Overview of Biological Monitoring for Isocyanates

Summary

Isocyanates are respiratory sensitisers, the major cause of occupational asthma in Great Britain. Occupational exposure to isocyanates must be well controlled. In the case of spray painting, the controls normally needed are a spray booth and respiratory protective equipment (RPE). COSHH requires the assessment of exposure control efficacy. This paper proposes the use of biological monitoring as an essential element of assessing the control of exposure. As biological monitoring is the most practical method for measuring exposure when RPE is part of the control measures, it also suggests a biological monitoring guidance value.

Isocyanates are absorbed by inhalation, and converted to amine-protein or amine-haemoglobin adducts. Urinary elimination of protein adducts occurs within a few hours). The measurement of isocyanate-derived diamines in urine provides a sensitive means of assessing exposure. This approach has been applied in both field studies of occupational exposure to reacting isocyanate mixtures and in volunteer studies involving the more commonly used pure diisocyanates. The peer-reviewed literature is briefly summarised in Part A. There is a clear relationship between inhalation exposure to pure HDI, TDI and IPDI and the elimination of the respective diamines released by hydrolysis from protein adducts in urine. Field studies show that analysis of urine samples for these diamines has sufficient sensitivity to help assess the adequacy of controls. Studies by HSE have shown that where good control practice measures are in place, the levels of isocyanate-derived diamines are below 1 μmol/mol creatinine and this is proposed as a biological monitoring guidance value.

Introduction

1 For regulatory purposes, all isocyanates are regarded as respiratory sensitisers. The publication "Occupational Exposure Limits" EH40 lists Workplace Exposure Limits (WELs) for total isocyanate at 0.02mg/m^3 (8-hour TWA) and 0.07 mg/m^3 (15 minute short-term exposure limit), accompanied with a "Sen" notation. These are not health-based limits – it is not possible to identify a no-effect threshold.

2 The more commonly used isocyanates are:

- Hexamethylene diisocyanate (HDI) and polymers, used in paints and coatings;
- Toluene diisocyanate (TDI), eg used for flexible polyurethane foam;
- Methylenediphenyl diisocyanate (MDI), used for rigid foam and adhesives; and
- Isophorone diisocyanate (IPDI), also used with HDI in 2 pack isocyanate based paints.

While there are uses for naphthalene diisocyanate (NDI) and tosyl isocyanate (TsI), these are less common.

3 Exposure to isocyanate by inhalation is most likely for HDI and IPDI due both to their volatility, and to application by spraying. Polymeric paint preparations are based on dimers, trimers or short-chain polymers based on HDI and IPDI, with isocyanate terminal groups: these polymeric forms have much lower volatility than the monomers. Such preparations are common, but contain small amounts of monomeric diisocyanate. However, spraying creates inhalable aerosols.

TDI and MDI are less volatile. Isocyanate can also be released by spraying and cutting or heating partly or fully cured polyurethane foams, films, etc.

Metabolism

4 The metabolism of isocyanates in humans is poorly understood. Isocyanates are very reactive with compounds containing active hydrogen, eg hydroxyl, sulphydryl and amino groups in proteins. It is likely that isocyanates react with tissues with which they come into contact, rather than being absorbed and distributed as free isocyanate molecules.

5 No free amines have been reported in plasma or urine after exposure to diisocyanate compounds. However, toluene diamine (TDA), methylenediamine (MDA), hexamethylene diamine (HDA) and isophorone diamine (IPDA) have been released from adducts after acid or alkaline hydrolysis.

6 Several authors report TDI - protein adducts in plasma and urine in volunteer studies (Skarping et al 1991, Persson et al 1993, Lind et al 1996 & 1997) and of MDI (Dalene et al 1996, Sepai et al 1996).

Haemoglobin adducts of TDI have been reported in plasma from workers (Lind et al 1997) or of MDI (Schutze et al 1995, Sepai et al 1995), but no such adducts were found in volunteers exposed to HDI or to IPDI (Brorson et al 1990, Tinnerberg 1995).

Absorption by inhalation

7 Analysis of the isocyanate-derived diamines after hydrolysis of urine from volunteers inhaling known concentrations to isocyanate showed that for TDI, 15 to 20% of the dose of 2,4-TDI and 17 to 23% of the dose of 2,6-TDI is absorbed and excreted as TDA adducts in urine (Brorson et al 1991).

Skarping et al (1991) found similar values – 8 to 14% of the inhaled dose of 2,4-TDI and 14 to 18% of 2,6-TDI.

Tinnerberg et al (1995) found 39% (range 9 to 94%) of an inhaled dose of HDI and 27% (range 19 to 46%) of a dose of IPDI to be excreted in urine as hydrolysable adducts.

8 Isocyanates do not carry a skin notation in EH40, though they are capable of skin sensitisation. Being reactive, they are expected to bind to skin. However, direct skin contact with spray products is limited to a few percent of the total deposition on the sprayer’s coverall (HSE, 2002), and by using disposable protective gloves.

Elimination

9 For TDI, the initial elimination half-life of adducts in urine is 1.9 hours for 2,4-TDA and 1.6 hours for 2,6-TDA, with both isomers having a second half-life around 5 hours (Brorson et al 1991, Skarping et al 1991). The half-lives for the elimination of TDA–adducts in plasma are around 10 days and 21 days (Lind et al 1996 & 1997).

For HDI, the half-life for elimination of HDA-adducts in urine is 2.5 hours (range 1 to 4.3) and for IPDI, IPDA-adducts, 2.8 hours (range 1.7 to 4.7 hours) (Tinnerberg et al 1995).

Biological Monitoring

10 Several volunteer and occupational studies show a good correlation between airborne concentrations of isocyanates and their hydrolysable adducts in urine (TDI: Rosenberg & Saviolainen 1986b, Brorson et al 1991, Maitre et al 1993, Persson et al 1993, Kaaria et al 2001; HDI: Maitre et al 1996, Tinnerberg 1995; IPDI: Tinnerberg 1995).

Peak excretion occurs at the end of exposure for TDI (Skarping et al 1991, Brorson et al 1991), and for HDI and IPDI (Tinnerberg 1995). Coupled with the short half-life, this suggests sample collection at the end of exposure to reflect exposure over the previous 2 to 4 hours.

11 Field studies have shown the utility of biological monitoring for detecting low-level exposure to isocyanates (including polymeric isocyanates). Persson et al 1993 was able to detect exposure to TDI based on measurements of TDA hydrolysable adducts in urine, for workers exposed to concentrations at 1 to 10% of the Swedish exposure limit (40 $\mu g/m^3$).

A series of studies have shown that measurement of MDA in hydrolysed urine can detect exposure to MDI at levels below the detection limit for air monitoring (Skarping et al 1996, Schutze et al 1995, Sepai et al 1995, Kaaria et al 2001).

Rosenberg & Saviolainen (1986a) showed that 15-minute exposures to 2.8 $\mu mol/m^3$ of functional isocyanate from HDI [equivalent to 118 $\mu g/m^3$] could produce 63 µmol HDA/mol creatinine in urine collected 30 minutes after exposure. That is about 100 times greater than the detection limit for HDA in urine.

12 Poor controls can result in detectable levels of TDA-adducts in urine (Maitre et al 1993) and HDA-adducts in urine (Williams et al 1999). However, biological monitoring has shown there to be no HDA-adducts detectable in urine when exposure to HDI was properly controlled (Rosenberg & Saviolainen 1986a, Williams et al 1999).

13 Urinary diamine adducts do not occur in populations that are not exposed to diisocyanates or diamines (eg in epoxy adhesives).

Analytical method

14 All the reported analytical methods begin with hydrolysis to liberate amines from urinary conjugates.

Sulphuric acid hydrolysis is frequently used to generate HDA, TDA, MDA and IPDA (Skarping & Dalene 1995, Rosenberg & Saviolainen 1996a, Tinnerberg et al 1997, Williams et al 1999, Sakai et al 2002). Hydrochloric acid hydrolysis is also reported (Tiljander et al 1989, Sandstrom 1989, Dalene et al 1990, Dalene et al 1994b, Sepai et al 1995, Kaaria et al 2001, Skarping et al 1991 Maitre et al 1993).

Three authors report using alkaline hydrolysis to HDA and IPDA, followed by HPLC-MS (Skarping et al 1994b, Dalene et al 1994, Tinnerberg 1995), to TDA followed by HLPC with electrochemical detection (Carbonnelle 1996) and to TDA followed by CG-MS (Sennbro et al 2003).

Unpublished work by the Health and Safety Laboratory (HSL) has shown interfering / artefact peaks when alkaline hydrolysis to HDA was used with GC-MS.

15 Acid hydrolysates are made alkaline, extracted into an organic solvent and derivatised with pentafluoropropionic (PFP) anhydride (Skarping et al 1991, 1994b & 1994c, 1995, Tiljander et al 1989, Marand et al 2004) or heptafluorobutyric anhydride Dalene et al 1990, Maitre et al 1993, Rosenberg & Saviolainen 1996a, Kaaria 2001, Williams et al 1999). This is followed by separation and detection by GC-MS with selected ion monitoring in either the electron impact or negative ion chemical ionisation. Detection limits are in the range 0.5 to 5 nmol/l and coefficients of variation are typically <5% for within day and <12% day to day.

16 A few methods are based on HPLC with either electrochemical detection (Carbonnelle et al 1996) or LC-MS of the PFP derivatives of HDA and IPDA (Skarping et al 1994b, Tinneberg 1995, Marand et al 2004) or trifluoroethylformate derivatives (Dalene et al 1994). One report concerned analysis of TDA by atmospheric ionisation LC-MS without derivatisation (Sakai et al 2002).

17 The method used by HSL involves acid hydrolysis followed by solvent extraction of the basified solution and formation of the heptafluorobutyryl derivatives. Separation and detection is by negative ion chemical ionisation mass spectrometry. The limit of quantitation is 5 nmol/l (approximately 0.5 μmol/mol creatinine) and the reproducibility (CV) is 5% within day and 12% from day to day (Williams et al 1999).

<u>Note</u>

The limit of detection (LoD) for the method is 1 nmol/l – approximately 0.1 μmol/mol creatinine (assuming an average creatinine value of 8.8 mmol/l), based on 3x background noise. The Limit of Quantitation (LoQ) is 5 nmol/l (0.5 μmol/mol creatinine) based on 5x LoD. The analytical precision of the method, expressed as the day to day coefficient of variation, is <12% at 200 nmol/l.

18 The short half lives of the metabolites in urine means that samples should be collected at the end of a period of exposure. Unpublished work at HSL showed a decline in detectable levels in urine samples spiked with TDA if these are not acidified. Samples spiked with HDA, MDA and IPDA are more stable. Samples from exposed workers containing TDA adducts may be more stable than the free amine; however, HSL considers it prudent to acidify the samples with citric acid after collection, to keep them refrigerated, and to analyse them as soon as possible.

Studies by HSE & HSL

19 HSL has analysed urine samples for diisocyanate metabolites since 1997, storing the results in the Biological Monitoring Database (BMDB). Samples were taken in HSE investigations, though increasingly these come directly from occupational health providers and small firms.

Most samples have no contextual occupational hygiene or control information. Many samples have no specific indication of the type of diisocyanate, so all samples are analysed for all four of the principal diamines - TDA, Had, MDA and IPDA.

20 Table 1 summarises data from the BMDB where HSL has contextual data, either because the samples were part of a HSE study or through information supplied with samples. The mean and 90% values show that compared with reported studies in the literature, occupational exposure to diisocyanate in the UK appears to be low.

However, the table includes data from workplaces with poor control. There is widespread poor compliance with good control practice in motor vehicle repair using HDI (see para.21). The values found for IPDA are raised by samples from a single company with poor control, and for MDA, the data include a site recycling rubber using an MDI based glue with poor control –(HSE investigation, Baldwin 2003).

Table 1 Summary of biological monitoring data for known diisocyanates, µmol/mol creatinine (all industries, including those with poor control)

Analyte	No of samples	Mean value (µmol/mol)	90% value (µmol/mol)	Max. Value (µmol/mol)
2,6-TDA	489	<0.5	<0.5	7
2,4-TDA	489	<0.5	<0.5	10
HDA	1350	0.6	1.4	78
IPDA	239	2.6	4	140
MDA	699	0.63	1	50

21 HSE's occupational hygienists observed that where exposure controls were deficient, urinary diamine would normally be detected and this would prompt an investigation of the cause. But where all controls were working correctly, including behavioural controls, no significant urinary diamine would be found (ie below LoQ, 0.5 µmol urinary diamine / mol creatinine).

22 Recent occupational hygiene studies and investigations by HSE concern exposure to HDI during spray painting of vehicles (Piney 2000, Bagon and Sandys 2003). While air monitoring has an important role in assessing the effectiveness of control measures (such as an enclosed spray booth) and the spread of airborne contamination, the only practicable way of measuring personal exposure to isocyanates is biological monitoring, indicating the efficacy of both respiratory protective equipment (RPE) and behavioural controls. The results of biological monitoring showed that control of exposure to

diisocyanates was often poor, both for escape of contamination from the spray booth, and for sprayer behaviour.

In this study (195 samples), 21% of the samples exceeded 0.5 μmol HDA /mol creatinine and 9% exceeded 1.0 μmol/mol. For IPDA, 5 samples exceeded 0.5 μmol IPDA /mol creatinine. The 50th percentile value of the whole dataset was below 0.5 μmol/mol creatinine and the 90th percentile was at 0.8 μmol/mol creatinine.

23 The data from published volunteer and field studies are insufficient to give a clear biological monitoring guidance value based on inhalation exposure and the subsequent concentration of diamine in urine. The diamine levels found in urine after inhalation exposure at the WEL range from <10 for MDI (DFG 2003) to 30 for HDI and IPDI (Brorson et al 1990 , 1991) with other values in between (Maitre et al 1993 & 1996, & Tinnerberg 1995). Nevertheless the data are sufficient to show that isocyanate exposures found in the HSE studies are likely to be well below the WEL and that control of isocyanate exposure is possible. The data from the HSE studies could be used to propose a biological monitoring guidance value associated with "good practice". Exceeding such a value would be a trigger to examine the adequacy of exposure controls, it would not be associated with the likelihood of ill-health. Two case studies (Part B) help illustrate the point.

Proposal

24 Biological monitoring, based on the analysis of diamines in urine after hydrolysis of isocyanate-protein adducts, is a useful tool for assessing the efficacy of exposure controls for isocyanates. It is particularly relevant where control of exposure includes respiratory protective equipment. Biological monitoring based on collection of urine samples at the end of exposure is a simple way for employers and workers to assess the efficacy of control measures and should be promoted.

25 A guidance value is critical to the interpretation of the results of biological monitoring but for substances like isocyanates that do not have health-based exposure limits, a biological monitoring value equivalent to inhalation exposure at the WEL is not appropriate. Further, although published studies show a relationship between inhalation of monomeric diisocyanate exposure and isocyanate adducts in urine there are insufficient data to propose such a value. A biological monitoring guidance value based on a relationship with good occupational hygiene practice is more appropriate and possible based on HSE studies.

The urinary diamine concentrations reported in Table 1, while indicative of current exposure in many UK workplaces, reflect concentrations that have been found in recent surveys where good control practice was generally not observed. A biological monitoring guidance value lower than the 90% values reported in Table 1 is both desirable and possible. HSE's occupational hygienists have found that in workplaces observing good occupational hygiene practice, urinary concentrations below the LoQ, 0.5 μmol amine /mol creatinine are achieved.

26 A Biological Monitoring Guidance Value (BMGV) is proposed, based on the release of diamines after hydrolysis from HDI, TDI, MDI or IPDI protein conjugates, at a level of 1 μmol diamine/mol creatinine, in urine samples collected at the end of exposure.

The purpose of this guidance value is to prompt an investigation of the reason for exposure, and to remedy defects.

27 The draft Control Guidance Sheet at Part C illustrates the form that guidance is likely to take. Based on WATCH's decision that biological monitoring is appropriate to indicate good control the ACTS COSHH Essential Working Group has endorsed guidance on biological monitoring for isocyanates. Although the only known UK service provider is, at present, the Health and Safety Laboratory (HSL) it is likely that promulgation of a BMGV for isocyanates will stimulate other laboratories to provide a service.

Part D is a draft of additional guidance produced after discussions with key stakeholders for workers and employers.

PART A Reported studies

TDI

Occupational studies

Rosenberg & Saviolainen (1986b) looked at workers exposed to an 80:20 mixture of 2,4 and 2,6 TDI in the manufacture of polyurethane foam. Air monitoring showed the 2,6 TDI isomer was in the range 9 – 78 µg/m^3 during various tasks and was present in greater amounts than the 2,4 isomer and so urine was analysed for 2,6 TDA only. No individual urine values were reported but the authors describe a good correlation between the product of 2,6-TDI in personal air samples x 'sampling time' (not explained) and TDA in urine (y(nmol/mol) = 2.18 x (µmol x min x m^{-3}) –6.25, r = 0.9, n=10). Because the authors did not elaborate on 'sampling time' the relationship cannot be used to predict exposures for 8h TWA.

Maitre et al (1993) also looked at 9 polyurethane foam workers exposed to a 2,4 and 2,6 TDI mixture in an 80:20 ratio. There was no ventilation system and the workers wore no personal protective equipment. Their exposure levels varied from 9.5 to 94 µg/m^3 (8h TWA) and their end of shift urine samples had a mean 'total' TDA concentration of 15.7 (+/- 8.3) µg/g (range 6.5 to 31.7 µg/g creatinine with a linear relationship to airborne TDI levels (log (y) = 0.5795Log(x)+3.278 r = 0.91 of the log transformed data). This suggests inhalation for 8h of 38 µg/m^3 would give and end of shift TDA value of 18 µg/g.

Persson et al (1993) studied exposure to TDI over 48h in a factory producing polyurethane foam. Five exposed workers, 2 supervisors (laboratory worker and a manager) and 2 'volunteers' provided several urine and blood samples that were analysed for 2,4 and 2,6 toluenediamine by GC-MS. Workers only wore RPE when they expected high concentrations of TDI (eg in the curing tunnel). Air monitoring showed inhalation concentrations between 1% and 10 % of the Swedish TLV of 40 µg/m^3. The 5 workers showed the highest urinary elimination rate of TDA. Two workers and two supervisors had elimination rates of 20 – 70 ng/h (sum of 2,4 and 2,6 TDA assuming a creatinine excretion rate of 67 mg/h this is 0.3 to 1 µg/g) and 3 production workers had an average of 100 – 300 ng/h (1.5 to 4.5 µg/g).

Lind et al (1996) Looked at exposure of 11 workers to 2,4 and 2,6 TDI in two polyurethane foam plants. Airborne TDI exposures ranged from 0.4 to 4 µg/m^3 in one plant and 10 to 120 µg/m^3 in the second plant. Air sampling was carried out several days before the biological monitoring. Urine excretion of 2,4 TDA before the holiday ranged from 0.04 to 0.54 µg/h (0.5 to 7.4 µmol/mol) and 0.02 to 0.18 µg/h (0.3 to 2.5 µmol/mol) afterwards. The concentrations of 2,6 TDA were 0.18 to 0.76 µg/h (2.5 to 10.5 µmol/mol) before and 0.09 – 0.27 (1.2 to 3.7 µmol/mol) after the holiday. The half- time for urinary TDA ranged from 5.8 to 11 days.

Tinnerberg et al (1997) studied 4 exposed workers (and one 'volunteer') in a TDI foaming plant – the average air concentration was 29.8 µg/m^3 (12.5 – 79.9 n=12). The highest concentration was around 3mg/m^3. Exposure durations were typically 2-3h per day and workers used RPE only when occupied with preparation of the foaming apparatus that occasionally caused high exposures. 2,4 and 2,6 TDA were measured in urine and plasma after strong acid hydrolysis using GC-MS. Urinary TDA values ranged from none detected to 2 µg/mmol (18 µg/g or 16 µmol/mol) and varied greatly with time and exposure. Peak urinary TDA values were found shortly after exposure.

Kaaria et al (2001) in a method development paper looked at occupational exposure to TDI in 17 employees in 2 plants making flexible foam and found a trend for a linear correlation (r = 0.86) between urinary TDA and the product of the airborne TDI concentration and time. Urinary TDA in plant 1 ranged from 0.11 to 39 nmol/mmol and in plant 2, <0.05 to 7.1 nmol/mmol. Plant 1 had a high pressure moulding technique that released more TDI into the air. The ratio of 2,4 to 2,6 TDA in urine followed the pattern observed in air samples. 2,6 TDA was reported in non-occupationally exposed people (n=12, mean 0.01 µmol/mol range 0.003 to 0.05 µmol/mol)

Sakai et al (2002) in a method development paper described a small study of 13 workers exposed to TDI (a mixture of 2,4-and 2,6-TDI) during polymerisation of foam and 20 non-exposed subjects. TDA was not detected in urine (<1 µg/l, 8 nmol/l) of non-exposed subjects but exposed workers had TDA levels up to 250 µg/l and 63 µg/l for 2,6 and 2,4 TDA respectively.

Volunteer studies with TDI

Brorson et al (1991) exposed two volunteers to 3 concentrations of TDI (a mixture of 2,4- and 2,6 TDI in a 30:70 ratio) for 4 hours on separate occasions to 25 µg/m^3 50 µg/m^3 and 70 µg/m^3. TDA was measured in urine after hydrolysis and the accumulated amount excreted over 24h accounted for 15-19% of the dose of 2,4-TDI and 17-23% of the dose of 2,6-TDI. TDA was rapidly eliminated from urine with a mean initial half-life of 1.9h for 2,4-TDA and 1.6h for 2,6-TDA. The second phase the half-life was about 5 hours for both isomers. There was a linear relationship (r = 0.98) between inhalation concentration and cumulative urinary excretion. The excretion of 2,4- and 2,6-TDA after exposure to 20µg/m^3 2,4- and 2,6-TDI is estimated (from a figure) to be 0.8 and 1 µg/h. If a creatinine excretion rate of 67mg/h is used this would give a urinary concentration of 2,4 TDA of 12 µg/g and of 2,6-TDA of 15µg/g (or 11 µmol/mol and 14 µmol/mol respectively).

So after exposure to 40 µg/m^3 2,4 and 2,6 TDI the urinary 2,4 and 2,6 TDA values would be 25 µg/g and 31 µg/g respectively. Using the table of urine TDA found in end of exposure samples with their corresponding inhalation concentrations and using a linear regression, gives an alternative approach to predicting urinary concentrations. Using this method after exposure for 4h to 20µg/m^3 2,4- and 2,6-TDI the calculated 2,4- and 2,6-TDA concentrations are 9 and 13 µmol/mol creatinine respectively. After exposure to 40 µg/m^3 TDI, the urinary TDA values would be 18 and 27 µgmol/mol for 2,4- and 2,6-TDA respectively.

Skarping et al (1991) exposed five men to a mean air concentration of 40 (range 36 – 43) μg/m^3 total TDI for 7.5h. The isomeric composition was ca 48% 2,4-TDI and 52% 2,6-TDI (no amines formed by hydrolysis of TDI were detected in the air). The inhaled doses (pulmonary ventilation x exposure concentration x duration of exposure) of 2,4- and 2,6-TDI were ca.120 μg. Urine and blood samples were collected from the volunteers and after hydrolysis were analysed for 2,4- and 2,6-TDA by GCMS. The peak of excretion was in the 6 – 8h sample (end of exposure) with the rate of excretion for 2,4-TDA averaging 0.6 μg/h and that for 2,6-TDA averaging 1.0 μg/h. The average 2,4-TDA concentration in the 6-8h sample was 5 μg/l (range 2.8 – 9.6) and the values for 2,6-TDA were 8.6 μg/l (range 5.6 – 16.6). Only traces of 2,4- and 2,6-TDA were found in the samples collected after 24 h. The urinary elimination of TDAs showed a possible biphasic pattern with the initial rapid phase half-lives of 1.9h (range 1.1 – 2.5) for 2,4-TDA and 1.6h (range 1.1 – 2.2) for 2,6-TDA. The approximate half time for the second phase was ca. 5h for both compounds. The cumulative elimination of 2,4-TDA within 28h ranged from 8 to 14% of the estimated dose and for 2,6-TDA was 14-18%. No correlation was found between acetylator phenotype and 2,4- or 2,6- TDA in hydrolysed urine.

MDI

Occupational studies

Skarping et al (1996) studied exposure to MDI in a factory using isocyanate-based polyurethane glue. Isocyanate concentrations were measured in air and samples from 174 workers were analysed after hydrolysis by GCMS for MDA in urine and plasma. Employees were screened for work related respiratory symptoms and tested for specific immunoglobulin E (IgE) and IgG antibodies directed against conjugated human serum albumin. TWA concentrations of isocyanate in workers' breathing zones were low (MDI < 0.2 – 7; HDI 0.1 – 7; 2,6-TDI 0 – 1 μg/m^3). All subjects had detectable levels of MDA in plasma and urine and there were significant associations between estimates of exposure to thermal degradation products of MDI based glue and MDA in plasma (0.1 – 5.5 μg/l) and MDA in urine (0.04 – 5 μg/l). In cases of heavy exposure plasma MDA and urine MDA were associated with each other (r=0.64 p=0.0001), with work related symptoms (plasma MDA – P=0.03 Mann-Whitney U test), and serum concentrations of MDI specific IgG antibodies (r=0.26 p=0.0007). Unexpectedly high plasma and urine MDA concentrations were also seen in workers cutting textile – no reason given. MDA was only seen in plasma and urine after hydrolysis, there was no free MDA.

Skarping & Dalene 1995 in a method development paper reported a concentration of 2.3 μg/l MDA in a pooled urine sample from 10 workers exposed to MDI (details not given)

Schutze et al (1995) developed analytical methods for MDA in urine and from haemoglobin adducts. Although workers were exposed only to low levels of MDI (only 3 samples above the detection limit of 3 μg/m^3) MDA could be detected in most urine samples after hydrolysis (range 0.7 to 10 nmol/l). MDI exposure could be detected in

more workers by measurement of urine metabolites than by haemoglobin adducts or air monitoring.

Sepai et al(1995) studied twenty workers exposed to MDI during the manufacture of polyurethane products. Air levels were measured by personal and workroom samplers and were mostly below detection limits. Blood and urine samples were analysed for the presence of adducts and metabolites by GC-MS. The amount of MDA released after acid hydrolysis was 6.5 times greater than the sum of free MDA and acetyl-MDA suggesting the presence of other adducts/conjugates. MDA was detected as haemoglobin adduct in all of the 20 exposed workers. The levels ranged from 70 to 710 fmol/g Hb. Plasma protein adducts of MDA ranged from 0.25 to 5.4 pmol/ml.

Dalene et al (1996) found MDA in urine of 4 workers (0.1, 0.6, 2.05, 1.25 µg/l) exposed to thermal degradation products of MDI-based polyurethane during welding of pipes. This study also looked at plasma levels of MDA (after hydrolysis) and found MDA in 18 of 30 pipe layers who had been welding pipe during the 3 months prior to the study. They also reported no association between acetylator genotype and levels of MDA in plasma.

Kaaria et al (2001) in a method development paper reports detecting MDA in urine from 97% of workers (n=57) exposed to MDI moulding rigid polyurethane foam even though MDI in air was below the detection limit for the air method for 67% of the workers.

Volunteer studies with MDI

There are no reported volunteer studies with MDI

HDI

Occupational studies

Maitre et al (1996) studied 19 men exposed to HDI during monomer production. Airborne concentrations ranged from 0.3 to 97.7 $\mu g/m^3$. Urine HDA was measured in post shift samples after acid hydrolysis and values ranged from 1.36 µg/g to 27.7 µg/g and there was a linear association between HDI in air and urinary HDA (log (y) µg/g = 0.4396 log(x) $\mu g/m^3$ +0.4612 (r=0.698, p=0.001, n=19). The authors proposed a biological exposure index value of 19 µg/g in post-shift urine samples as an indicator of exposure to HDI monomer at an 8h TWA of 75 $\mu g/m^3$.

Williams et al (1999) looked at occupational exposure to HDI in sprayers employed in motor vehicle repair. Urine samples were collected at the beginning, middle and end of shift from 22 workers (11 sprayers, 4 bystanders and 8 unexposed) and analysed for HDA by GC-MS. HDI monomer was probably a small proportion of the total isocyanate: nevertheless HDA was found in the urine of 4 sprayers (maximum concentrations 11, 1, 1 & 3 µmol/mol) and one bystander (12 µmol/mol) but not other potentially exposed

workers. The study concluded that exposure to HDI could still occur in workers wearing full face air-fed personal protective equipment and working in a ventilated space probably due to poor fit of the RPE and/or removing the RPE at the end of spraying but while a significant concentration of HDI remained in the spray booth. Bystander exposure was thought to be due to the bystander going into the spray booth to talk to the sprayer. No HDA was found in any sample taken before shift or in unexposed subjects and the half-life of elimination was thought to be consistent with 1.5 to 2 hours.

Volunteer studies with HDI

Rosenberg & Saviolainen (1986a) looked at car painters exposed to HDI polymer-based paint in an exposure chamber. The average concentration of the HDI polymer was 34% and that of the monomer was 0.24% in the hardener. The concentration of inhaled functional NCO groups during a 15 minute exposure was 2.8 +/- 0.8 µmol/m^3 and the HDA concentration in urine was 63 +/- 33 nmol/mmol creatinine (65 µg/g) in samples collected 30 minutes after the end of exposure. This value is higher than might be expected from the concentration of HDI monomer alone and suggests that HDA may be coming from both monomeric and polymeric HDI. This study also reported a single occupational exposure where no HDA was found in the urine of a car painter spraying wearing an air-fed hood – the spray booth had an isocyanate monomer concentration of 0.2 µmol/m^3 but no isocyanate was detected inside the hood.

Brorson et al (1990) exposed 5 volunteers to 1,6 hexamethylene diisocyanate (HDI, 25 µg/m^3) for 7.5h and the inhaled dose was estimated to be around 100 µg. After acid hydrolysis 1,6 HDA was determined in plasma and urine by GC-MS (CI+) of the heptafluorobutyrate (HFB) derivatives. The cumulative urinary excretion of HDA accounted for 11 to 21% of the estimated inhaled dose. Urine samples collected at the end of exposure had an average HDA concentration of 0.02 mmol/mol creatinine (range 0.01 to 0.03 mmol/mol or 10 to 30 µg/g) and the peak excretion rate was 1.1 to 1.4 µg/h. The half time for elimination of HDA in urine was 1.1 to 1.4h. No specific IgE or IgG antibodies to HDI were detected before or after provocation. Assuming a linear response to the MEL of 40 µg/m^3 this could result in urinary HDA of 32 µmol/mol.

Tinnerberg (1995) exposed 3 volunteers at rest to 3 concentrations of HDI (11.9, 20.5, 22.1 µg/m^3) and IPDI (12.1, 17.7, 50.7 µg/m^3) on separate occasions for 2h in an exposure chamber. Inhaled doses were estimated from pulmonary ventilation x dose level x duration. All urine was collected and blood samples were taken before and half an hour after exposure and again the next day. HDA and IPDA were not found ‘free’ (before hydrolysis). After hydrolysis an average of 39% of the estimated dose of HDI was found in urine (range 9 – 94%) over 48h. The maximum urine concentration of HDA was in samples collected at the end of exposure (but no values were reported). The average elimination half-life of HDA in urine was 2.5h (range 1.0 – 4.3). No HDA was found in hydrolysed plasma.

Mixed exposures

Dalene et al (1997) investigated exposure to TDI and MDI in 15 female workers as a consequence of exposure to thermal degradation products of polyurethane based foam and glue respectively. The workers made parts for passenger areas of cars using textile, polyurethane foam and MDI-based glues. During spraying of the glue workers wore respiratory protection (type not specified) and rubber gloves. After the solvents evaporated the parts were heated under infra red lamps for 10-20 sec or with heat guns. No air monitoring was done. Urinary elimination rates were in the range <0.1 to 5.7 µg/h of 2,4 TDA and <0.01 to 3.5 µg/h of 2,6 TDA and <0.1 to 1.6 µg/h of MDA.

Littorin et al 2000 looked at respiratory symptoms in a cross sectional study of 152 workers exposed to TDI and MDI while gluing and heating polyurethanes. After hydrolysis MDA was found in plasma from 65% of workers. TDA was found in urine from 47% of workers. Urinary MDA was associated with work related symptoms from the airways (OR = 3.7) and plasma TDA with work related symptoms from the lower airways (OR=6.6). Nine women making arm-rests had higher exposure than other workers and had median urine MDA values of 3.3µg/l, 2,4-TDA 0.6µg/l and 2,6-TDA of 0.5µg/l. Five of the arm-rest workers had work related symptoms.

Rosenberg et al (2002) studied workers in 5 different polyurethane processing environments – grinding and welding in car repair shops, milling and turning polyurethane coated metal cylinders, injection moulding thermoplastic polyurethanes, welding and cutting polyurethane insulated heating pipes, jointing and welding and heat flexing of polyurethane floor covering. TDA and MDA were detected in urine samples from car repair shops and MDA from workers welding heating pipes. The isomer, 2,4-TDA, accounted for 80% of the TDA detected. The highest TDA and MDA concentrations were 0.79 and 3.1 nmol/mmol creatinine respectively. Low levels of the amines were found in non-occupationally exposed workers – 0.08 and 0.05 nmol/mmol (arithmetic means) for MDA and TDA respectively. Exposure to thermal degradation products of polyurethanes are often intermittent and short duration but can be detected by analysis of diisocyanate derived material in urine.

IPDI

Volunteer studies

Tinnerberg (1995) exposed 3 volunteers at rest to 3 concentrations of IPDI (12.1, 17.7, 50.7 $\mu g/m^3$) on separate occasions for 2h in an exposure chamber. Inhaled doses were estimated from pulmonary ventilation x dose level x duration. All urine was collected and blood samples were taken before and half an hour after exposure and again the next day. IPDA was not found ‘free’ (before hydrolysis). After hydrolysis an average of 27% of the estimated dose of IPDI was found in urine (range 19 – 46%) over 48h. The maximum urine concentration of IPDA was in samples collected at the end of exposure. A figure gave values for a single volunteer exposed to each of the 3 IPDI levels and reported excretion rates (read from the figure) of approximately 420, 780 and 720 ng/h.

Using an average excretion of creatinine of 67 mg/h this would give urinary IPDA concentrations of roughly 6, 12, & 11 µg/g (or 4, 8 and 7 µmol/mol) for each inhalation concentration. Taking a simple average of all three values extrapolated to 53 µg/m^3 gives an average urinary IPDA of 24 µg/g (16 µmol/mol). The average elimination half-life of IPDA in urine was 2.8h (range 1.7 – 4.7)

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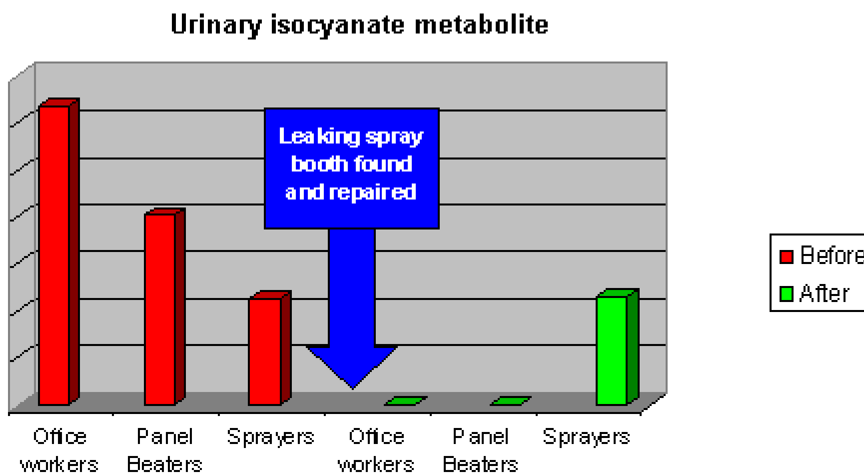
PART B Case Studies

Case Study A

An MVR site was investigated because there were reports of occupational ill health amongst workers. Biological monitoring (BM) showed that all staff, including administrative staff that never did any painting, had been exposed to isocyanates from the spray paints in use at the factory.

Investigation showed that the spray booth was leaking. Isocyanates from the booth were able to spread throughout the building, so everybody who worked there was exposed to them. This was an unexpected finding because the spray booth had been recently serviced, inspected and tested, and was thought to be working properly.

The leaking spray booth was repaired. Further BM showed that the spray booth was working properly, but one of the paint sprayers was still exposed to isocyanates because he had not received proper instructions and was not using his air-fed RPE correctly. His exposure was not as low as it should have been.



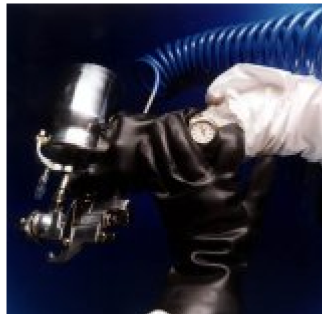
Case Study B

A biological monitoring survey was undertaken in a variety of motor vehicle repairers ranged from national dealerships to 'one man' operations. Factors that might lead to isocyanates exposure were identified. These included:

• Spraying above head height. Respiratory protective equipment sometimes does not fit properly when people tilt the head upwards;
• Lifting visors to take a closer look at the job quality. Wearers are then not protected;
• Entering a spray booth unprotected, before the correct 'clearance time' had elapsed;
• Unprotected workers entering spray areas to speak to protected colleagues during spraying.

Biological Monitoring allowed these risk factors to be identified. They might otherwise have gone unnoticed. It highlighted areas where improved health and safety procedures were needed.

Sprayers should not lift their visor to check on the job



Sprayers should always know and observe clearance times for booths and ventilated spaces

Never allow an unprotected person to approach a sprayer while spraying

PART C

4

SPECIAL

Special advice DRAFT

Measuring exposure to isocyanates by Biological Monitoring

40X

Essentials Logo

This guidance sheet is for **employers**. It will help them comply with the requirements of the Control of Substances Hazardous to Health Regulations 2002 (COSHH) by helping them check on the effectiveness of the controls that protect workers' health.

This sheet covers the points you need to have clear when using biological monitoring to measure isocyanate exposure.

Reference

Williams N, Jones K, Cocker J (1999).
Biological monitoring to assess exposure from isocyanate use in motor vehicle repair.
Occup. Environ. Med. 56 pp. 598 - 601.

Useful Addresses

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email hslinfo@hsl.gov.uk

Introduction

This control sheet provides general advice on the points to consider when measuring exposure to isocyanates by biological monitoring.

Isocyanates often occur in:

- 2-pack spray paint, lacquer, underseal and varnish.
- Some glues and adhesives.
- Foam and plastic production.
- Hot work on polyurethane foam, cured paint or plastic.

Relatively low levels of exposure to isocyanate may cause asthma. Exposure should be as low as is reasonably practicable. Even people working nearby can be affected. Biological monitoring is one practical way to assess exposure, and the effectiveness of control measures which include respiratory protective equipment (RPE).

Biological Monitoring

Measuring exposure by biological monitoring is a sure way to know if exposures are properly controlled. Companies that set-up their control measures properly (including proper training and instruction of workers directly involved) can get isocyanate exposures down to low levels.

Biological monitoring works by measuring the breakdown products of isocyanate in urine. This means taking samples of urine from people who may be exposed.

Control of exposure includes:

- Work process, work organisation and worker behaviour to minimise exposure.
- Engineering controls such as spray booths and enclosed systems.
- Air-fed respiratory protective equipment (RPE).
- Excluding other people from areas where isocyanates are used.

Biological monitoring will tell if people have breathed in isocyanate - if so, the control measures are not working properly.

Urine sampling

The Health and Safety Laboratory (HSL) offers a commercial service to measure isocyanate breakdown products in urine. HSL charges about £50 per sample, which includes collection kit, packing and instructions. Other laboratories may be able to offer equivalent services.

- Urine samples should be collected immediately the task or shift has finished.

Interpreting results

Biological monitoring gives no information about health – it is simply a measure of exposure. You need to explain this clearly to everyone taking part, and get their informed consent.

Why are the results high? Results need interpreting. HSL can advise on simple practical ways of setting up a biological monitoring programme, and can help in interpreting the results.

Quality assurance

Any laboratory offering a service should participate in a relevant Quality Assurance scheme.

PART D

Slide used in Safety and Health Awareness Days - Motor Vehicle Repair and 2 pack paints.

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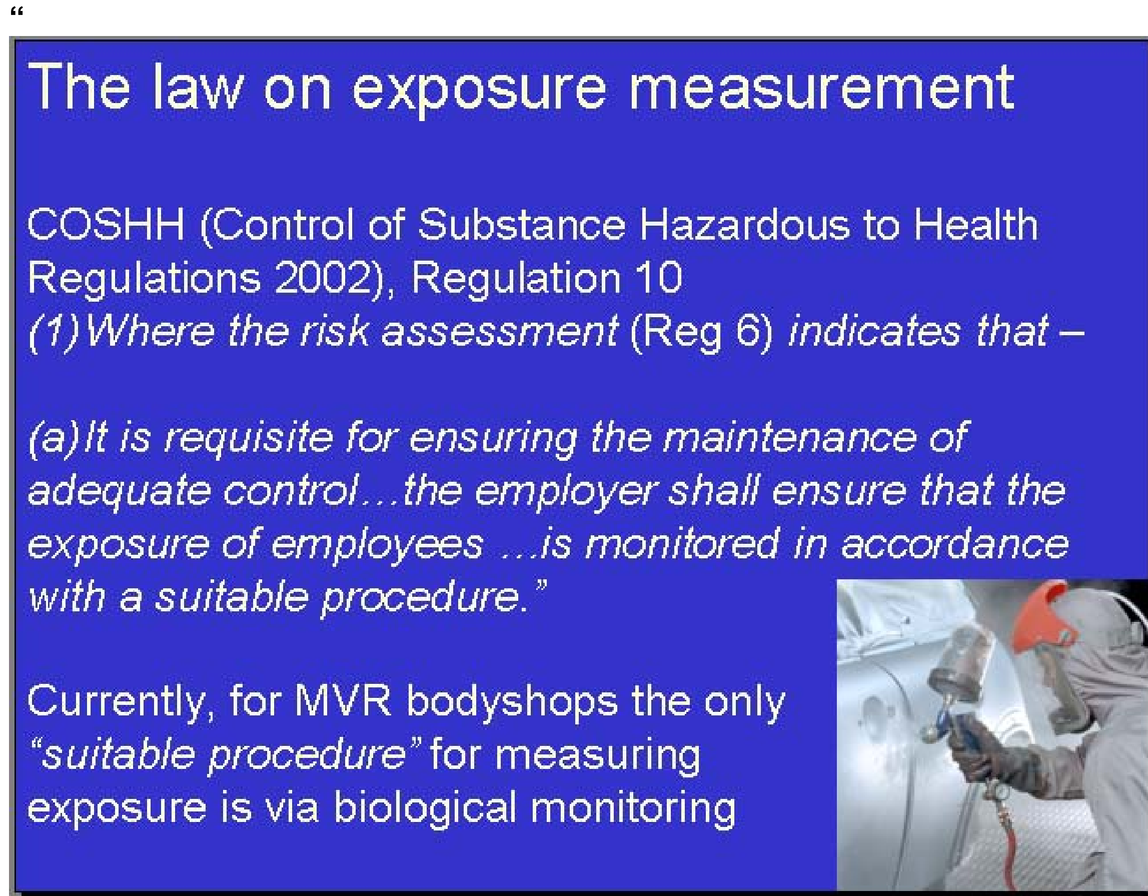

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PART E Guidance for workers & Employers and occupational health professionals

What is the problem with isocyanates?
Isocyanates can cause asthma. They are the biggest cause of work-related asthma in the UK. Not everyone working with isocyanates gets asthma but HSE estimates that there are over 150 new cases each year. People with asthma caused by isocyanates have to change jobs to protect their health. Because of this exposure to isocyanates should be well controlled and the controls should be checked to make sure they are working.

Why analyse urine ?
If the exposure controls are not working and isocyanates enter the body they are metabolised or broken down and eliminated in urine. The level of these isocyanate metabolites in urine is an indicator of how much isocyanate has been absorbed and how well the controls are working.

In some jobs, like spraying 2-pack paints, control relies on air-fed masks and measurement of isocyanate metabolites is a simple way of checking that the mask fits, works and is used properly.

Is it compulsory?
No. Giving a urine sample is voluntary but it is in the worker's own interest to make sure their health is being protected by good control of exposure. In some cases (like paint spraying) it may be very difficult to check any other way that the controls are working.

How should samples be collected ?
Collecting samples of urine to assess exposure is usually called biological monitoring. HSE has produce guidance booklets on how to set up a biological monitoring programme[1,2]. Briefly, everyone needs to know:

- why samples are being collected (to check exposure to isocyanates),
- when they should be collected (at the end of exposure),
- how often (once or twice a year unless results there is a problem,
- What will be measured (isocyanate metabolites only not drugs or alcohol etc)
- Who will see the results (it makes most sense if the workers let management see the results so they can work together to sort out any problems)
- What happens if the results show controls are not working as well as they should (try and find out why, sort it and then check again)

Someone needs to manage the process and usually this would be the occupational health provider (workers using isocyanates are required to have annual health surveillance). An occupational hygienist or a manager could also manage the programme perhaps with initial help from an occupational physician.

An important aspect is that the worker(s) understand what is being done and why (i.e. they can give informed consent).

Samples should be collected into 30ml plastic bottles containing 0.5g of citric acid. The bottles should be firmly closed and labelled with the worker's name, and date of collection.

Samples should be sent to the laboratory in appropriate packaging (usually supplied by the laboratory) with details of the tests required (isocyanate) and where to send the results (probably on a form supplied by the laboratory)

What do the results mean?

The laboratory will be report the results something like this:

Name	HDA µmol/mol creatinine	TDA µmol/mol creatinine	IPDA µmol/mol creatinine	MDA µmol/mol creatinine
Mr Smith	2	<1	<1	<1
Guidance value	1	1	1	1

HDA is hexamethylendiamine the metabolite of hexamethylenediisocyanate (HDI)
TDA is toluenediamine the metabolite of toluenediisocyanate (TDI)
IPDA is isophoronediamine the metabolite of isophoronediisocyanate (IPDI)
MDA is methylenedianiline the metabolite of methylenediisocyanate (MDI)

The levels of HDA, TDA etc are reported as 'µmol/mol creatinine'. Creatinine is found in everyone's urine and adjusts the level of HDA, TDA etc to compensate for very dilute or very concentrated urine.

The guidance value is 1 and in the example above for TDA, IPDA and MDA the level (<1) is less than the guidance value showing either no exposure or well-controlled exposure. For HDA, the level reported above is 2 and is above the guidance value.

What do I do if I have a result above the guidance value?

If a biological monitoring result is above the guidance value it may mean that the methods of controlling exposure to isocyanates are not working as well as they should. If the result is the first from a worker then it is sensible to ask for another sample for confirmation. It is also sensible to look at how the worker may be exposed to isocyanates and whether the controls are working as intended. It should be noted that because the guidance value is based on the value found in 9 out of 10 samples in places with good control it is likely that 1 in 10 values will be above the guidance value even in places with good control.

Will anything else be measured?

No – the laboratory will not analyse the urine sample for anything else

How often should monitoring be done?

If results are below the guidance value, sampling only needs to be done once per year unless working practices change or new workers are employed. If the results exceed the guidance value, a repeat sample should be taken. If this sample is also positive, further samples should be taken after implementing changes to improve control of exposure until results are below the guidance value.

1) Biological Monitoring in the workplace A guide to its practical application to chemical exposure HSE 167

2) 2) Biological Monitoring in the workplace Information for employees on its application to chemical exposure HSE books INDG245 (free)

BIOLOGICAL MONITORING for ISOCYANATES
Guidance on laboratory methods

Biological Monitoring Guidance value:

1 µmol isocyanate derived diamine/mol creatinine in urine collected at the end of exposure.

Method for Isocyanated derived amines in Urine

❒ ***Sample Collection***

Time: Urine samples should be collected at the end of exposure – the urinary half-life is around 2 hours. Equipment: polystyrene universal container (30ml) containing 0.5g citric acid

❒ ***Description of Suggested Method***

Internal standards (100 µl of heptane diamine 1µM and ethylenedianiline 5µM) are added to urine (2 ml), acidified with concentrated sulphuric acid (200 µl), tubes are capped and incubated at 100 ^{o}C for 90 min. After cooling sodium hydroxide (2ml, 10M) and diethyl ether (4ml) are added and the contents mixed for 20 min. After centrifugation 3ml of each ether layer is removed to a clean tube and the solvent removed under nitrogen. The residue is derivatised with heptafluorobutyric anhydride (50 µl) in toluene (500 µl) in closed tubes at 55°C for 1h. After cooling the derivatisng reagent is removed under nitrogen and reconstituted in toluene (100µl). Injections (1µl) are made splitless (350°C, 30 sec) into a capillary column (30m x 0.3 mm BP5 1µm) at 150 ^{o}C increasing at 10 ^{o}C/min to 240 ^{o}C then 20 ^{o}C/min to 300 ^{o}C. Detection is by mass spectrometry with negative ion chemical ionization (methane) monitoring ions at m/z 488, 449, 462, 495 and 542 from 4 to 10 min and m/z 571 and 585 from 11 to 15 min.

❒ ***Sample Transport to laboratory***

At ambient temperature, should arrive within 48h of collection.
If any delay anticipated, store at –20°C
Samples sent through the postal system must comply with Post Office regulations.

❒ ***Analytical Evaluation***

Precision
- within day <5% RSD at 200 nmol/l
- day to day <12% RSD at 200 nmol/l

Detection limit
- 3 x background - 1 nmol/l

Calibration range
- typically 50 - 300 nmol/l

Sample Stability
-2 days at ambient, > 3 months at –20 ^{o}C

Analytical interferences
-None known

❒ ***Other Information***

Elimination half-time

- -Half-life for HDI and TDI is around 2 hours. The half-life for MDI is much longer (over 50 hours has been reported for chronic exposures) and so previous days' exposures will influence results.

Confounding Factors
Exposure to free hexamethylene diamine, toluenediamine, isophoronediamine and methylene dianiline will also contribute to their respective urinary diamine levels and may confound assessment of exposure to the isocyanates.

Unexposed Levels
< 0.5 μmol /mol creatintine

❒ ***Quality Assurance***

- Internal QC must be established.
- External QA - available from HSL

May 2005

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