groups were then derivatized with HFBA, to obtain the aminoisocyanate derivatives. In previous methods where TAI was derivatized with DBA and ET, standards for determination of aminoisocyanates were obtained by collecting thermal degradation of TDI-foam in toluene-DBA, and derivatize the amine-groups with ET after sampling. Aminoisocyanate derivatives can also be prepared from the corresponding diisocyanate in a two-step reaction. In paper V a method for the synthesis of aminoisocyanates derivatized with DBA and ET in reference solutions is presented. Isocyanates were dissolved in an organic solvent, usually isoctane, and reacted in two steps, with ethanol and DBA. The reaction of an isocyanate group with ethanol results in the same urethane derivative as is obtained when an amine group is reacted with ET. The amount of reagent added in the first step was just sufficient to derivatize one isocyanate group on each diisocyanate. After completion of the first reaction the second reagent was added in excess, to derivatize the remaining isocyanate groups. For synthesis of HAI, MAI and 2,6-TAI, DBA was added in the first step, then ethanol was added. The amount of derivatives in the reference solution was determined using LC-CLND as described in chapter 3 for preparation of isocyanate-DBA reference solutions. Identification of the derivatives was
performed using TOF-MS. From 2,4-TDI it is possible to obtain two types of aminoisocyanate derivatives, with the amine group either on position 2 or position four. One of the isocyanate groups on 2,4-TDI, probably the group in position four, reacts faster with the added reagent than the other group. By varying which reagent is added first, both types of derivatives can be obtained. Small amounts of the diamine-ET and diisocyanate-DBA derivatives were also found in the prepared reference solutions (figure 10).

Figure 10. LC-CLND chromatograms from the synthesis of 2,4-TAI-ET-DBA. Peak A is the chromatographic front, B 2,4-TDA-ET, C 2,4-TAI-ET-DBA isomer 1, D 2,4-TAI-ET-DBA isomer 2, E 2,4-TDI-DBA. To the mixture in the lower chromatogram ethanol was added before DBA, while DBA was added first in the top chromatogram.

When solutions where the aminoisocyanate derivatives were the dominating compounds were obtained, diamine-ET and diisocyanate-DBA derivatives were added, so all of the compounds in the reference solutions had the same concentration, to facilitate when the solutions were used as analytical standards.

4.5.2 Analysis

Amines derivatized with different fluorinated anhydrides have often been analyzed using gas chromatography. The derivatives formed are suitable for analysis with for example electron capture detector\textsuperscript{166,168} (ECD) which is very sensitive for compounds containing halogens, or thermionic specific detector\textsuperscript{81,150} (TSD), which is sensitive towards nitrogen containing compounds. Anhydride-amine derivatives of this type has often been analyzed using GC, but it is also possible to use LC-methods for separation of the derivatives.\textsuperscript{172}

LC methods for underivatized amines use buffered mobile phases for the separation. To obtain retention on a reversed-phase column it is necessary to
keep the amines deprotonized. Methods where free amines are analyzed by LC are all developed for aromatic amines, so UV-detection have been chosen for detection.\textsuperscript{80,81,167} Electrochemical detection was also used in two of the methods.\textsuperscript{81,167}

One advantage with derivatization of amines before LC analysis is that active groups are made less active. This minimizes the possibilities for adsorption in the analytical system. Alternatively, that retention is lost due to charging of the amine group. The use of buffered mobile phases can also be avoided and this is an advantage for some types of detectors, for example mass spectrometers. \textsuperscript{169,170} LC-UV analysis have been used for amines derivatized with acetic anhydride.\textsuperscript{169,170} For analysis of amines and aminoisocyanates derivatized with ET LC-MS has been used for analysis.\textsuperscript{143} The urea bond present in the aminoisocyanate derivatives is prone to degradation during GC-analysis, so this type of derivatives is better suited for LC analysis at ambient temperature.

In paper V LC-MS was used for analysis of amines and aminoisocyanates in air samples. For amines and aminoisocyanates deuterated amine-ET derivatives were used as internal standards. Air samples were also simultaneously analyzed for isocyanates. The fragmentation patterns of amine derivatives contained molecular ions, [M+H]\textsuperscript{+}, sodium adducts, [M+Na]\textsuperscript{+}, and the fragments [M-46]\textsuperscript{+} and [M-92]\textsuperscript{+}, that represents neutral loss of one or two ethanol. The fragmentation pattern for aminoisocyanate derivatives was recognized from both amine derivatives and isocyanate derivatives, that is except for molecular ions [M+H]\textsuperscript{+} and sodium adducts, [M+Na]\textsuperscript{+}, [M-46]\textsuperscript{+} [M-129]\textsuperscript{+}, [DBA+H]\textsuperscript{+} and [DBACO]\textsuperscript{+} can be found in the spectra. The instrumental detection limits for the aminoisocyanates was as good as for isocyanate-DBA-derivatives analyzed by the single quadropole, about 2-4 fmol. The detection limits for amine derivatives was about ten times higher.

The amine-ET derivatives have retention times close to the monoisocyanates, and aminoisocyanates derivatized with ET and DBA elutes in the time window between their corresponding diamines and diisocyanates on reversed-phase LC. Thus air samples could be analyzed for the presence of amines and aminoisocyanates with the same analysis time as for isocyanates only. However, to achieve separation between the two different isomers of 2,4-TAI, some modifications of the mobile phase were made. Instead of using 95/5/0.05 (% v/v/v) acetonitrile/H\textsubscript{2}O/formic acid as the strong eluent, the acetonitrile was replaced by a mixture of 75/25 acetonitrile/methanol (% v/v).

The excess DBA, present in the air samples also reacts with ET in the work-up procedure. The DBA-ET derivative is also eluted during the chromatographic analysis as a broad overloaded peak, but fortunately this derivative elutes between the last 2,4-TAI-ET-DBA and MAI-ET-DBA, and the determination of these peaks is not disturbed.
5 Organic Acid Anhydrides

5.1 Chemical properties

Acid anhydrides are formed by reaction of two carboxylic groups, with elimination of water. Anhydrides can be formed by reaction between two acid molecules, for example acetic anhydride formed by reaction of two molecules acetic acid, or when two carboxylic groups in a diacid react with each other, for example maleic acid forms maleic anhydride. In the production of polymers only anhydrides from diacids are used, because they are difunctional and can be used for crosslinking. Anhydrides are reactive towards water, amines and alcohols. They are also strong oxidizers. The anhydrides studied in paper VI, maleic anhydride (MA), phthalic anhydride (PA), tetrahydrophthalic anhydride (TA) and cis-hexahydrophthalic anhydride (HA) are all solids at room temperature. Structures are given in table 9.

Table 9. Organic acid anhydrides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Vapor pressure (Pa)</th>
<th>CAS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td><img src="image" alt="MA structure" /></td>
<td>21.3 (20°C)</td>
<td>108-31-6</td>
</tr>
<tr>
<td>PA</td>
<td><img src="image" alt="PA structure" /></td>
<td>1.3 (20°C)</td>
<td>85-44-9</td>
</tr>
<tr>
<td>TA</td>
<td><img src="image" alt="TA structure" /></td>
<td>1.3 (20°C)</td>
<td>85-43-8</td>
</tr>
<tr>
<td>HA</td>
<td><img src="image" alt="HA structure" /></td>
<td>1 (20°C)</td>
<td>13149-00-3</td>
</tr>
</tbody>
</table>
5.2 Industrial applications

Organic acid anhydrides are important industrial chemicals, and used in many chemical processes. In production of polymers, anhydrides are used as hardeners and curing agents for epoxy, alkyd or polyester resins. Compared to amines, which also can be used as curing agents for epoxy resins, anhydrides need heat for the curing reaction.\textsuperscript{152} This makes them more expensive to use in production, but it is easier to control the properties of the formed resin.\textsuperscript{177} Besides from production of resins anhydrides are found in several different applications. Phthalic anhydride is also used for production of plasticizers, dyes, pharmaceuticals and pesticides.\textsuperscript{178,179} Maleic anhydride is predominantly used for production of polyester resins. It is also used in the synthesis of other chemicals as fumaric acid and hexahydrophthalic anhydride. In the production of the sweetener aspartame maleic anhydride serves as a raw material.\textsuperscript{180} Tetrahydrophthalic anhydride and hexahydrophthalic anhydride are used in different anhydride-epoxy resins.\textsuperscript{181} Hexahydrophthalic anhydride is also used in the production of insecticides and rust preservatives.\textsuperscript{179} Other anhydrides used in the industry are for example trimellitic anhydride, tetrachlorophthalic anhydride and methyltetrahydrophthalic anhydride.\textsuperscript{177,179}

5.3 Exposure and health effects

Several reports about exposure to acid anhydrides have been published. The effects of anhydrides on workers has often been studied during production of different resins, where for example PA\textsuperscript{182,183}, MA\textsuperscript{182,184}, HA\textsuperscript{185} or other anhydrides are used in the production. Anhydrides are often used as solids in the production of resins. Dust containing anhydrides lead to exposure via the airways or skin.

Besides from production exposure to anhydrides can occur during application of powder paints\textsuperscript{186}, or during thermal degradation of paints for example during welding or flame cutting of painted steel.\textsuperscript{187,188,189} In \textit{paper VI} generation of different anhydrides during heating of different coatings was demonstrated.

Because of their reactivity and oxidizing properties organic acid anhydrides are irritants to human airways, eyes, skin and mucous membranes. Besides from being direct toxins, exposure to acid anhydrides may also cause hypersensitivity that for example leads to the development of occupational asthma.\textsuperscript{177} This has been shown for example for exposure to PA\textsuperscript{183} and HA.\textsuperscript{185} Rhinitis and chronic bronchitis are other symptoms associated with workplace exposure. Trimellitic anhydride causes influenza-like symptoms, or may lead to pulmonary hemorrhage.\textsuperscript{177} Trimellitic or phthalic anhydride
may also cause allergic alveolitis. Anhydrides are also suspected to cause contact dermatitis and urticaria.

Methods have been developed for biological monitoring of organic acid anhydrides. In the human body the anhydrides are hydrolysed to their corresponding diacids, and these can be found in urine or blood as biomarkers of anhydride exposure. Methods for determination of phthalic acid and hexahydrophthalic acid in urine and hexahydrophthalic acid and methylhexahydrophthalic acid in plasma have been presented. Antibodies (IgE and IgG) are also sometimes found in workers exposed to anhydrides. Presence of antibodies shall only be regarded as biomarkers of exposure, since the connection between antibodies and disease is weak.

In Sweden the TWA values for PA is 2 mg m\(^{-3}\). For MA it has been set to 1.2 mg m\(^{-3}\). No limit values are given for TA and HA, but because they are known to cause hypersensitivity, they may only be handled by authorized laboratories.

### 5.4 Air sampling

A lot of different air sampling methods for determination of airborne anhydrides have been developed since the early eighties. If the development of sampling devices and collection methods is compared with the development of isocyanates sampling it can be noted that there are few similarities between the anhydride methods. For isocyanate methods developed at the same time almost all methods contain an amine reagent for the isocyanate derivatization. The anhydride methods presented and reviewed here uses derivatization during sampling, collection of anhydrides as such and hydrolysis of the anhydrides during sampling. Anhydrides are reactive compounds, but they are not as reactive as isocyanates, and methods where derivatization occurs during the collection are less common. Due to higher exposure limits the development of new air sampling methods for anhydrides has not been as extensive as for isocyanates.

#### 5.4.1 Filters and solid sorbent sampling

Glass fiber filters have been a common choice for collection of airborne anhydrides. If the filters used are unimpregnated, the extraction usually takes place in aqueous solutions to hydrolyze the anhydrides to corresponding acids. Glass fiber filter methods that involves hydrolysis before analysis have been presented for MA, PA, HA and TMA. In the method for HA, the acid formed during hydrolysis was derivatized with pentafluorobenzylbromide (PFBBBr) before analysis.

OSHA has published several methods for different airborne anhydrides using impregnated glass fiber filters for collection and derivatization of the
anhydrides upon collection. One method uses filters impregnated with 1-2PP for collection of acetic anhydride (AA). 1-2PP is a reagent that has also been used for sampling and derivatization of isocyanates. The other reagent used for filter sampling with impregnated filters is 3,4-dimethoxybenzylamine, also known as veratrylamine. Four almost identical methods have been presented for sampling of MA, PA, TMA and AA. When the methods for TMA and AA was published it had been found that addition of di-n-octylphthalate to the filters improved the contact between the reactants.

In an earlier method published by OSHA, MA was collected using a solid sorbent, tenax, coated with p-anisidine for derivatization of the anhydride. Tenax as an adsorbent for anhydrides has also been used in a method developed by Health and Safety Executive (HSE) in Great Britain. The method has been evaluated for sampling of PA, TMA and tetrachlorophthalic anhydride. After sampling the anhydrides are extracted and hydrolyzed in aqueous solutions. Sampling on tenax has also been performed for MA, in a method where the anhydride is determined as such, i.e. without prior derivatization or hydrolysis. In some methods a glass fiber filter has been placed in front of the tenax-tube to prevent large particles from entering. In the PA-method the anhydrides are analyzed as such, while the corresponding acid is esterfied with methanol in the presence of boron trifluoride (BF\textsubscript{3}) in the TMA method.

XAD-2 is another type of sorbent that has been used for anhydride sampling. Methods has been developed for HA, TA and methyltetrahydrophthalic anhydride. In all the methods the anhydrides are analyzed without modification.

Sampling of TMA on PVC-copolymer filters has been published by Plassis and co-workers and the National Institute for Occupational Safety and Health (NIOSH). Both the methods use esterfication of the acid of TMA, with methanol/BF\textsubscript{3}.

A somewhat unusual sampling device, a spiral glass trap has been developed by Pfäffli. The spiral glass trap has been used for determination of PA and chlorendic anhydride. The sampler has been built for fume sampling, and to be able to separate gases and particles. A filter in front of the spiral glass trap collects particles, gaseous anhydrides are collected on the walls of the spiral glass trap and anhydrides not collected in the two first stages are collected on a tenax-tube that is used as a back up. After sampling the different parts of the sampler are extracted, and anhydrides are determined as such.

5.4.2 Impinger or bubbler sampling

In all the impinger and bubbler methods found in the literature, some aqueous solution is used in the impinger, and the anhydrides collected are imme-
diately hydrolyzed. NIOSH has a method for MA, where anhydrides are collected in distilled water\textsuperscript{214}, A similar method for MA was published a few years earlier by Geyer and Saunders\textsuperscript{194}, where the anhydrides were collected in dilute phosphoric acid. For collection of HA\textsuperscript{208} and TA\textsuperscript{196} two methods using 0.1 M NaOH in bubblers has been presented by Jönsson and coworkers.

In \textit{paper VI} it was investigated if it was possible to use an isocyanate method\textsuperscript{53,73} using impinger sampling for screening of organic acid anhydrides as well. Anhydrides are reactive towards amine reagents, and there are possibilities for isocyanates and anhydrides to occur in the same exposure situations for example during thermal degradation of polymeric coatings, that often consists of several different layers. It was found that with some modifications of the isocyanate method anhydrides and isocyanates could be collected simultaneously. One modification of the existing method was that instead of pure toluene as a solvent for the DBA reagent a mixture of toluene and acetonitrile was used instead. This increased the recovery of TA and HA. In pure toluene the recovery of these anhydrides was just about 20 \%.

\section*{5.5 Sample Determination}

For the determination of anhydrides several different methods have been used. The for anhydrides corresponding acids that have UV response, LC with UV detection has been performed\textsuperscript{194,195,197,204,214,215}. Also the methods where derivatizing reagents has been used for anhydride sampling, LC-UV has been the method of choice\textsuperscript{199,200,201,202,203}.

GC has also been used for sample analysis. Various detection principles have been used, such as flame ionization detection (FID)\textsuperscript{196,208,209,210,211}, electron capture detector (ECD)\textsuperscript{205,206,207,212,213}, MS\textsuperscript{196,213} or nitrogen phosphorous detector (NPD)\textsuperscript{198}.

In \textit{paper VI} organic acid anhydrides derivatised with DBA were determined by LC-MS. A quadropole MS was used, with electrospray ionization. The ability of anhydrides to react with DBA was discovered when scanning of isocyanate air-samples was performed. Early eluting peaks were suspected as anhydrides, and it was confirmed by reacting pure anhydrides with DBA in solution and comparing the mass spectra. Anhydrides were also derivatized with di-n-propylamine (DPA) and di-n-ethylamine (DEA). The same fragmentation pattern as for the DBA derivatives were obtained. Initially positive electrospray ionization was used. However, when quantification of anhydrides should be performed, it was found that calibration graphs were non-linear. When the ionization was switched to the negative mode, linear calibration graphs and a gain in selectivity were obtained. In the positive mode protonated molecular ions and sodium and potassium adducts were seen. Some of the anhydride derivatives also showed more complex
adducts, and an ion at m/z = M+64 is probably [M+Na+CH$_3$CN]$^+$, that is an adduct with sodium and acetonitrile. Increasing the cone voltage lead to more fragmentation, and [M+H-18]$^+$ (loss of water) and [M+H-129]$^+$ was seen, as well as the [DBA+H]$^+$ ion. The two last ions are also very common in the fragmentation patterns of isocyanate-DBA derivatives. Switching to negative ionization, the spectra was dominated by [M-H]$^-$, the molecular ion without one proton at low cone voltages. At higher cone voltages fragmentation was increased and ions like [M-H-44]$^-$ (loss of carbon dioxide) and [M-H-129]$^-$ (loss of DBA) were seen in the spectra.

Figure 11. Chromatograms and negative ion spectra for the studied anhydride-DBA derivatives. A: Maleic anhydride-DBA, B: Phthalic anhydride-DBA, C: Tetrahydrophthalic anhydride-DBA, D: cis-hexahydrophthalic anhydride-DBA.
The instrumental detection limits for the anhydride-DBA derivatives in the negative ionization mode ranged from 10-30 fmol. For SIR determination acquisition using two functions simultaneously was performed. One function was set for acquisition of the negative anhydride-DBA ions, while the other function was used for acquisition of positive ions of monoisocyanate-DBA derivatives. As internal standard during the MS analysis amines derivatized with trifluoro acetic anhydride and PFPA were used. These internal standards are not ideal, in that the resemblance with the analyzed derivatives are small, but the derivatives used had similar retention times and a good response in the negative ionization mode.

For the chromatography of the anhydride-DBA derivatives it was found that it was necessary to use formic acid in the mobile phase, to keep the derivatives uncharged. Exclusion of formic acid removes the proton on the carboxyl group, resulting in decreased retention times, and the anhydride derivatives were eluted with the chromatographic front.

Standards of anhydrides-DBA derivatives were obtained by derivatization of anhydrides with DBA in solutions. The concentration of the derivatives in solution was determined using LC-CLND, as previously described for the synthesis of isocyanate standard solutions. In paper VI a number of different paint samples were heated, and the thermal degradation products were analyzed for anhydrides. Phthalic anhydride was the dominating anhydride, present in most of the paint samples, but in some paint samples it was also possible to detect maleic and hexahydrophthalic anhydride.
The sampling of airborne chemicals is justified for many reasons and several interest groups are involved. Workers have a justified interest to be informed about possible health risk at workplaces. Workers want to be ensured that the work is safe. They very often demand independent measurements and opinions. Sometimes they want data and information regarding different kinds of legal actions when e.g. workers are believed to have been sick due to the work. The companies (employers) have a great interest to make sure that workers do not become ill due to the work, in addition to the concern of workers health. It is expensive to replace sick workers and the production will be affected. It is also of importance for the employer to demonstrate that all is done to make the workplace safe. Many things in working life are regulated by different kinds of laws. The authorities have a direct responsibility to enforce current legislation. This results in practice in inspections of workplaces and e.g. demands of measurements of airborne chemicals.

However, exposure assessment by air sampling is a very demanding procedure. One random sample does not mean much. Several samples are necessary to obtain a representative overview. A complete study involves many samples, takes long time and is very costly. If the exposure is complex, a lot of efforts need to be done and a battery of different methods must be applied.

Nowadays, most professional work in the industry is performed in a way that meets demands from ISO standards. Subcontractors also have a demand to fulfill the same requirements. Contract laboratories and industrial hygiene consultants are such subcontractors. This results in standardized protocols and procedures. It has many advantages but there is a conflict with the scientific community. Scientists are dedicated to find out new things and to develop new methods and procedures. Results are in most cases presented in peer reviewed papers, but this is not always sufficient as scientific papers are read only by a limited group of people. The time to implement new scientific achievements into national or international standards is very long. In practice, many present standards are obsolete.

Today, there are many powerful analytical instruments commercially available. The modern instruments are much better than the ones that were available a few years ago. In the pharmaceutical industry such instrumentation are routinely used. In the field of work environment chemistry there are many examples of laboratories with obsolete equipment. The equipment
used in this thesis is up to date and the author has experienced the necessity to have these kinds of equipment available to solve the aims of this thesis.

The aims of this thesis have essentially been fulfilled. Many new scientific questions were found during the work. The field of thermal decomposition of plastics is complex. The focus has been on isocyanates, amines and anhydrides, but there are many more compounds to take into account regarding health risks. There is an immediate need for future studies as many workers are exposed and may be of great risk.

There are unsolved problems regarding air sampling of reactive compounds. The denuder-impactor sampler has so far mostly been applied to sampling of monomeric isocyanates. But isocyanate aerosols also consist of more compounds such as reaction products between isocyanates and polyols. Is it possible to also sample isocyanates that have partly reacted with polyols?

Reversed-phase chromatography has been shown to have limitations regarding larger isocyanate compounds. Should other chromatographic methods as gel permeation chromatography, where other solvents can be used, be considered as an alternative?

Further, sampling of amines and aminoisocyanates using dry sampling is still an unsolved problem. There is almost no knowledge on how amines and aminoisocyanates are distributed in reactive aerosols. In addition, sampling of anhydrides has not yet been studied with the new sampler.

Even though “wet” sampling methods as the impinger-filter may be cumbersome to handle, they should still be regarded as very important for the development of new methods and reagents. It is the simplest and most effective way to bring reactants and reagents together.
7 Conclusions

In this thesis methods for air sampling and determination of isocyanates, amines, aminoisocyanates and anhydrides generated during production or thermal degradation of polymers such as polyurethane (PUR) or epoxy have been developed. The methods are based on derivatization of reactive groups with di-n-butylamine (DBA). Isocyanates and aminoisocyanates form stable urea derivatives with DBA. Anhydrides form amide derivatives with DBA. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) enabled a reduction of instrumental detection limits 100 times, to as low as 10 attomoles for isocyanate-DBA derivatives. Methods for preparation and characterization of reference solutions of technical grade isocyanates, aminoisocyanates and anhydrides have been developed. A nitrogen-selective LC-detector enabled the quantification of the DBA-derivatives. By using deuterium labeled DBA it was possible to synthesize internal standards for all studied isocyanates.

A novel sampler is developed. The sampler consists of a denuder in series with a three-stage cascade impactor and an end filter. The sampler made it possible to reveal the distribution of isocyanates between gas and different particle size fractions. As a reference method, simultaneous sampling was performed using an impinger filled with DBA in toluene, connected in series with a glass fiber filter. There was a good agreement between the denuder impactor sampler and the reference method. It was found that during thermal degradation of PUR, isocyanates were associated to particle size fractions (<1 µm) that may penetrate to the lower airways. The distribution of isocyanates in aerosols during 8 minutes changes noticeably. Gas phase TDI and MDI became associated to small particles (<1 µm). This type of sampler can be an effective tool to study how different isocyanates can reach different parts of the human airways.

Isocyanater är reaktiva ämnen som kan irriterar luftvägarna och orsaka astma. Flera aromatiska aminer och isocyanater är klassade som möjligt cancerframkallande. Anhydridrider är irriterande och kända allergener. Särskilda tillstånd krävs för hantering av flera typer av isocyanater, aminer och anhydridrider. Små partiklar (0.1-5 µm) har egenskapen att kunna nå långt ner i andningsvägarna och orsaka ohälsa. Det är därför viktigt att kemiskt kunna studera dessa små partiklars sammansättning, men metodik har saknats.

I arbetslivet exponeras många för termiska nedbrytningsprodukter av polymorer. Exponering förekommer inte bara i plastindustrin, utan även inom många andra områden. Heta arbeten där termiska nedbrytningsprodukter bildas förekommer i bilverkstäder, byggarbetsplatser och vid borttagning av färg. Även andra plastmaterial kan avge isocyanater vid uppvärmning såsom bakelit och fenol/formaldehyd/urea-harts som finns i mineralull. Även i hemmiljö kan man bli exponerad om man upphettar plast eller färg. Isocyanaterna förekommer både i gas- och partikelform.

Isocyanater är reaktiva ämnen. Vid luftprovtagning måste därför isocyanatgrupperna skyddas, för att de inte skall reagera med andra ämnen annars underskattas halten. I avhandlingen görs detta genom att isocyanaterna reagerar med en amin, di-n-butylamin (DBA) redan vid insamlingen. DBA reagerar också med organiska syraanhydridrider, och denna princip har utnyttjats för luftprovtagning av dessa ämnen.

Eftersom isocyanater är mycket giftiga har de mycket låga gränsvärden. Därför har analysmetoder med extremt låga detektionsgränser utvecklats.
Vätskekromatografi (LC) med masspektrometrisk (MS) detektering kan nu bestämma isocyanater i attomolmängder med en hög selektivitet. Detta är nödvändigt när man ska analysera flera ämnen samtidigt i ett och samma luftprov. Den utvecklade analysetekniken har använts för analys av flera undersökta plasttyper. För en säker bestämning har stor vikt lagts på att ta fram nödvändiga standardlösningar. LC-MS-analys kräver att standarder finns för samtliga bestämda ämnen. För detta ändamål har en LC kvävselektiv detektor, chemiluminescent nitrogen detector (CLND) använts.


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