



Ensuring improved isocyanate exposure assessment to better protect health

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Isocyanate exposure is one of the leading causes of occupational asthma in Great Britain (GB). Exposure can also cause dermatitis and irritation of the eyes, nose and throat. Isocyanates are widely used in industry, particularly in spray painting, adhesives, flexible foam and polyurethane resin production. Dutyholders must ensure that effective control measures are in place to protect workers. Dutyholders may use air monitoring to demonstrate that airborne isocyanate levels are below the workplace exposure limit (WEL). Dutyholders may use biomonitoring of workers to ensure that exposure control measures are effective. These established HSE monitoring methods have some drawbacks for users, for example, a license is needed to use the air monitoring method, MDHS 25/4, because it uses a restricted chemical. Also, the HSE urine biomonitoring method does not distinguish between exposure to isocyanates and some other chemicals.

This report describes research to evaluate the suitability of potential alternative monitoring methods. It will be of interest to technical specialists undertaking isocyanate sampling and/or analysis. The research included a literature review, laboratory studies, and tests at three volunteer sites (3D printing, a foundry, and aerospace). The researchers' conclusions are: (1) MDHS 25/4 continues to be the most suitable approach to demonstrate compliance with the WEL. (2) HSE's methods for air monitoring and biomonitoring continue to be the most suitable for use in GB and may be adaptable as alternative isocyanates come to market. (3) Other monitoring methods may be appropriate in certain circumstances but are unlikely to be universally applicable for the measurement and analysis of all isocyanates.

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Ensuring improved isocyanate exposure assessment to better protect health

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Site visits for this study were reviewed by the Health and Safety Executive's Research Ethics Panel which operates under delegated authority from the University of Sheffield Medical School Research Ethics Committee (Reference: REACT_ERAC_171019, date 17/10/19).

Key Messages

Isocyanates continue to be one of the leading causes of occupational asthma in Great Britain (GB), and in the period 2015-2019, were the second most identified cause. They were also the leading identified cause for Industrial Injuries Disablement Benefit (IIDB) cases. Isocyanates are widely used in industry, particularly in spray painting, coatings and adhesives, flexible foam and polyurethane resin production.

For dutyholders to demonstrate effective control of isocyanate exposures requires suitable monitoring methods for both the working environment and for workers themselves. Existing methods have several drawbacks, therefore, this research aimed to evaluate other approaches to address these. Possible exposures from using alternative isocyanates and new processes that might generate isocyanates were measured at two workplaces. This is a technical report aimed at those undertaking sampling and/or analysis for isocyanates in workplace settings.

The Health and Safety Executive (HSE) air monitoring methodology (MDHS 25/4, 2014) uses a chemical that is restricted by the Home Office. This presents some barriers to use so other methods were investigated. The research found that the HSE method remains the most viable method for demonstrating compliance with the GB Workplace Exposure Limit, while another method (ASSET EZ4-NCO Sampler™, 2012) may be more practical in certain circumstances.

The HSE biomonitoring method for isocyanates measures the corresponding amines in urine. However, the measurement is not specific to isocyanates. The biomonitoring methods research found that the current HSE approach may be adaptable for use with other isocyanates and has been successfully demonstrated in one instance. A new urine biomarker, specific to one of the more common isocyanates, was measured in real samples. Screening techniques may be viable, making analysis cheaper and quicker, however there is a lack of evidence to support this.

Overall, the work has concluded that:

- The existing methods for both air monitoring and biomonitoring remain the most suitable and may be adaptable as other isocyanates come to market.
- Other techniques for both air monitoring and biomonitoring may be appropriate and useful in certain circumstances.

Executive Summary

Background and Aims

Isocyanates continue to be one of the leading causes of occupational asthma in Great Britain (GB). This is despite considerable improvements, particularly in the motor vehicle repair sector because of HSE's interventions. In the period 2015-2019, isocyanates were the second most identified cause of occupational asthma, and the leading identified cause for IIDB cases. Isocyanates are widely used in industry, particularly in spray painting, coatings and adhesives, flexible foam and polyurethane resin production.

Exposure monitoring is necessary to reach an informed and valid judgement about risk, to ensure that a Workplace Exposure Limit (WEL) is not exceeded or as a check on the effectiveness of controls. Exposure to isocyanates can be monitored in different ways and can involve personal monitoring (measuring the amount of substance in a worker's breathing zone), background air monitoring or biological monitoring. A competent person should be able to determine which procedure is suitable for which situation.

For dutyholders to demonstrate effective control of isocyanate exposure requires suitable monitoring methods. Existing methods have several drawbacks; this research aimed to evaluate other approaches to address these. Possible exposures from using alternative isocyanates and new processes were measured at two workplaces.

This is a technical report aimed at those undertaking sampling and/or analysis for isocyanates in workplace settings.

In GB, the WEL for isocyanate-containing materials is distinct as it is based on the total of a chemical functional group (-NCO), rather than a single compound. The current HSE air monitoring methodology, Methods for the Determination of Hazardous Substances (MDHS) 25/4, uses a chemical that is restricted by the Home Office. This restriction means that users require a licence, and this is a barrier to use.

The HSE biomonitoring method for isocyanates measures the corresponding amines in urine. However, these amines are also commercially used chemicals, so their measurement is not specific to isocyanates.

There were three main objectives of the work:

- Evaluate alternative air monitoring methods that could still provide measurements suitable for comparison with the GB (WEL) but avoid restricted chemicals and enable a more straightforward analysis;

- Develop methods for biomarkers that are specific to isocyanate exposure; and
- Evaluate example new isocyanates and use scenarios for their potential exposures.

Methods

For the air monitoring methods, a literature review was conducted, and the practicality of promising candidate methods were investigated in the laboratory. Methods were also tested in the field where possible. Available quality assurance schemes were reviewed.

Several strands were developed for the biomonitoring methods including expanding the current approach to other isocyanates, exploring specific biomarkers, and investigating the use of screening techniques to simplify analysis. A literature search on relevant effect biomarkers was also conducted.

Three site visits (aerospace, foundry and 3D printing) were undertaken to explore new isocyanate uses and potential exposure scenarios, and to evaluate the alternative methods identified in this project.

Findings

Air Monitoring

From the literature review, two alternative methods were chosen to be investigated further. One involves a commercial sampler (ASSET EZ4-NCO Sampler™), and some analytical standards are available. Although the sampling is more practical, safer and requires no licensing, the analysis is at least as challenging as the existing method. It may be suitable in cases where demonstrating compliance with the GB WEL is not required, for example, checking effectiveness of controls. The other method (1,8-Diaminonaphthalene (DAN) method) has potential as a straightforward analysis however, the method is not validated, and practical issues were encountered when using the method.

The existing HSE method (MDHS 25/4) remains the most viable approach for demonstrating compliance with the GB WEL and is adaptable to other isocyanate-containing compounds as demonstrated in one of the site visits.

There is no external quality assurance scheme that offers a full assessment of the current MDHS 25/4 method; it is, therefore, difficult for dutyholders and HSE to assess laboratories for their performance in this challenging analysis.

Biomarkers

An adaptation of the existing HSE method was successfully applied to some samples from workers using a larger, less volatile isocyanate, proving that the “corresponding amine”

biomarker approach is adaptable. The work also demonstrated that these alternative isocyanates can still be absorbed into the body, therefore presenting a respiratory sensitisation hazard.

A new urine biomarker, specific to one of the more common isocyanates MDI (methylene diphenyl diisocyanate), was measured in real samples. Several screening approaches were developed that could make the analysis quicker and cheaper. Promising methods were identified but significant improvements in sensitivity would be required to be practical for use.

A literature review revealed no specific, reliable effect biomarkers for isocyanates that were in common use, however several may warrant further investigation.

Site Visits

Three site visits were undertaken; with MDI being used in processes at all three sites. One company was also using a newer alternative isocyanate. Exposures to the alternative isocyanate were detected using the existing HSE air and biomonitoring methods. This confirms that the current approaches may be adaptable as alternative isocyanates come to the market.

Isocyanate exposures at one site allowed the comparison of one of the new air monitoring methods with the existing MDHS 25/4 method. Whilst the samples using the new method showed broad agreement with the HSE method, considerable variability was noted.

One site was a 3D printing studio. Although, the visit was only a single assessment of one workplace, the very low levels of MDI detected, despite ten printers running simultaneously for three and a half hours, indicated that the isocyanate exposure in this scenario is likely to be low. Previous HSE research ([RR1146](#), 2019) has identified operating practices to minimise emissions from desktop 3D printers.

Conclusions

The work has concluded that:

- The existing HSE methods for both air monitoring and biomonitoring remain valid and may be adaptable as alternative isocyanates come to the market.
- Other techniques may be appropriate in certain circumstances but are unlikely to be universally applicable for the measurement and analysis of all isocyanates.
- An external quality assurance scheme for air monitoring would be valuable to assess laboratories for their performance in providing analysis suitable for demonstrating compliance with the GB WEL.

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1. Introduction

1.1 Project Background

Isocyanates continue to be widely used in industry and remain a significant cause of occupational asthma in GB (HSE, 2022a). It is clear that isocyanate-containing products are evolving for example, using "blocked" technologies, bespoke structures, and larger or different species. HSE's knowledge so far indicates that these newer products may be marketed as 'safer', and indeed, some are also available to consumers. Some of these products that contain prepolymers or polyisocyanates are not required to be labelled with the warning 'H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled' and this may therefore lead to an under-appreciation of the hazards of these products. Notwithstanding that, they can still present a significant exposure to isocyanates if not adequately controlled.

As well as the problem identified above, there are also known issues with the current HSE air monitoring method - Methods for the Determination of Hazardous Substances 25/4 [MDHS 25/4, (HSE, 2014a)], in terms of measuring compliance with the GB Workplace Exposure Limit (WEL) for isocyanates. This method involves the use of a chemical, 1-(2-methoxyethyl)piperazine (1,2-MP), that is restricted by the Home Office, meaning that users require a licence. This requirement has created issues for users of MDHS 25/4, during both sampling and analysis since they must comply with the licensing rules. There are also challenges to users when trying to fully characterise exposures – hence the use of a simpler method (based on a single, "total reactive isocyanate group" (TRIG) marker, if possible) would serve to improve regulatory compliance. There continues to be a practical issue when trying to characterise isocyanates present as aerosols. Here, using impingers is currently the only proven effective means of sampling, but they are not ideal for taking personal samples as they involve using hazardous solvents, and are usually of glass construction.

Biomonitoring for isocyanates has grown significantly in GB because of awareness of HSE's guidance in this area including, for example, the biological monitoring guidance value (BMGV) published in EH40 (HSE, 2020), a Control of Substances Hazardous to Health (COSHH) Essentials sheet [i.e., G408, (HSE, 2022b)], and specific recommendations provided to workers in the motor vehicle repair sector (HSE, 2014b). However, studies have demonstrated the potential for confounding by amines used alongside, or formed during use of isocyanates (Jones et al., 2017). Therefore, the development of more specific biomarkers would be invaluable in such instances. Currently, there are no suitable, proven, "effect" biomarkers for isocyanates (an immune response is inconsistent, for example), but there may be on-going developments in this area.

This work was undertaken to update HSE on the current and future exposure issues in this field, to help ensure its ability to respond appropriately to any concerns involving new technologies and uses of isocyanates. It also sought to provide a robust evaluation of the most appropriate exposure measurement techniques, considering their compliance, specificity, and aspects of practicality.

1.2 Proposed Approach

There were three main objectives of the work:

- Evaluate alternative air monitoring methods that could still provide TRIG measurements but avoid restricted reagents and enable a more straightforward analysis.
- Develop methods for more specific exposure biomarkers.
- Evaluate example new isocyanate uses and use scenarios for their potential exposures.

For the air monitoring methods, a literature review was first conducted, and then promising candidate methods were identified and investigated in the laboratory as to their practicality. The methods were also tested alongside MDHS25/4 during site visits, where possible. Available quality assurance schemes were reviewed for their suitability for TRIG analysis.

Several research strands were developed for the biomonitoring methods including, exploring new, specific biomarkers, expanding the “corresponding amine” approach to other isocyanates, and investigating the use of immunoassay techniques to simplify analysis. A literature search on relevant effect biomarkers was also conducted.

Finally, a number of site visits were conducted to explore new isocyanate uses and exposure scenarios, and to evaluate the alternative methods explored in this project.

2. Air Monitoring

2.1 Background

In GB, the WEL for isocyanate-containing materials is distinct as it is not based on a single substance (except for methyl isocyanate), but rather on the chemical functional group -NCO. The WEL is set relatively low at 0.02 mg -NCO/m³ (8-hour time-weighted average (TWA) reference period; HSE, 2020), and hence, necessitates the use of a sensitive method to first stabilise the isocyanates, and then quantify -NCO to ng levels of recovered mass per sample. The method MDHS 25/4 (HSE, 2014a), has been established for many years and is recommended by HSE for determining compliance with the WEL. Whilst there are also several established International Organization for Standardization (ISO) methods for the determination of isocyanates, they often apply to individual, or small groups of isocyanate substances, and not the sum of -NCO in the work environment. The sum of -NCO is also referred to as 'total reactive isocyanate group' (TRIG). As it is apparent that isocyanate substances with substantially different chemical structures present a similar occupational asthma health hazard (HSE, 2001), the international community is becoming more interested in adopting methods capable of measuring TRIG. For example, the European Chemicals Agency has proposed "a NCO group (R-N=C=O) approach for all diisocyanates" (ECHA, 2020).

MDHS 25/4 is arguably the only true TRIG method. It, like each of the international standard methods, involves fixing the different isocyanate substances that may be present in the sample with an amine-based derivatising agent, then separating (using chromatography), and detecting the derivative formed. As several interfering substances can also be detected, it is necessary to confirm which of the detected substances are isocyanates. This is not usually a problem where the isocyanate species present are simple, and common diisocyanate monomers such as, hexamethylene diisocyanate (HDI), or toluene diisocyanate (TDI); with these, the chromatographic retention times are easily recognised. However, the current trend is for isocyanate products to be formulated with less volatile polymeric isocyanates, to minimise inhalation exposures. These newer isocyanates also often have more complex structures as their uses become more specialised. Although, some information may be available from the product safety data sheets and accompanying Chemical Abstracts Service (CAS) numbers, the specific isocyanate species contained are often not listed. This can result in omissions during analysis, resulting in significantly underestimated exposures.

MDHS 25/4 uses a combination of two different detectors in the analysis to confirm which of the separated substances are isocyanates; currently no other method can do this. There are methods available that use tandem mass spectrometry (MS/MS), which can be set up

to selectively detect specific substances, including isocyanates, but analytical standards need to be available. However, only the specified substances will be detected, and any isocyanate species not specified will not be detected. Again, this could result in exposures being significantly underestimated.

It is important to ensure that all relevant phases (aerosols and vapours) are sampled appropriately for the exposure scenario being monitored. Where aerosols are likely to be present, impingers should be used in conjunction with an impregnated filter as the filter alone is likely to significantly underestimate aerosol exposures.

In addition to the issues identified above it is apparent from dealings with HSE's occupational hygienists and external laboratories that some commercial laboratories are not implementing MDHS 25/4 fully and are only analysing monomers. This is likely to severely underestimate TRIG exposures in many scenarios. Currently, there is no external quality assurance scheme designed for MDHS 25/4 analysis. Therefore, this means that laboratories cannot readily demonstrate their competency in determining TRIG exposures using MDHS 25/4.

The primary issue with current air monitoring for isocyanates, is that MDHS 25/4 involves the use of 1-(2-methoxyethyl)piperazine (1,2-MP), a chemical that is restricted by the Home Office under The Misuse of Drugs Regulations 2001¹. This means that users require a licence, and this creates issues for users of MDHS 25/4, who must comply with the licensing rules. Within GB, the continued use of 1,2-MP within MDHS 25/4 is under a Group Authority Licence (GAL), held by the British Occupational Hygiene Society (BOHS). This allows qualified occupational hygienists (they must be a member of the professional faculty of BOHS) to receive sampling kits and return samples to laboratories for analysis, without the need to have an individual licence (which would be prohibitive on cost and administrative grounds²). However, discussions with BOHS suggest that the management of the GAL is onerous, and they may not commit to maintaining it long-term. The analytical laboratories must also be licensed (or exempt), to handle 1,2-MP.

The work strands in relation to air monitoring were:

1. Explore the restriction of 1,2-MP with the Home Office.
2. Investigate options for an MDHS 25/4-compliant quality assurance scheme.
3. Evaluate alternative practical air monitoring methods that still provide TRIG measurements, as required by the GB WEL (HSE, 2020).

¹ <https://www.legislation.gov.uk/uksi/2001/3998/regulation/2/made>

² <https://www.gov.uk/guidance/controlled-drugs-licence-fees>

2.2 Home Office Restriction

A letter was drafted to the Home Office to explain the impact of the current regulation on isocyanate measurement and, potentially, exposure control within GB. The letter was sent from Professor Andrew Curran (HSE's Chief Scientific Adviser) to Professor John Aston (Chief Scientific Advisor to the Home Office), in 2019. Despite a positive conversation between the two, there has been no further progress on this issue and the Home Office retains the restriction on 1,2-MP for all uses.

Separate discussions held between BOHS and the Home Office (also in 2019), confirmed that filters, and/or stabilising solutions containing 1,2-MP would need to be classed as an "exempted product" to be used without a licence³. One of the criteria for an exempted product is, "the controlled drug in any component part is packaged in such a form, or in combination with other active or inert substances in such a manner, that it cannot be recovered by readily applicable means". The formulations used do not conform to this requirement and cannot be readily adapted.

The conclusion is that if 1,2-MP is to continue to be used in a method for measuring isocyanates, then a licence or exemption will be required (within GB), for those handling such material, both during sampling, and analysis.

2.3 Options for an external Proficiency Testing scheme for MDHS 25/4

As previously mentioned, the WEL for isocyanates is based on the TRIG. Measurement is achieved by using MDHS 25/4, but it is apparent from dealings with HSE's occupational hygienists and external laboratories that some commercial laboratories are not implementing MDHS 25/4 fully, and are only analysing monomers, which is likely to severely underestimate total -NCO exposures [as required for measuring compliance with the GB WEL, (HSE, 2020)] in many 'in use' scenarios. One way of improving the performance of laboratories undertaking MDHS 25/4, is through a Proficiency Testing (PT) scheme.

HSE scientists previously ran a PT for MDHS 25/4, ceasing in 2010/11. It provided participating laboratories with test samples for analysis using MDHS 25/4, or the International Organization for Standardization (ISO) 16702:2007 (ISO, 2007). The test samples consisted of filters impregnated with the derivatising reagent, 1,2-MP and spiked with 1,2-MP derivatised isocyanates. The isocyanates used were monomers of common diisocyanates [methylene diphenyl diisocyanate (MDI), HDI and TDI], prepared from

³ <https://www.legislation.gov.uk/ukxi/2001/3998/regulation/2/made>

commercially available material of at least technical grade, and subsequently purified. On occasion, purified oligomers of MDI and HDI were prepared using commercially available formulations e.g., Desmodur N3300, and Bevedan. The attempt to use oligomers was not particularly successful and was therefore not pursued. To be of genuine value, any scheme would have to incorporate oligomers. This scheme was withdrawn when HSE collaborated with LGC Limited to set up the Air Stacks & Emissions Scheme (AIR PT)⁴.

An isocyanate PT scheme would be one way of improving the quality of TRIG analysis and is a necessity to satisfy accreditation bodies. An existing scheme, the Institut de recherche Robert-Sauvé en santé et en sécurité du travail⁵ (IRSST) ISO-CHEK scheme, does provide 1,2-MP derivatised isocyanates, and HSE have used this as a replacement for the previously run HSE scheme. It is, however, not designed around MDHS 25/4, since it does not provide the test samples as 1,2-MP impregnated filters, which would be preferable. Nonetheless, participation in this scheme would provide assurance that the 1,2-MP analysis, which is central to MDHS 25/4, is being conducted appropriately. Unfortunately, discussions with the scheme organisers in 2022, indicated that they no longer provide oligomer test samples, and the scheme does not appear to be publicly available.

Currently, there is an absence of any suitable PT schemes demonstrating the ability of laboratories to adequately undertake TRIG analysis using MDHS 25/4 (or other methods). Discussions with appropriate PT scheme providers (such as IRSST and LGC Limited), may be helpful in trying to reinstate a suitable scheme, particularly if other regulators (such as ECHA), also move to TRIG-based exposure limits.

2.4 Evaluation of alternative air monitoring methods

2.4.1 Literature Review

A literature review was conducted to assess any available methods for their viability against HSE's criteria of measuring TRIG, and not using a restricted derivatisation reagent, as well as an evaluation of the practicability of potentially suitable methods. This review has been published as a commentary (McConnachie and Johnson, 2023).

From the aforementioned review, two methods were considered as potentially viable alternatives to MDHS 25/4, and worthy of further investigation; the dibutylamine (DBA)

⁴ www.lgcstandards.com

⁵ www.irsst.qc.ca

method [ISO 17734-1:2013 (ISO, 2013)], and the novel diaminonaphthalene (DAN) method originally developed by NIOSH⁶.

- The DBA method uses a commercially available dry sampler claimed to have comparable performance to impinger/filter sampling.
- The DAN method, although not yet validated, has the potential to be a true TRIG method that gives only one analyte to determine. This, therefore, eliminates the necessity to fully characterise each isocyanate species in the products being measured, making it potentially far more practicable.

There were other methods identified that could have been considered, but they all involved piperazine compounds, which are also likely to be restricted in GB by the Home Office (due to the broad scope of the regulation) or could be restricted in the future.

Consequently, none of these methods were considered suitable alternatives to MDHS 25/4.

The first stage of method evaluations required the identification and purchase of the necessary reagents. Where reagents were not commercially available, or were of insufficient purity, it was necessary to prepare them in the laboratory. The second stage was to set up the DBA and DAN methods.

The DBA method was tested alongside MDHS 25/4 at the site visits conducted (see further detail in Section 4 of this report). Unfortunately, practical issues with the DAN method prevented it from being assessed alongside the DBA, and MDHS 25/4 methods.

2.4.2 DBA method (ISO 17734-1:2013)

The DBA method (ISO 17734-1:2013), has previously been studied by HSE scientists (Kenny, 2016). It uses readily available DBA (rather than a piperazine) to derivatise isocyanates. The sampling can be performed with a commercially available denuder-type sampler [ASSET EZ4-NCO Dry Sampler™ (Brown et al., 2012)], eliminating the need for an impinger. It is claimed that the results from using an ASSET EZ4-NCO sampler are comparable to those from an impinger/filter combination. Due to the absence of both volatile solvents and a glass impinger, it would also offer a safer method of carrying out personal sampling. This would be a particular advantage when sampling aerosols as these currently necessitate the use of an impinger. The results from earlier HSE work were encouraging in terms of sensitivity, selectivity and extraction efficiency. It was, however, concluded that the reliability of the results from extracting the ASSET EZ4-NCO sampler was variable depending on the isocyanate species. The DBA method may, therefore, not be a suitable alternative for the measurement of all isocyanate species. Hence, a more in-

⁶ www.cdc.gov/niosh/

depth comparison between the ASSET EZ4-NCO sampler and impinger methods with real workplace air samples was recommended.

The DBA method is not a TRIG method as it primarily uses MS/MS for the determination of individual isocyanate species rather than total –NCO, as is required for comparison with the GB WEL. The method does attempt to resolve this by using chemiluminescent nitrogen detection, a nitrogen-specific detector that produces a linear and equimolar response to nitrogen. However, this detector is not readily available and could be vulnerable to interferences.

To make the DBA method a true TRIG method would require all the isocyanate species present to be known and determined individually. The contribution of –NCO from each species could then be calculated and summed. One way to establish which isocyanate species are present is to characterise the bulk isocyanate products. This firstly requires the determination of the total –NCO content using a titration method such as BS EN 1242:2013 (BSI, 2013). Each isocyanate species in the bulk product is then qualitatively and quantitatively analysed, as far as possible, by their reaction with DBA and subsequent analysis by high performance liquid chromatography with MS/MS detection (HPLC-MS/MS). The products' safety data sheets can be used in the characterisation, but the specific isocyanate species present are often not listed.

If the sum of –NCO from the characterisation equals that determined by titration, a full characterisation has been successful. It is only really possible to characterise a particular species if a calibration standard is available. If the characterisation sum is lower than the titration, then not all the isocyanate species have been accounted for. While several DBA urea calibration standards are commercially available there are many that are not, and these will have to be prepared in cases where the sum of –NCO from characterisation is less than the titration result. It may be possible for the difference between the two values to be accounted for by applying a correction factor to the air sample results, but this has the potential to introduce errors.

In summary, using the DBA method would simplify sampling for isocyanates, in that it is harmonised through a commercial supplier, is intrinsically safer, and does not depend on the use of a restricted substance. However, the reality is that although, the sampling would be more straightforward, the subsequent analysis remains at least as challenging as for MDHS 25/4.

2.4.3 1,8-Diaminonaphthalene (DAN) method

Information about the DAN method was kindly provided (via personal communication) by Dr Dhimiter Bello (Associate Professor, Department of Public Health at the University of Massachusetts, Lowell, USA). Dr Bello provided a number of unpublished reports detailing the development of this novel method. He also participated in telephone conferences and a meeting to assist our study of this method. The method has been evaluated by IRSST

using MDI and good performance reported (Puscasu et al., 2017); however, a more recent paper by the same team (looking at on-site performance for TDI), reported problems (Aubin et al., 2020).

The derivatising agent used in this method, 1,8-diaminonaphthalene (DAN), undergoes a two-step reaction with isocyanates. Firstly, a reaction occurs between one of the amino groups with an isocyanate group to form a urea, and secondly, a ring closure with the urea reacting with the other amino group. The key advantage of the method is that the reaction sequence results in the formation of a single analyte - perimidone, regardless of the isocyanate species present (Figure 1). Determination of this analyte is used to determine the TRIG.

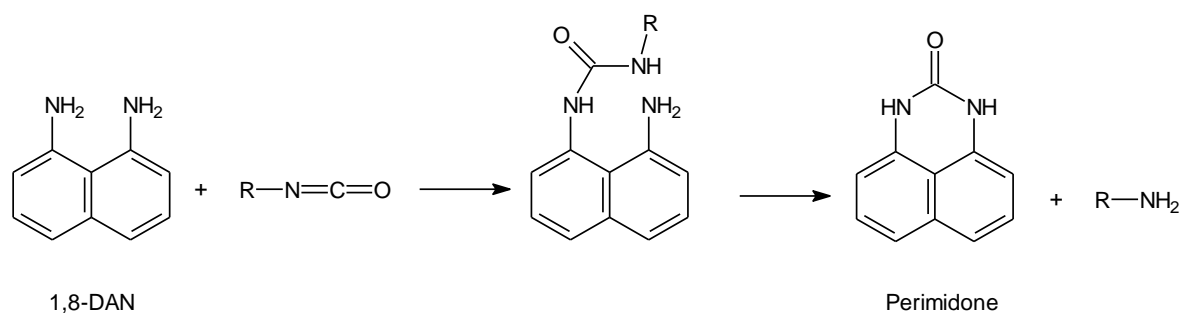


Figure 1 Reaction scheme of the DAN method giving rise to perimidone for analysis.

If this so described reaction could be shown to work equally for all isocyanate groups regardless of the isocyanate species (e.g., aromatic, aliphatic or large polymeric structures), then no lengthy characterisation, or chromatographic interpretations would be necessary.

One problem initially encountered with this method was that perimidone was not readily available commercially. It had to be either custom synthesised (often prohibitively expensive) or synthesised in the laboratory. Synthesis of perimidone was found to be difficult and time consuming with the apparatus available, and only small quantities with low yields were achievable. This, in turn, made synthesising a deuterated version for use as an internal standard unfeasible. Different methods of purifying perimidone were investigated (including, for example, vacuum sublimation and acid base extraction), but a convenient, efficient method was not achieved.

Another problem is that DAN is unstable. A 99% (by weight) pure sample purchased commercially became discoloured (indicating deterioration) within weeks, and therefore, had to be recrystallized each time before use. A small amount of pure white crystals of DAN could be stored under nitrogen at -22°C but became equally discoloured readily during use.

There were also practical problems with the impingers and treated filters used for the DAN method. The impinger solution uses dimethyl sulfoxide (DMSO) as the solvent, which has a freezing point of 18.5°C. Keeping materials cool during site visits meant that the solutions would freeze and needed to be thawed out on site. As with the pure DAN, the treated filters and solutions would discolour within days and quickly became unusable.

In 2022, a commercial source for perimidone was identified, although price and delivery times may still be an issue. This would aid with the adoption of this method although other issues, e.g., the stability of DAN, remain. A commercial source for DAN has also been identified making it potentially possible to receive small quantities (e.g., 5g), of “fresh” DAN as required.

The principle of the DAN method, i.e., only a single analyte to quantify, is of value. However, given that the method is not yet validated, and the practical issues encountered, it cannot provide a practical solution in the short term. However, further evaluation with the now-available commercial reagents may be worthwhile.

3. Biomarkers

3.1 Specific urine biomarkers

The current predominant biomonitoring method for diisocyanates (Cocker et al., 2017) is to measure the corresponding diamine in urine, e.g., hexamethylene diamine (HDA) for HDI. Whilst this method is relatively straightforward, it does not distinguish between diisocyanate exposure and the corresponding diamine exposure; some of which are formed during isocyanate-using processes (Jones et al., 2017). Amines are also commonly used industrial chemicals e.g., 4,4'-methylenedianiline (MDA, which is the corresponding amine for MDI), has uses as a hardener in epoxy resins and adhesives (ATSDR, 1998).

More specific biomarkers, based on haemoglobin or albumin adducts, have been proposed and reported in the literature (Gries and Leng, 2013; Sabbioni et al., 2016). These require a blood sample to be taken and are therefore, invasive and require specialist collection staff. However, as albumin is broken down and excreted in urine, the possibility of specific metabolites being excreted in urine was previously hypothesised; the work has been developed further in this project.

Work carried out previously (unpublished) at HSE into MDI lysine conjugates (MDI-Lys), yielded promising results with both MDI-Lys and its acetylated version, acMDI-Lys, present in urine samples from workers exposed to MDI.

In the work reported here, the MDI-Lys method was improved, and further samples were analysed (Nwoko et al., 2022). The work was also extended to look at whether the equivalent conjugates were present in the urine of persons exposed to HDI and TDI. Urine samples from workers were collected with informed consent either as part of routine surveillance (HSE, 1997), or under ethical approval from HSE's Research Ethics Panel, which operates under delegated authority from the University of Sheffield Medical School Research Ethics Committee (Reference: REACT_ERAC_171019, date 17/10/19).

3.1.1 MDI

A characterised (confirmed structure, analysed purity) standard of acetyl-MDI-lysine (molecular weight 412.5) was commissioned from HelloBio⁷. Calibration standards were prepared in 2 mL urine from a 2 µmol/L analyte solution. Samples were extracted using Agilent Bond-Elut Plexa PCX, 60mg, 3 mL solid phase extraction cartridges and analysed

⁷ www.hellobio.com

by HPLC-MS/MS. Separation was achieved on a Luna C18 150 x 2mm, 5 μ column (Phenomenex, UK) using a gradient mobile phase of methanol and 20 mmol/L ammonium acetate with 0.1% acetic acid. The mass spectrometer was operated in positive multiple reaction mode monitoring the transitions 413/241 and 413/106.

Samples from 55 MDI casting workers (all samples were from a single company), were analysed for acetyl-MDI-lysine and compared to results for urinary MDA, analysed by the standard gas chromatography-mass spectrometry (GC-MS) method (Cocker, 2017); a weak, but statistically significant ($p < 0.001$), positive correlation ($r^2 = 0.377$) was observed (Figure 2).

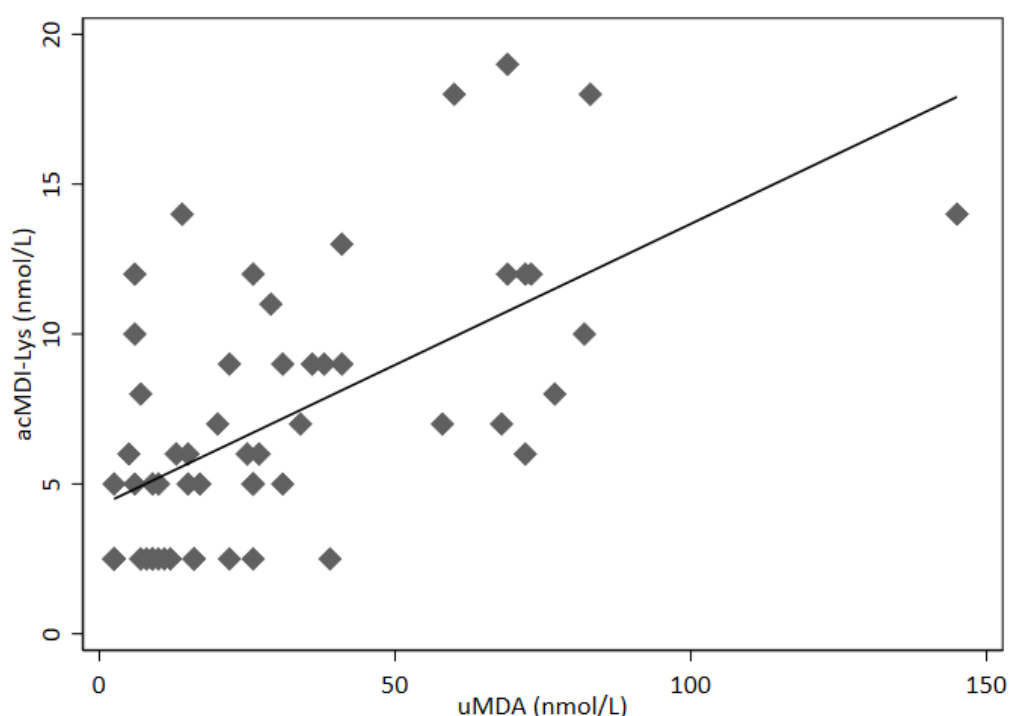


Figure 2 Linear regression correlation of urinary methylene dianiline (uMDA) and urinary acetyl-MDI-lysine (acMDI-Lys) (line: $y = 0.0941x + 4.2655$, $r^2 = 0.377$, $N=55$). Results below the limit of quantitation (LOQ) represented as half the LOQ (2.5 nmol/L).

The positive correlation and the lack of positive acMDI-Lys in control samples provided evidence that urinary acMDI-Lys may be a suitable and specific biomarker for assessing MDI exposure. However, more data are required looking at other exposure scenarios and defining a sampling strategy. Further details are available in the associated publication (Nwoko et al., 2022).

3.1.2 HDI

Standards of HDI-Lysine (HDI-Lys), and its acetylated version (acHDI-Lys) were previously commissioned from Sheffield Hallam University. Structure was confirmed by mass spectrometry and Nuclear Magnetic Resonance spectroscopy (NMR), but purity was not analytically determined (and likely was not sufficient for an analytical standard). Samples that were received by HSE Science and Research Centre for HDA analysis that showed positive results by GC-MS, were identified as potential samples to be investigated for HDI-Lys conjugates. The same extraction method applied for MDI-Lys work was applied to these samples.

Detection and quantitation were performed in negative Multiple Reaction Monitoring (MRM) mode. Mass transitions monitored for HDI-Lys were m/z 287/145 and for acHDI-Lys m/z 329/145.

An initial analysis of a small set of HDA-positive samples ($n = 5$), demonstrated no evidence of HDI-Lys conjugates, and further method validation was not carried out. There are reasons why HDI-Lys conjugates in urine may not have been found. One is that the method / instrument may not have been sensitive enough for HDI-Lys detection. For exposure to MDI, lower recovery of MDI-Lys in mice has been reported (Wisnewski et al., 2019), when exposed through inhalation, than when exposed via dermal exposure. The inhalation pathway required repeated exposures on successive days for levels to accumulate to detectable amounts. Therefore, it may be that HDI-Lys will not be present at significant amounts after acute exposures, and this is why no samples tested were detectable. Most HDI exposures are through spray paint where exposures greatly exceed the WEL, and the inhalation route is likely to dominate despite the use of respiratory protective equipment (RPE). It may also be because HDI-Lys or acHDI-Lys are not the main conjugates being eliminated in urine; for example, it has been reported for MDI (Wisnewski et al., 2019), that another conjugate (MDI-dilysine) is present in mice urine at higher concentrations than MDI-lysine.

3.1.3 TDI

TDI lysine (TDI-Lys) conjugates were also investigated. A custom synthesised standard of 2,6-TDI-Lys had been commissioned from Sheffield Hallam University; again, structure was confirmed, but purity was not analytically determined. The 2,4-TDI isomer and the acetylated versions were not synthesized. The same extraction and analysis methods were used as described for HDI-Lys above (section 3.1.2). Mass spectrometer parameters were optimised for TDI-Lys and the mass transition monitored in negative MRM was 293/145. A small number ($n = 4$) of samples positive for toluenediamine (TDA - a biomarker of TDI exposure) by GC-MS analysis following acid hydrolysis were taken through the lysine method.

Initial analysis of the TDA positive samples ($n = 4$), was promising and so further validation of the method was carried out. The LOQ was determined at 0.1 nmol/L (assuming the standard to be 100% pure) and the method intra- and inter-assay coefficients of variation (CVs) were 4.3% ($n = 10$), and 10.0% ($n = 30$, 3 runs) respectively. The four samples previously analysed had quantifiable 2,6-TDI-Lys present (see Figure 3b for an example) and these correlated strongly ($r^2=0.9997$) when compared with 2,6-TDA results by GC-MS analysis.

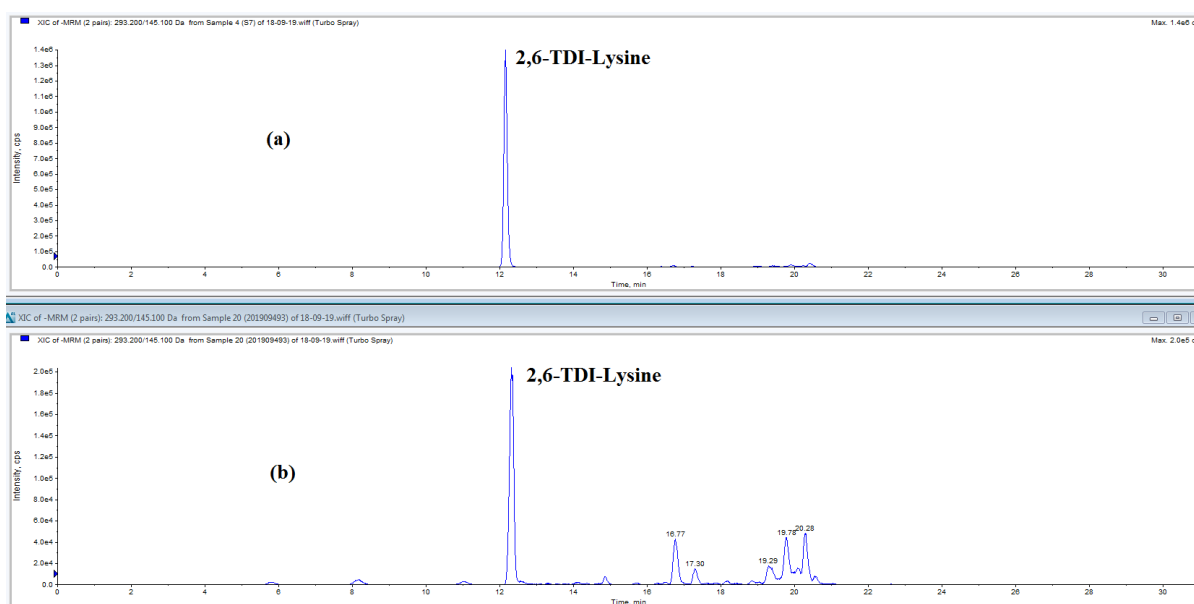


Figure 3 Chromatogram of 2,6-TDI-Lysine in (a) spiked urine sample, nominally 100 nmol/L and (b) positive TDA urine sample (TDI-Lys = 17.4 nmol/L (nominally), TDA by GC-MS was 85 nmol/L).

This is a very small dataset, and more samples are needed to draw final conclusions as to whether these conjugates may be viable for exposure assessment. In our experience, positive TDA samples are rare [although we have observed elevated results in foam blowing scenarios (Jones et al., 2017)], and most commercial analysis at HSE is for HDI or MDI exposures. To progress the work further, a new custom synthesis of the standard(s) would be required to authenticate purity and because of long-term stability concerns.

3.2 New isocyanates - tris(p-isocyanatophenyl) thiophosphate (TIPTP)

In addition to the well-known and widely used isocyanates [MDI, TDI, HDI, and to a lesser extent isophorone diisocyanate (IPDI) and naphthalene diisocyanate (NDI)], there are

newer isocyanates being introduced to the market. Some of these are 'blocked' isocyanates where the –NCO group is blocked by another chemical (e.g., 2-butanone oxime, MEKO), until released by heating. Others are larger and less volatile isocyanates such as tris(p-isocyanatophenyl) thiophosphate (TIPTP, molecular weight 465). Although still classified as respiratory sensitisers, inhalation exposures are expected to be lower due to their higher boiling points (e.g., 589.3°C for TIPTP⁸). It should be noted however, that skin contact with isocyanates can also elicit respiratory sensitisation (Bello et al, 2007).

TIPTP was being used on one of the sites visited as part of this project and therefore, some preliminary method development was undertaken.

There is no reported biological monitoring method for TIPTP isocyanate. It was theorised that metabolism could be similar to diisocyanates and the equivalent amine (in this instance, a triamine as opposed to a diamine), would be a useful biomarker to target. If the isocyanate was fully hydrolysed to the triamine this would be tris(aminophenyl) thiophosphate (TAPTP, CAS: 52664-35-4). This was available to purchase as an industrial chemical in large quantities, but there was no analytical reagent available. Attempts were made to hydrolyse the bulk product, Desmodur RFE, containing TIPTP (provided by the work site visited, see section 4.2) to produce a usable standard for analysis by HPLC-MS/MS.

Out of three possible amine derivatives (one to three -NCO groups converted to amine) only one mass was evident in positive ionisation mode. A peak at m/z 388 was observed, most likely from all three of the isocyanate groups hydrolysing to the amine, to produce a molecule with a mass of 387 a.m.u [M+H]. Fragmentation of this molecular mass (388) indicated that the triamine may have been formed, although not all fragments could be explained. This may be due in part to the technical mix used which may have contained interfering constituents. The transition of 388/108 had the largest response and was used for quantification.

Samples were extracted using the standard diamine preparation (Cocker et al., 2017). After the diethyl ether extraction, the solvent was evaporated, and the resulting residue was suspended in 2 mL of acetonitrile. A basic chromatography method was set up using a Zorbax SB-Aq 2.1 x 50 mm column (Agilent, UK), and mobile phases of water and acetonitrile. A simple gradient was employed with a run time of 13 minutes.

A total of 64 urine samples were collected from 15 workers from a single site, with each worker providing a pre- and post-shift spot urine sample; some workers provided samples more than once. The method described above was used to analyse the samples for the presence of the triamine. The method developed was used as a screen and only one aliquot of each urine sample was analysed. Most samples (78%) had no detectable trace of the ion transitions monitored. Two samples had prominent peaks for the 388/108

⁸ https://www.chemsrc.com/en/cas/4151-51-3_509529.html

transition ($\geq 3:1$ signal to noise), supported by several qualifier ions, which eluted at the same retention time as blank urine spiked with the standard solution (Figure 4). Both samples were from the same individual who worked in a decanting area.

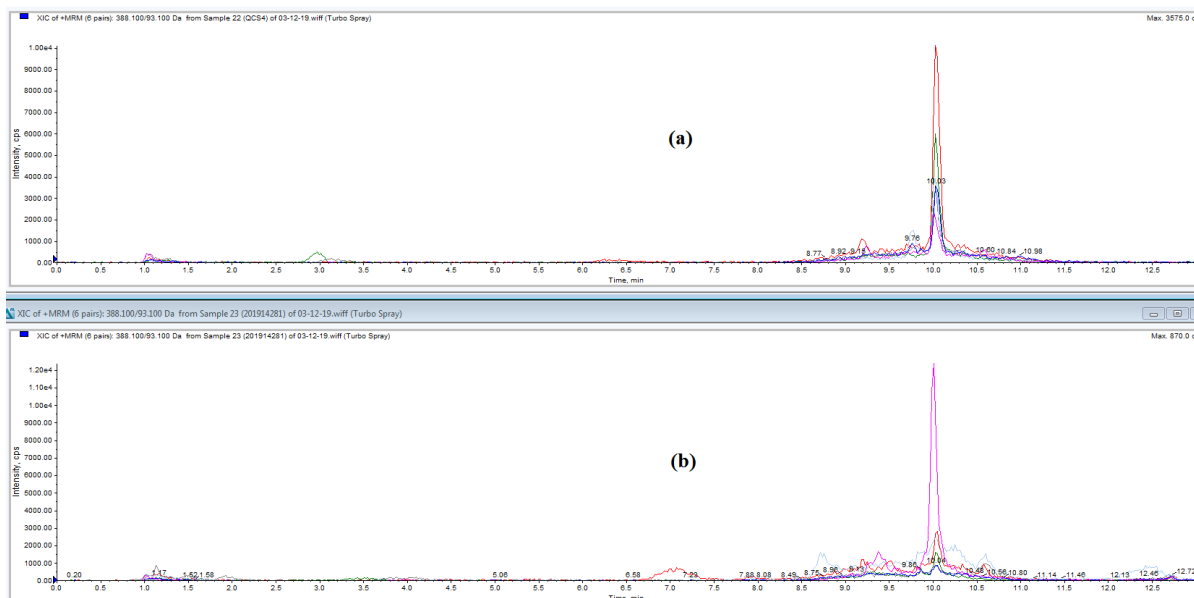


Figure 4 Chromatogram of the presumed triamine of TIPTP in (a) spiked urine sample, (b) urine from a potentially exposed worker.

Peaks observed in two urine samples from the worker in the decanting area suggested exposure to TIPTP, especially when considering that the personal air sample for this worker had significant levels of TIPTP (NCO 6665 ng, $>15 \mu\text{g}/\text{m}^3$, 8-hour time-weighted average (8h TWA)).

At a later date, it was possible to purchase a standard of TAPTP from Alfa Chemistry. The method above was confirmed with this standard although samples were resuspended in 10% acetonitrile (the starting gradient), prior to injection. Deuterated-MDA was added as an internal standard. A small batch of quality control material was prepared (46 samples analysed across four runs); the coefficient of variation was high ($>30\%$) indicating that deuterated-MDA may not be the most appropriate internal standard (structurally not similar to TIPTP). The two positive samples from the decanting worker were reanalysed; one of these showed measurable levels of TAPTP. Further samples from another company using very small amounts of product ($\sim 50 \text{ mL}$ at a time), containing TIPTP were all determined as 'none detected' for TAPTP.

3.3 Immunoassay screening methods

As well as developing new biomarkers, the possibility of a screening assay for the current diamine metabolites was also considered. About 90% of all samples (>4000 per year), analysed by HSE scientists for diisocyanate metabolites are 'none detected'. The GC-MS analytical method (Cocker et al, 2017), is labour intensive and time consuming. A screening method could potentially focus quantitative analysis on those samples only where there is evidence of exposure, cutting costs and improving availability and turnaround time.

Antisera-based assays offer the possibility of measuring metabolites of various isocyanates in accessible bodily fluids. We employed two main strategies to investigate this:

1. Development of a screening competitive immunoassay, capable of detecting small metabolites of specific diisocyanates, at relevant levels in urine samples from workers. Importantly, the focus was on measuring one of the major metabolites of an isocyanate.
2. Development of an immunoassay capable of detecting when reactive diisocyanate has conjugated to human serum albumin (HSA), so producing a modified HSA. Several identified papers confirm that diisocyanates react with HSA, the most abundant extracellular protein. There are a number of lysine residues where this most likely occurs. Although largely retained in circulating blood, a fraction of any normal and modified albumin will be excreted into urine.

3.3.1 Urine metabolite screening

All antisera were commercially raised using either Harlan Laboratory UK Ltd (Loughborough, UK) or Pettingill Technology Ltd (Oxford, UK). Polyclonal antisera in rabbits were raised to carrier proteins modified with:

- (a) HDI
- (b) MDI and
- (c) N-Ac-MDI-Lysine.

Antisera were purified by protein A columns to give immunoglobulin fractions. In practice, the antisera (1993, 1994, 2094, 2095, see Table 1), were designed to recognise the free diamine metabolites (HDA and MDA).

HSE research has shown that some metabolites of MDI in urine are lysine conjugates, particularly the N-acetylated form, i.e. N-Ac-MDI-lysine [also referred to as acMDI-Lys, (Nwoko et al., 2022)]. Antisera HS0010 & HS0011, were therefore raised against N-Ac-

MDI-Lysine (Table 1), with the conjugation strategy of coupling to the carrier protein through the carboxy group of lysine, and the multiple amino groups of cationised bovine serum albumin (BSA).

Table 1. Characterisation of the eight polyclonal antisera raised in rabbits.

Hapten	Carrier protein	Identifier	Notes
MDA	succBSA ^a	1993 & 1994	Hapten & carrier chemically conjugated at 20:1 molar ratio
HDA	succBSA	2094 & 2095	Hapten & carrier chemically conjugated at 20:1 molar ratio
HDI	cBSA ^b	3845 & 3846	Hapten & carrier allowed to react at a 20:1 molar ratio
N-Ac-MDI-Lys	cBSA	HS0010 & HS0011	Hapten & carrier chemically conjugated at 20:1 molar ratio

^a succinate-BSA conjugate, ^b cationised-BSA conjugate

All antisera (see Table 1) were tested in competitive immunoassays to measure the respective low molecular weight metabolites of MDI and HDI. These assays need to be able to detect down to the low nmol/L levels, based on the current GC-MS method (Cocker et al., 2017). Such a detection limit in urine (5 nmol/L) is challenging for a competitive immunoassay, as a dilution of the urine of at least ten-fold is necessary to eliminate the adverse effects of urine on antisera reactions. Currently the GC-MS method involves an acid hydrolysis step (0.2 mL conc. sulphuric acid added to 2 mL urine, boiling and then neutralisation with alkali before analysis). This suggests an even more aggressive sample matrix in which the immunoassay has to function is needed. All the concentrations discussed here (section 3.3), are in buffer systems, not in urine, which will to an extent degrade the assay detection limits.

The titres of the raised antisera against the hapten moiety, were tested by the standard method of exploring the binding of serial dilutions of the antisera against a microtitre plate coated with the respective haptens coupled to an irrelevant protein - in this case Beta-lactoglobulin (BLG). Detection used anti-rabbit HRP (horseradish peroxidase), and TMB (3,3',5,5'-tetramethylbenzidine) colour development. All the antisera showed titres (defined as the dilution giving 50% absorbance of maximal binding), between 1/12K and 1/50K dilutions. This suggests that an immune response was raised in the rabbits and all the antisera may have the potential to be useful within an immunoassay.

In the competitive immunoassay scheme, the analyte competes with a limited amount of primary antisera for an appropriate hapten coated on the wells of a microtitre plate. After incubation and washing, the quantity of antisera bound to the plate is detected by anti-rabbit antisera labelled with HRP, that develops colour with the substrate TMB. In essence, greater concentration of analyte leads to less colour development. Lower detection limits can be achieved by two approaches (which are not mutually exclusive).

(a) reducing the amount of hapten coated on the microtitre plates, and increasing the dilution of the primary antisera, while keeping an appropriate level of developed signal by amplification; and

(b) by coating the plate with a heterologous⁹ antigen with an addition of the antigen of interest, causes less binding (and therefore, a stronger signal) to the coating antigen, due to the competition.

Figure 5 shows the initial, non-optimised ELISA (enzyme-linked immunosorbent assay) using HS0011 raised against NAc-MDI-LYS-cBSA. NAc-MDI-LYS-BLG was used as the microtitre plate coating antigen to eliminate antisera binding to the carrier protein. The antisera appear to react equally to NAc-MDI-LYS, and the unacetylated MDI-LYS, without any large reaction to NAc-MDA, or free MDA. A similar result was found utilising antisera HS0010.

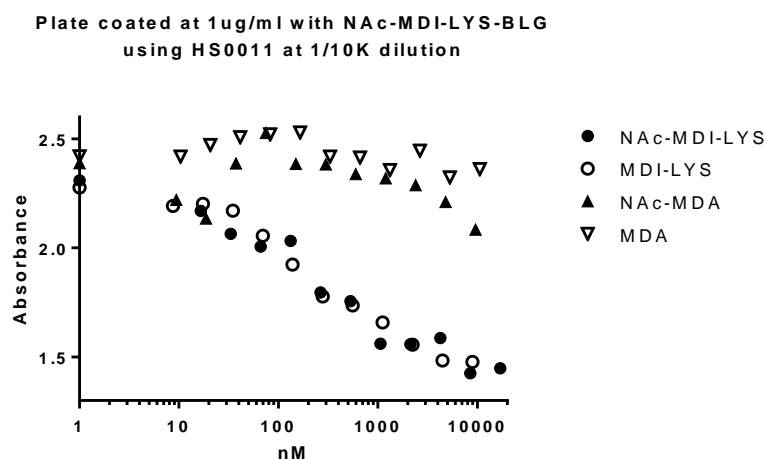


Figure 5 Results of a competitive immunoassay employing NAc-MDI-LYS-BLG as a coating antigen, and HS0011 as antisera.

⁹ structurally a little different to the antigen used to raise the antisera, and therefore, the primary antisera also respond to this antigen but more weakly

These results suggest that immunoassays employing HS0010 or HS0011 could have potential as screening tools for MDI-LYS conjugates found in urine, if adequate sensitivity could be achieved. A synthesised polyvalent HRP system (Poly HRP 80; SDT, Germany) was used at 1/5K to amplify the colour developed in the reaction. Refining the methods suggested that HS0011 antisera had modest advantages over HS0010, so further work focussed on HS0011.

Further work was undertaken using the principle of a heterologous antigen as a coating antigen on the wells. Where the heterologous coating antigen was employed, greater displacement by the antigen of interest (NAc-MDI-LYS) occurred, indicating greater sensitivity. Refinement of this format suggested a detection limit of around 1 nmol/L using buffer systems (see Figure 6), interestingly both NAc-MDI-LYS and MDI-LYS gave identical responses over the range 1-170 nmol/L.

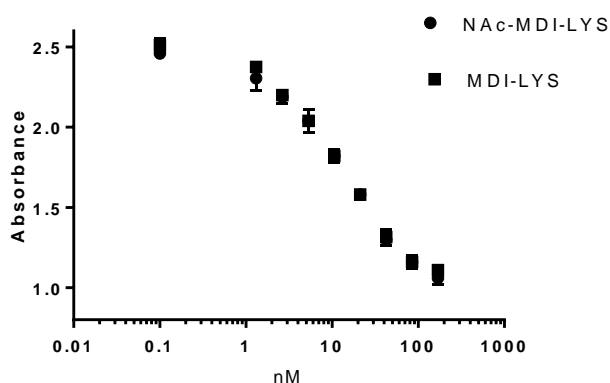


Figure 6. Competitive immunoassay for NAc-MDI-LYS & MDI-LYS, using NAc-MDA-BLG as a coating antigen and polymeric HRP signal amplification.

Similar work was done measuring HDA and MDA using antisera 1993, 1994, 2094, 2095 3845, and 3846 in indirect competitive immunoassays. A summary table of the experimental work is shown below (Table 2). The detection limits were achieved by using the optimisation approach (a) identified earlier. It may still be possible to gain increased sensitivity by employing a heterologous coating antigen [optimisation approach (b) identified earlier].

Given the unremarkable structure of HDA, it was surprising that a useful antisera titre and a competitive immunoassay were achieved using the antisera 2094 and 2095. Interestingly pentanediamine (PDA), with only five methylene groups, shows a significant decrease in sensitivity. It may be that using a PDA-irrelevant protein as the coating antigen may be able to gain significant increases in sensitivity towards HDA.

Table 2. Summary of detection limits and comments on competitive immunoassays for MDA and HDA using various antisera.

Antisera	Target	Detection limits in assay buffer	Antisera Titre [#]	Comments
1993	MDA	0.5 nmol/L (MDA) 25 nmol/L (NAc-MDA) >500 nmol/L (HDA) 200 nmol/L (diphenyldiamine)	1/25K	This looks most promising to develop a screening assay for “total” MDA, after neutralisation of the acid hydrolysis of urine samples.
1994	MDA	5 nmol/L (MDA) 100 nmol/L (NAc-MDA) >500 nmol/L (HDA) 250 nmol/L (diphenyldiamine)	1/30K	About 10x less sensitive than 1993 antisera.
2094	HDA	4 nmol/L (HDA) >500 nmol/L (MDA) 130 nmol/L (pentanediamine)	1/12K	Potentially useful although further sensitivity gains are needed.
2095	HDA	5 nmol/L (HDA) >500 nmol/L (MDA) >500 nmol/L (diphenylamine) 180 nmol/L (pentanediamine)	1/15K	Potentially useful although further sensitivity gains are needed.
3845	HDA	10 nmol/L (HDA)	1/45K	Less sensitive than 2094 or 2095 antisera
3846	HDA	15 nmol/L (HDA)	1/50K	Less sensitive than 2094 or 2095 antisera

[#] antisera dilution giving 50% maximum binding using antigen coupled to “irrelevant” protein.

The GC-MS procedure using acid hydrolysis to convert urine metabolites to total MDA or HDA would be an aggressive matrix for an immunoassay, so it is likely that a 1/20K -1/50K dilution would be necessary. Based on antisera 1993, it is likely that a limit of detection of only 10-25 nmol/L in urine would be achievable without further improvements to the

sensitivity of the immunoassay (compared to a target of 5 nmol/L, as per the GC-MS method).

A suggested next step would be to investigate how acid hydrolysed and neutralised urine would affect these immunoassay constructs. This could be achieved by running hydrolysed/neutralised urine from a non-exposed subject as a diluent for standards.

3.3.2 Modified HSA

Both in-vivo and ex-vivo published experiments (Sabbioni et al, 2016, Wisnewski et al, 2019), have shown that isocyanates as reactive molecules, form adducts with albumin and, in particular, available lysine (LYS) groups in albumin. Albumin is the most abundant extracellular protein circulating in blood and an available source of amino groups for isocyanates to couple to. Thus, the detection of albumin-isocyanate conjugates may be an alternative approach to measuring isocyanate exposure and uptake. While the half-life of human serum albumin (HSA) is about 21 days¹⁰, approximately 0.1% of all circulating serum albumin is continuously excreted via the kidneys into the urine. Thus, the detection of isocyanate conjugated to albumin in urine is a potential non-invasive, but specific means of monitoring exposure over extended periods.

It is likely that the extent of modified albumin will be very small in relative terms to the amount of unmodified albumin, so very sensitive and specific means of detecting modified albumin in urine are necessary. Two analytical approaches were tested: a non-competitive sandwich ELISA assay, and an automated Western blot system.

3.3.2.1 Non-competitive sandwich ELISA assay

Using a non-competitive, sandwich immunoassay¹¹ (as shown in the Figure 7), we have shown that one of our antisera to HSA can detect in a sandwich immunoassay at least 10⁶-fold lower than the level of HSA in urine (2-4 mg/L, usually or 30-65 nmol/L). A conjugate between HSA and MDI was formed with a molar ratio of 2-5:1 MDI:HSA. This was to be used as a surrogate standard and standardised in terms of protein concentration by BCA (bicinchoninic acid) reagent. Antisera 1993, 1994, HS0010 and HS0011 were used, together with several antibodies to HSA, to investigate general sandwich immunoassay conditions to give the best sensitivity for MDI-HSA. MDI-HSA is presumed to be formed during exposure to MDI and excreted in urine and was therefore the target analyte here.

¹⁰ https://en.wikipedia.org/wiki/Human_serum_albumin

¹¹ where one antisera detects the isocyanate moiety, and the other antisera detects HSA

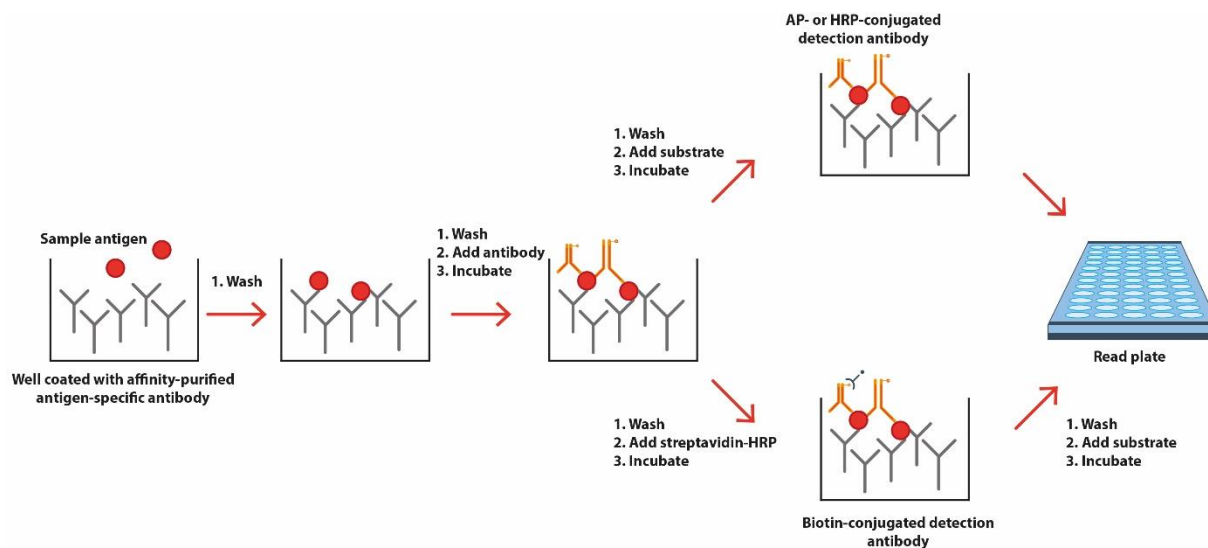


Figure 7. Scheme for non-competitive immunoassays.

The level of MDI-HSA in a urine sample from an exposed worker would be much lower than the level of unmodified HSA. It was therefore assumed that using isocyanate antisera as the initial capture antibody would be the most sensitive approach, because the excess unmodified HSA would be removed after the initial binding step. Microtitre plates were coated at 2 ug/mL of 1993, 1994, HS0010 and HS0011, using standard procedures. Serial dilutions of the MDI-HSA conjugate were used as standards, followed by a two-hour incubation at room temperature. The microtitre plate was then washed and dilutions of anti-HSA were added for a further one-hour incubation. After washing, a 1/5K dilution of anti-rabbit polyclonal antisera labelled with peroxidase was added and incubated for 30 minutes. After plate washing, slow TMB was added to develop a colour reaction. Initial experiments suggested that using a polyclonal anti-HSA gave very significant background absorbance, probably due to some cross-reactivity with the cBSA and succBSA that were used as carrier proteins to raise the antisera. A monoclonal anti-HSA together with anti-mouse HRP labelled antisera, gave significantly reduced background absorbance, and an anti-rabbit antisera labelled with polymeric peroxidase (SDT Germany) was employed to give enhanced sensitivity. The results are shown in Figure 8.

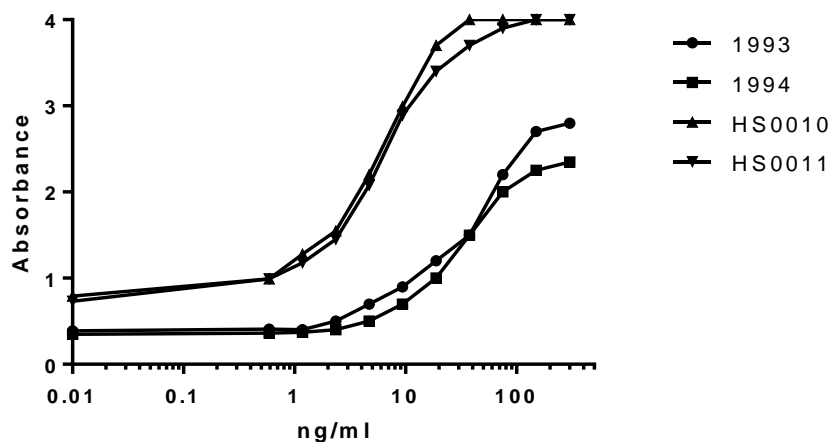


Figure 8. Non-competitive, sandwich assay for MDI-HSA construct in standard assay buffer showing the differences between the use of four different antisera for microtitre plate coating.

Using coating antisera HS0010 and HS0011, gave the best sensitivity (0.8-0.9 ng/mL), albeit with a high blank absorbance that will in practice, impact on the detection limit of the method. Unfortunately, the immunogen had been prepared using cBSA for HS0010 and HS0011, before an assay was conceptualised for an albumin-conjugate. An alternative carrier protein, such as BLG would have given greater sensitivity (even though cBSA is structurally very different to HSA). Assays based on coating antisera 1993 and 1994, gave less sensitive assays (3-8 ng/mL), with less signal generated per unit of standard. This is probably because the chemical structure with the MDI-LYS construct better reflects what happens when this isocyanate reacts with HSA.

Currently it is unclear whether this proposed conjugate exists in the urine of exposed workers. Dialysis and concentration of urine from exposed workers could be employed in future work to investigate this using the assay based on HS0010 or HS0011 coating antisera.

3.3.2.2 Automated Western blot system

An alternative analytical approach is using an automated Western blot system. Detection of a very low level of modified MDI-HSA (through a shift in the molecular weight), was shown not to be practicable due to the low molecular weight difference of adducted MDI. Also, the protein detection system would not have the necessary sensitivity. Therefore, effort focussed on an approach whereby the proteins, including MDI modified albumin, in samples applied to a capillary are separated, and antisera against the isocyanate moiety can then be automatically applied to the capillary with any binding detected by a sensitive

chemiluminescent reagent. Such a strategy only needs antisera relevant to the isocyanate in question, and the previous work suggested that HS0010 and HS0011 are the most appropriate antisera.

Initial experiments were conducted using either HS0010 or HS0011 (at 1/1k dilution), with secondary anti-rabbit-HRP (1/5K), as detection antisera, then applying (as sample) the MDI-HSA conjugate “standard”. These showed detection limits of 1-2 ng/mL, which is 1/1000 -1/2000 lower than the usual level of HSA found in urine. Signals were then explored from standard curves of MDI-HSA with relevant concentrations of unmodified HSA to replicate possible urine samples. The detection limits for the MDI-HSA conjugate degraded to around 30-40 ng/mL; this was due to a much high background signal, presumably from the added HSA. Further work would be needed to reduce this background signal for this to be a viable approach.

3.4 Effect biomarkers

A brief literature review was undertaken to evaluate progress on the development of biomarkers of effect caused by isocyanate exposure that may be useful to deploy. Such effect biomarkers would be based on the mechanism(s) for how isocyanates can ultimately cause (immunologic) occupational asthma (OA).

Among those high and low molecular weight agents associated with causing OA, isocyanates contribute a significant proportion of cases of this respiratory disease (Maestrelli et al., 2020). Most OA involves a type 1 hypersensitivity mechanism - an immediate reaction that involves immunoglobulin E (IgE) mediated release of antibodies against the antigen. However, for isocyanates, specific IgE is only sporadically detected in sensitised workers (Tarlo and Lemiere, 2014). This suggests that in workers sensitised to isocyanates, the disease mechanism is independent of an IgE-mediated pathway. To prove this, a mouse model of MDI-mediated asthma was used to identify the biological pathways which may contribute to the development of asthma (Wisnewski et al., 2020). Mice deficient of IgE were exposed to MDI before eosinophils from their airway fluid were quantified. Gene expression in lung tissue was also analysed. Based on the changes seen in eosinophils and gene expression, the model showed IgE-independent asthma-like pathology following MDI exposure. Maestrelli et al. (2020), highlights that for many of the low molecular weight causes of OA (e.g., diisocyanates, acid anhydrides, reactive dyes, platinum salts), the mechanisms involved in respiratory sensitisation are not yet well characterised.

Wisnewski et al. (2022), carried out investigations during the post-mortem of a worker whose death was thought to have been caused by OA. This was done to determine whether exposure to MDI contributed to this workplace fatality. Sections of the worker's

lung tissue were assessed to identify if serum MDI-specific IgE, and IgG were present. Based on several physical factors it was determined that asthma was the cause of death and that there were significantly elevated levels of MDI-specific IgE and IgG in the lung tissue. As a result, OA induced by MDI exposure was determined as the cause of death. This indicates that diisocyanate-induced OA may not be totally independent of an IgE-mediated response.

The literature suggests that both, largely non-characterised, and IgE-mediated mechanisms may be involved in the underlying respiratory sensitisation, and the development of OA arising from exposure to isocyanates.

A large retrospective cohort study of subjects with OA, mostly induced by isocyanates or flour, and documented by specific inhalation challenge, has been reported (Vandenplas et al., 2019). This work suggested different phenotypes in terms of clinical, functional and inflammatory characteristics, between high and low molecular weight sensitisers.

Suojalehto et al. (2021), looked at the molecular mechanisms associated with occupational asthma induced by flour, isocyanates and welding (type of welding not stated). Nasal biopsy and blood sampling was used and sets of biomarkers were identified which were unique to each exposure type. The work showed that isocyanate-induced asthma was not IgE-associated. For each of the occupational agents assessed, five differentially expressed genes were selected to accurately identify the exposure endotype (downregulation of TST, TMEM55A, SPHAR, and DNAJA1 together with upregulation of ANO6 for isocyanate-induced asthma). This could provide a basis for the development of diagnostic biomarkers in the future, although the data appeared to be more distinct for flour and welding than for isocyanates, where some overlap with control samples was still observed.

Cytokines have been investigated for some time as to their viability as a biomarker of effect for occupational asthma. Several cytokines are associated with the inflammatory response of asthma including interleukins (i.e., IL-4, IL-5, IL-13 and IL-6). IL-4 has been studied most widely as it is involved in the development of allergy inflammation (Steinke and Borish, 2001). Cytokines are most often measured in plasma samples, which is invasive. Other options that have been studied are exhaled breath condensate (EBC) and urine. In the study by Chi et al., (2016), EBC was collected from 23 asthma patients that were treated with Budesonide (an anti-inflammatory drug) for 12 weeks. IL-4 was analysed before treatment, and at the 8- and 12-week stages; the mean concentration decreased gradually throughout the study which correlated with positive lung function tests. IL-6 was also analysed but did not show the same effect. It may be that observing an increase in IL-4 in EBC could be an early effect biomarker indicating the possible development of asthmatic symptoms. This would most likely require establishing a baseline for healthy individuals, which may differ greatly from person to person. It has been reported that assays for cytokines are lacking in their reliability and validation (Blindow et al., 2015).

Urine analysis for cytokines would be a less invasive approach than taking plasma samples, and more practical than EBC sampling. A study in 2015 (Nobles et al., 2015), studied the relationship between many interleukin cytokines (including IL-4) in plasma and urine in a healthy population. No relationship was seen between the two matrices, and it was concluded that urine would not be a good alternative to plasma.

Cytokines have been investigated as predictors of many sources of inflammation from bladder cancer (Margel et al., 2011), to strenuous exercise (Sugama et al., 2013). It is likely that there are too many confounders to use cytokines independently as effect biomarkers for occupational asthma.

IL-13 is thought to induce the expression of the extracellular matrix protein, periostin, by the epithelial cells of the bronchi (Kraft, 2011). Periostin can contribute to subepithelial fibrosis with other extracellular matrix proteins (Takayama et al., 2006). Periostin has been implicated in the development of asthma and other allergic diseases (Matsumoto, 2014).

A study was conducted by Lee et al. (2018), to determine the role of periostin in the mechanisms of airway inflammation and remodelling in TDI-induced occupational asthma. The serum periostin levels were compared between TDI-induced occupational asthma subjects, asymptomatic TDI exposure controls, non-occupational asthmatics, and normal general population controls. The mean serum level of periostin (127.6 ± 79.5 ng/mL), in TDI occupationally exposed subjects with asthma was higher than in occupationally exposed subjects without asthma (100.9 ± 33.5 ng/mL, $p=0.001$), non-occupationally exposed subjects with asthma (75.3 ± 34.6 ng/mL, $p<0.001$), and the general population control (58.7 ± 29.8 ng/mL, $p<0.001$). These results indicated that increased levels of periostin may contribute to the progression of occupational asthma in people exposed to TDI. The authors concluded that periostin could potentially be used as a serological marker for representing phenotypes of TDI-induced occupational asthma. Periostin has not however, been implicated in occupational asthma induced by exposure to other isocyanates (i.e., HDI, IPDI and MDI).

Measurement of fractional exhaled nitric oxide (FeNO) is a non-invasive technique to evaluate airway inflammation and may be useful in the diagnosis of occupational asthma. Concentration levels of FeNO, measured as parts per billion (ppb) in breath increase with airway inflammation; however, FeNO will respond to many causes of airway inflammation and there are many confounding factors, such as smoking, diet, exercise, air pollution, gender, height and age (Bjermer et al., 2014) among others. This means that a single set of FeNO measurements may be hard to interpret in terms of the occupational context. It has been observed however, in a study by Ferrazzoni et al. (2009), that levels of FeNO increased after specific inhalation challenges with isocyanates at 24-hour ($p < 0.05$), and 48-hour ($p < 0.005$) intervals, for persons identified with having isocyanate-induced asthma. The same correlations were not seen in persons with rhinitis induced by isocyanate exposure ($n = 3$), or in 24 non-sensitized controls. A rise in FeNO levels at 24

hours also correlated with sputum eosinophil changes ($p = 0.001$). This work was contradicted by another study amongst bakery and hairdressing workers (Florentin et al., 2014), where it was concluded that FeNO measurements alone cannot be considered a useful screening tool for the detection of occupational asthma. It is possible that different causal agents may affect FeNO levels differently; the Ferrazzoni paper (Ferrazzoni et al., 2009), specifically investigated isocyanate exposures.

The delayed response of FeNO to allergenic exposures (noted by Bjermer et al., 2014), and other contributing factors means that establishing an individual baseline may be necessary, and that serial measurements may provide an overall better diagnostic tool. By taking multiple FeNO readings over a period of weeks it may be possible to measure an increase in FeNO levels in workers suffering from OA on their return, after a period away from work. A case study of a baker, observed an increase in daily FeNO levels from their time off work (~10 ppm), to their time at work (~75 ppm max), over a 1-month period (Merget et al., 2015).

A study by Klusáčková et al. (2022), which assessed the health effects of exposure to diisocyanates on workers in a car factory, used FeNO to monitor any subsequent airway inflammation. FeNO levels were measured for 26 exposed workers and 9 control (office) workers over two consecutive days. No significant difference was found between the two groups; but despite this, five exposed workers did have elevated FeNO levels (>50 ppb). These same workers had previously reported health problems in the workplace. Based on this information, there may be some value for the use of FeNO as an effect biomarker in diisocyanate related occupational asthma.

Although, being easy to sample (as a non-invasive quick breath test), the value of the FeNO test in detecting short-term exposure impacts during a work-shift, or a workweek is unknown, as to date it has primarily been used as a treatment efficacy evaluation tool. FeNO measurements may prove useful in the diagnosis of occupational asthma, and aid in treatment protocols; indeed, the American Thoracic Society has issued a clinical practice guideline (Dweik et al., 2011), but even here, further work is needed to define its use. Despite the use of FeNO in occupational medicine, there is nonetheless, no accepted value for its use to diagnose occupational asthma. After occupational exposures there is no consensus on an accepted magnitude of change in levels of FeNO (Oŕtelea et al., 2022). There is, however, general agreement on the optimal time to measure FeNO - i.e., 24 hours after an exposure. HSE has participated in a European project (HBM4EU¹²), where FeNO was measured as part of a suite of biomarker analytes in workers exposed to diisocyanates (Jones et al., 2022); results are due to be published in 2023.

¹² <https://www.hbm4eu.eu/>

4. Site Visits

Three companies, using different processes, were approached to participate in the study:

- An aerospace company using isocyanates in several processes,
- A foundry casting process and
- A college with multiple 3D printers in a workshop.

4.1 Sampling Methods

Where possible, sampling was undertaken using both MDHS 25/4 (using filter and impinger), and the DBA method [ISO 17734-1 1 using the ASSET EZ4-NCO dry sampler (Merck Life Science UK Limited, UK)].

4.1.1 Filter and impinger (MDHS 25/4)

For static location samples, a measured volume of air was drawn [at a pump rate of 1 litre/minute ($L \cdot min^{-1}$)] through a glass impinger containing 1-(2-methoxyphenyl)piperazine (1,2-MP) absorbing solution backed with a glass-fibre filter impregnated with 1,2-MP reagent. For personal samples, only a glass-fibre filter impregnated with 1,2-MP reagent was used (for safety reasons). The solutions were then extracted and concentrated, and analysed by HPLC-MS/MS.

4.1.2 ASSET EZ4-NCO sampler (ISO 17734-1 2013).

The ASSET EZ4-NCO sampler is a sampling tube containing dibutylamine (DBA) impregnated glass-fibre filters housed in a denuder and filter cassette. Air was drawn through the sampler at a pump rate of $0.2 L \cdot min^{-1}$. After sampling, the interior filter medium was removed from the sampler and the DBA-isocyanate based derivatives extracted and analysed using HPLC-MS/MS.

Bulk samples of the products used were obtained and characterised where possible (BSI, 2013).

4.2 Aerospace company

The company was an aerospace component manufacturer and supplier.

The most widely used isocyanate product at the site was Desmodur RFE, which contains approximately 28% isocyanate [(tris (p-isocyanatophenyl) thiophosphate, TIPTP, CAS No: 4151-51-3]. This was the hardener for many of the 2-part adhesives used throughout the factory. Thermoplastic polyurethane chips were heated, extruded and rolled into sheets of material for manufacturing flotation devices and fuel cells.

The company also provided three different coloured samples of solid thermoplastic polyurethane chips for analysis. These were extruded at 185°C. The safety data sheets indicated an operational temperature range of 177°C to 232°C for hot melt processes, and that decomposition may occur if heated above the recommended maximum, possibly generating isocyanates. To determine what isocyanates may be generated, the chips were analysed before the visit using pyrolysis GC-MS. This analysis indicated that MDI may be released from these polyurethane chips.

Results of the analysis are summarised below.

- Except for one sample, all TRIG results for personal samples were lower than 1 $\mu\text{g}\cdot\text{min}^{-3}$ (8h TWA), i.e., low compared to the WEL of 20 $\mu\text{g}\cdot\text{m}^{-3}$ (8h TWA).
- A single personal sample from an operative in the decant room was the exception. A TRIG result of 15 $\mu\text{g}\cdot\text{m}^{-3}$ (8h TWA), mainly from TIPTP, was determined, which is much closer to the WEL. Only one personal sampler could be fitted to the operative - an MDHS 25/4 filter, so a direct comparison with an ASSET EZ4-NCO sampler was not possible. Urine samples from this worker also demonstrated potential exposure.
- Low levels (<1 $\mu\text{g}\cdot\text{m}^{-3}$) of -NCO from TIPTP were detected from several processes. However, these were too low to make a reasonable comparison between the MDHS 25/4, and ASSET EZ4-NCO samplers. In general, at low levels, the ASSET EZ4-NCO sampler would appear to be less sensitive for measuring TIPTP than an MDHS 25/4 sampler. The ASSET EZ4-NCO sampler uses a lower flow rate than the MDHS 25/4 samplers; therefore, less sample was captured by the former samplers.

4.3 Foundry casting

At the foundry, a resin (containing MDI) and an activator were mixed, and then combined with dry sand to form a sand core. Molten aluminium (up to 750°C) was added to the sand core to cast the relevant component. During casting, other materials could be added at various temperatures as the component cooled. Given the high temperature of the molten metal, there was the potential to vaporise MDI from the sand core during this process.

An initial sampling round identified TRIG results for personal samples up to 8 $\mu\text{g}\cdot\text{m}^{-3}$ (8h TWA), compared to the WEL of 20 $\mu\text{g}\cdot\text{m}^{-3}$ (8h TWA). A number of isocyanate substances

were identified with the general order of contribution being phenyl isocyanate (PHI), p-tolyl isocyanate, o-tolyl isocyanate, 4,4 MDI, 2,4 MDI and 3-ring MDI. A repeat sampling exercise was undertaken using static samplers only (to allow impingers to be used for aerosol sampling for MDHS 25/4), to compare the MDHS 25/4 and ASSET EZ4-NCO samplers. Results were compared for MDI and PHI.

For MDI, the correlation was driven by one higher exposure sample pair although even this result (up to $3.8 \mu\text{g}\cdot\text{m}^{-3}$), was still comparatively low. Including this sample, the correlation had a linear least squares regression of $r > 0.9987$, with the ASSET EZ4-NCO sampler, determining the exposure to be about 60% lower than when using the MDHS method. If this high exposure point is excluded (Figure 9), then some variability in the comparison is observed. Some samples taken using the ASSET EZ4-NCO samplers measured levels more than twice as high as those measured with the MDHS 25/4 method whereas others measured half the concentration (compared to using the MDHS 25/4 method).

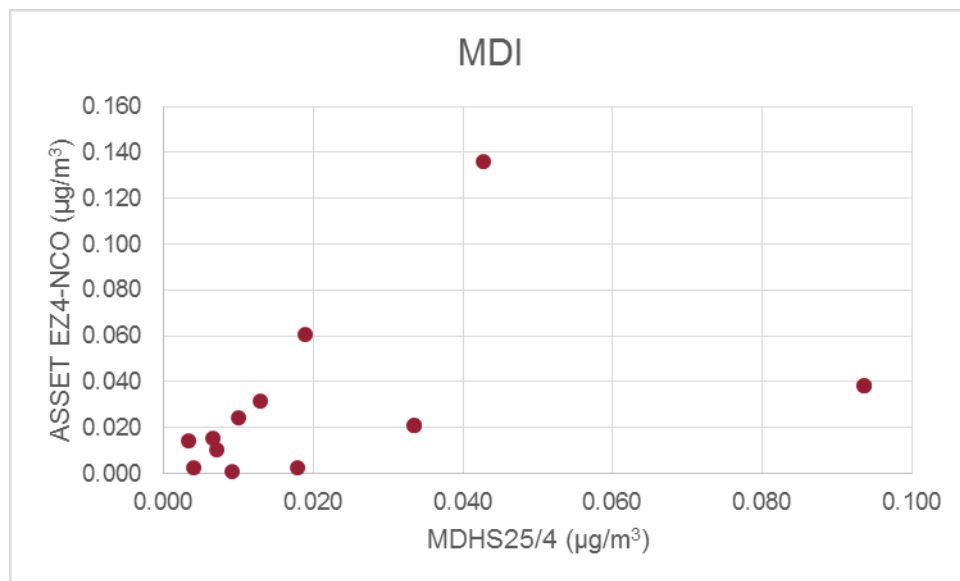


Figure 9. Comparison of MDHS25/4 and ASSET EZ4-NCO samplers for MDI. The higher exposure point is excluded.

PHI results compared better between the two samplers, with a correlation of $r > 0.7260$, and the ASSET EZ4-NCO sampler quantifying results about 20% higher than for the MDHS 25/4 sampler (Figure 10).

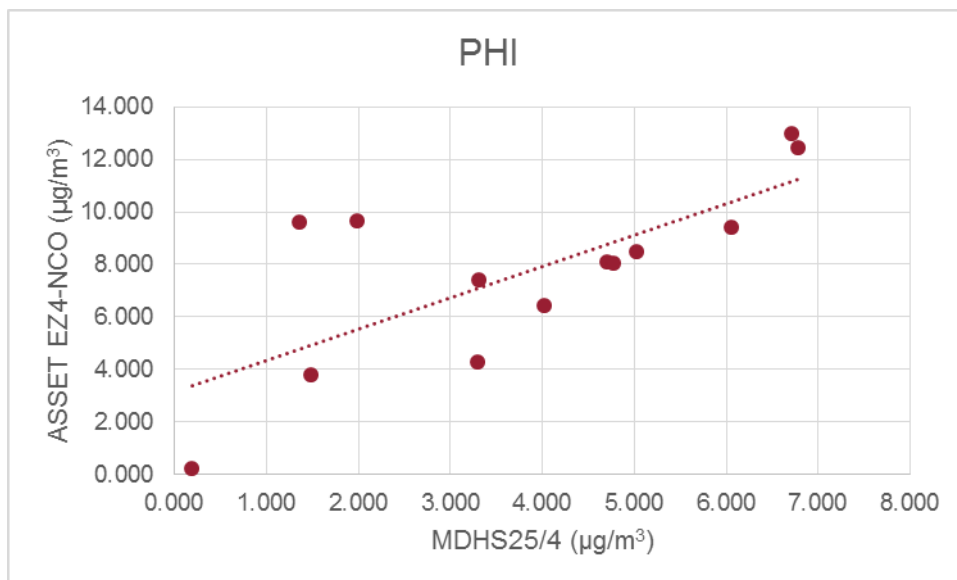


Figure 10. Comparison of MDHS 25/4 and ASSET EZ4-NCO samplers for PHI.

4.3 3D printing

Previous work (HSE, 2019), indicated that isocyanates could be released from certain 3D printing filaments when heated. This work was conducted under laboratory conditions so it was not known how these findings might apply to in-use scenarios in workplaces.

A visit was conducted to a 3D printing workshop that agreed to set up their printers using filaments supplied by HSE scientists. This filament was Ninjaflex®¹³, which was characterised (by pyrolysis gas chromatography with mass selective detection) before the visit to confirm the species of isocyanate that may be liberated during heating. MDI was found to be released from this filament. The workshop and its layout can be seen in Figure 11.

The Ninjaflex filament cable was fed into ten printers (numbers 3, 4, 5, 6, 8, 14, 15, 16, 20 and 23, see Figure 11), and these switched on. Static sampling was carried out whilst the printers were in operation. Samplers were located adjacent to these ten printers (see Figure 11, sampling locations are marked with a red star). Sampling durations were between 208 and 210 minutes.

¹³ www.ninjatek.com

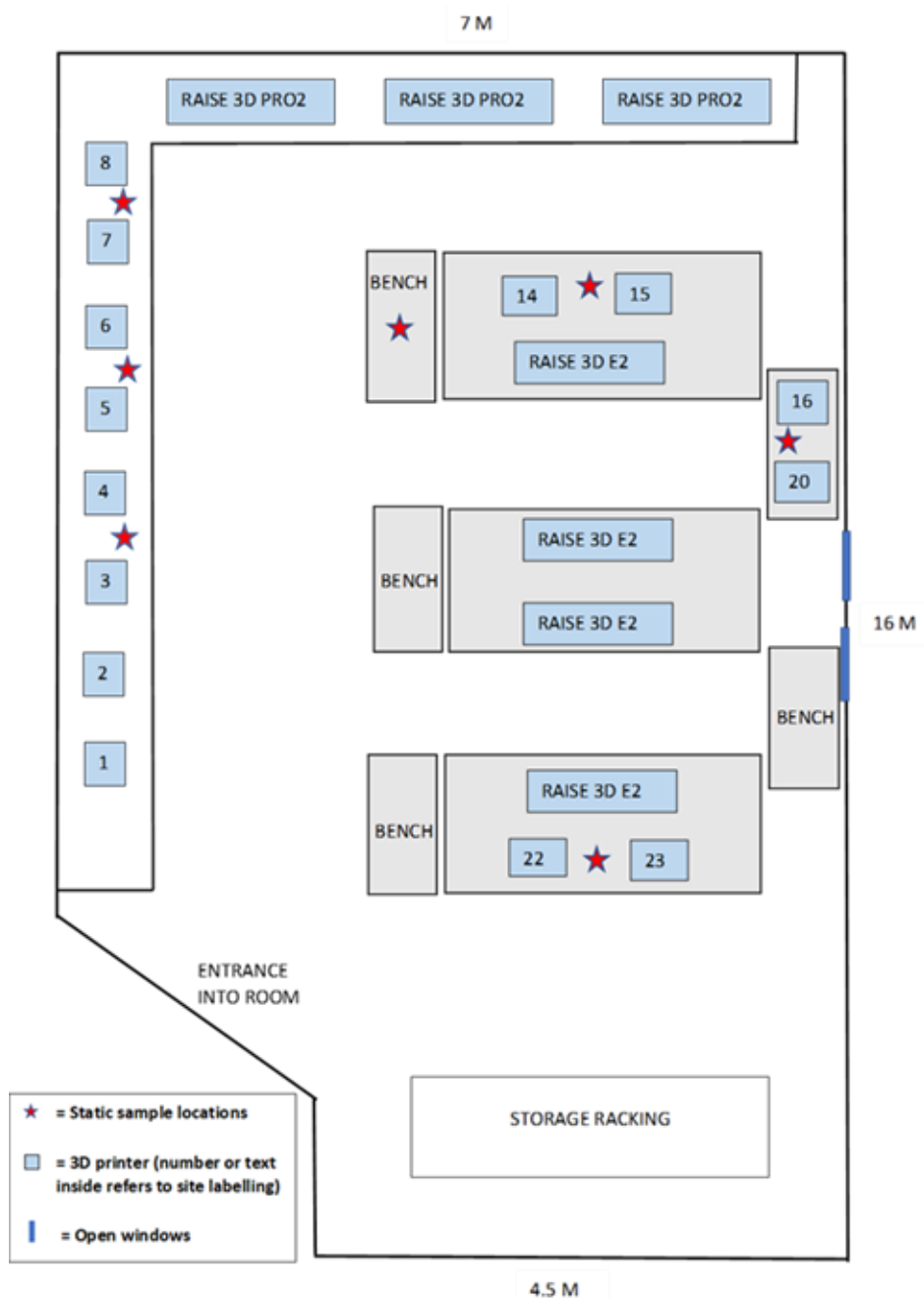


Figure 11. Plan of the 3D printing workshop, sampling locations are marked with a red star.

The MDI isomers, 4,4-MDI and 2,4-MDI, and also PHI, were detected but at very low levels which were lower than the LOQ for the analytical methods (total NCO results were about one thousandth of the WEL). The 2,4-MDI isomer was not seen in the ASSET EZ4-NCO sampler analysis possibly due to co-elution with 4,4-MDI.

Although, a quantitative comparison of the sampling methods was not possible due to the low levels detected, MDHS 25/4 proved to be the more sensitive method for MDI at this low exposure level; this is primarily due to the sampling volume used for each method.

4.4 Test chamber evaluation

In addition to the site visits, a test chamber experiment was undertaken. A small number of samplers were sited within the test chamber containing a TDI atmosphere (primarily monomer). Diffusion tubes containing TDI were placed in a vapour generator at 70 – 80°C and a flow of nitrogen passed through the heating tube. The isocyanate-laden nitrogen was then diluted with humidified air with the resulting flow passing into a glass chamber where the sampling devices were situated. The concentration inside the chamber was monitored in real time using a ChemLogic Portable (CLPX) paper tape device.

The ASSET EZ4-NCO samplers were run at flow rates of 200 mL.min⁻¹, 500 mL.min⁻¹, and 850 mL.min⁻¹; MDHS 25/4 samplers were run at 2 L.min⁻¹. The sampling duration for all samplers was 266 minutes. There was good agreement between the two types of sampler; in addition, there was good reproducibility between the samplers of the same type, even when running at different flowrates (ASSET EZ4-NCO samplers). The results are presented in Table 3.

Table 3. Comparison of MDHS25/4 and ASSET EZ4-NCO samplers for TDI in a test chamber.

	MDHS 25/4* (mg.m ⁻³)	ASSET EZ4-NCO (mg.m ⁻³)	ASSET EZ4-NCO flow (mL.min ⁻¹)
	0.036	0.036	200
	0.036	0.035	500
	0.037	0.035	850
	0.038	–#	–#
Mean	0.037	0.035	
Relative standard deviation (%)	2.7%	2.5%	

* All MDHS 25/4 samplers run at 2L.min⁻³. # Only three ASSET EZ4-NCO samplers used.

5. Conclusions

5.1 Air monitoring

Discussions with the Home Office have indicated that an exemption for the use of 1,2-MP in the context of MDHS 25/4 is unlikely.

The reintroduction of a quality assurance scheme for laboratories performing MDHS 25/4 would be possible, but more evaluation is required to determine whether it would be cost-effective, or viable as a scheme in the long term. It could be discussed with the current available scheme provider as to whether impregnated filters may be provided as part of their existing scheme. There would be a requirement to include oligomeric species in any proposed scheme. Currently, it is therefore difficult for dutyholders and regulators to assess laboratories for their performance in this challenging analysis.

The two alternative methods evaluated in this project have their own limitations. Although sampling for the DBA method is more straightforward (commercial supplier, no restrictions on purchase or use), the analysis remains challenging and is arguably more demanding on the analyst than the current MDHS 25/4 method. For the DAN method, the principle is of value i.e., only a single analyte to quantify; however, the method is not currently validated, and practical issues were encountered with the availability and stability of the reagents.

The continued use of MDHS 25/4 is dependent on the BOHS maintaining a GAL, and their long-term commitment to this is uncertain. To prepare for the possibility that the GAL is not maintained, investigation could continue into how the DBA method could be adapted to be a TRIG method. Further evaluation of the DAN method, using the now commercially available reagents, could also be pursued as a less demanding, and more accessible method.

The use of MDHS 25/4 continues to present challenges for the personal sampling of aerosols where the use of impingers, for safety reasons, is not advised. To avoid underestimation of exposures where aerosols are likely to be present, the use of static samplers (both with and without impingers) is proposed as a means of “field calibration” of the likely contribution of aerosols to the overall exposure.

5.2 Biomarkers

The lysine biomarkers show promise for being more specific than the diamines for TDI and MDI, although properly characterised standards are required to investigate TDI further. At

the current time, HDI-lysine does not look viable. Within the previously mentioned European project (HBM4EU), looking at occupational exposures to diisocyanates, samples from workers exposed to MDI have been analysed for acMDI-Lys (Jones et al., 2022); the results are due to be published in 2023.

It seems likely that a biomarker for TIPTP (the corresponding triamine), has been detected. This finding demonstrates that the use of the “corresponding amine” of newer isocyanates on the market can potentially serve as biomarkers, even though detailed metabolism data may be unavailable. This detection also indicates systemic uptake is possible despite the larger size and lower volatility, indicating that there is still potential for respiratory sensitisation.

A screening method for urine would still be valuable, given the low rates of positive samples. Several potentially workable approaches were demonstrated although, further development is required to demonstrate the applicability to urine samples.

There continues to be no specific, reliable effect biomarkers for isocyanates; however, the scientific literature should continue to be monitored. The biomarkers, IL-4 in exhaled breath condensate, serum periostin, and FeNO may warrant further investigation. The biomarkers, IgE, IgG, and FeNO have been measured in the aforementioned, HBM4EU project and may provide further information in due course.

5.3 Site visits and sampler comparisons

Three site visits were undertaken, with MDI being present at all three sites. The aerospace company also used TIPTP, a larger, less volatile isocyanate. Exposures to TIPTP were detected using MDHS 25/4, including one that was approaching the WEL. This confirms the broad applicability of TRIG measurements using MDHS 25/4 – i.e., it is adaptable to new, and different isocyanates being used in the workplace.

The 3D printer visit was only a single assessment of one workplace, so the findings obtained may not reflect all such printing scenarios. However, the very low levels of MDI detected (below LOQ; despite ten printers running simultaneously for three and a half hours), indicates that the exposure to diisocyanates in this scenario is likely to be low. Previous HSE research has identified operating practices to minimise emissions from desktop 3D printers (HSE, 2019), which informed the Consortium of Local Education Authorities for the Provision of Science Services guidance (CLEAPSS, 2019).

The test chamber comparison showed that there was good agreement between the two different samplers (MDHS 25/4 and ASSET EZ4-NCO), in a controlled environment. This comparison sampled a predominantly monomer-based vapour atmosphere at significant exposure levels (i.e., about twice the WEL).

The field-based evaluations were less clear-cut, with some exposures too low to draw firm conclusions; the casting visit showed that comparisons could be variable. Further work would be required to determine the key factors in these differences (for example, isocyanate species, presence of aerosols).

6. References

ATSDR. (1998) Toxicological Profile for 4,4'-methylenedianiline. Available at: <https://www.atsdr.cdc.gov/ToxProfiles/tp122.pdf> (accessed 02/05/2023).

Aubin S, Hamdi EM, Joly A. (2020) On-site comparison of the OSHA 47, Asset EZ4-NCO, Iso-Chek, DAN, and CIP10 methods for measuring methylene diphenyl diisocyanate (MDI) at an oriented-strand board (OSB) factory. *J Occup Environ Hyg* 17: 560-573.

Bello D, Herrick CA, Smith TJ, Woskie SR, Streicher RP, Cullen MR, Liu Y, Redlich CA (2007). Skin exposure to isocyanates: reasons for concern. *Environ Health Perspect* 115(3): 328-35.

Bjermer L, Alving K, Diamant Z. (2014) Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med* 108: 830-841.

Blindow S, Preisser AM, Baur X. (2015) Is the analysis of histamine and/or interleukin-4 release after isocyanate challenge useful in the identification of patients with IgE-mediated isocyanate asthma? *J Immunol Methods* 422: 35-50.

Brown J, Barrey E, Shimelis O, Schultz K. (2012) Analysis of Isocyanates Using the ASSET™ EZ4-NCO Dry Sampler. *The Reporter*, 30. Available at: https://theanalyticalscientist.com/fileadmin/tas/issues/App_Notes/Sampling_Analysis_Isocyanates.pdf (accessed 02/05/2023).

BSI. (2013) BS EN 1242:2013 Adhesives. Determination of isocyanate content. Available at: <https://shop.bsigroup.com/ProductDetail?pid=000000000030259192> (accessed 02/05/2023).

Chi CH, Liao JP, Zhao YN. (2016) Effect of Inhaled Budesonide on Interleukin-4 and Interleukin-6 in Exhaled Breath Condensate of Asthmatic Patients. *Chin Med J (Engl)* 129: 819-823.

CLEAPSS. (2019) G276 - 3D Printing in Schools and Colleges. Managing the Risks. Available at: <https://dt.cleapss.org.uk/Resource-File/3D-printing-in-schools-and-colleges-managing-the-risks.pdf> (accessed 02/05/2023).

Cocker J, Jones K, Leng G, Gries W, Budnik L, Müller J, Göen T, Hartwig A. (2017) Hexamethylene diisocyanate, 2,4-toluene diisocyanate, 2,6-toluene diisocyanate, isophorone diisocyanate and 4,4'-methylene diphenyl diisocyanate – Determination of hexamethylenediamine, 2,4-toluenediamine, 2,6-toluenediamine, isophoronediamine and 4,4'-methylenedianiline in urine using gas chromatography-mass spectrometry

[Biomonitoring Methods, 2017]. The MAK-Collection for Occupational Health and Safety. 1415-1435.

Dweik RA, Boggs PB, Erzurum SC. (2011) An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 184: 602-615.

ECHA. (2020) Opinion on scientific evaluation of occupational exposure limits for diisocyanates. Available at: <https://echa.europa.eu/documents/10162/4ea3b5ee-141b-63c9-8ffd-1c268dda95e9> (accessed 02/05/2023).

Ferrazzoni S, Scarpa MC, Guarnieri G. (2009) Exhaled nitric oxide and breath condensate pH in asthmatic reactions induced by isocyanates. *Chest* 136: 155-162.

Florentin A, Acouetey DS, Remen T. (2014) Exhaled nitric oxide and screening for occupational asthma in two at-risk sectors: bakery and hairdressing. *Int J Tuberc Lung Dis* 18: 744-750.

Gries W and Leng G. (2013) Analytical determination of specific 4,4'-methylene diphenyl diisocyanate hemoglobin adducts in human blood. *Anal Bioanal Chem* 405: 7205-7213.

HSE. (1997) Biological monitoring in the workplace: A guide to its practical application to chemical exposure. Available at: <http://www.hse.gov.uk/pubns/books/hsg167.htm> (accessed 02/05/2023).

HSE. (2001) Asthmagen? Critical assessments of the evidence for agents implicated in occupational asthma. Available at: <https://www.hse.gov.uk/asthma/asthmagen.pdf> (accessed 02/05/2023).

HSE. (2014a) MDHS 25/4: Organic isocyanates in air. Available at: <https://www.hse.gov.uk/pubns/mdhs/pdfs/mdhs25-4.pdf> (accessed 02/05/2023).

HSE. (2014b) Safety in isocyanate paint spraying INDG388(rev2). Available at: <http://www.hse.gov.uk/pubns/indg388.pdf> (accessed 02/05/2023).

HSE. (2019) RR1146 - Measuring and controlling emissions from polymer filament desktop 3D printers. Available at: <https://www.hse.gov.uk/research/rrhtm/rr1146.htm> (accessed 02/05/2023).

HSE. (2020) EH40/2005 Workplace exposure limits. Available at: <http://www.hse.gov.uk/pubns/priced/eh40.pdf> (accessed 02/05/2023).

HSE. (2022a) Work-related asthma statistics Great Britain, 2022. Available at: <https://www.hse.gov.uk/statistics/causdis/asthma.pdf> (accessed 02/05/2023).

HSE. (2022b) G408 - Urine sampling (biological monitoring) for isocyanate exposure measurement. Available at: <https://www.hse.gov.uk/pubns/guidance/g408.pdf> (accessed 02/05/2023).

ISO. (2007) ISO 16702:2007. Workplace air quality — Determination of total organic isocyanate groups in air using 1-(2-methoxyphenyl)piperazine and liquid chromatography. Available at: <https://www.iso.org/standard/42007.html> (accessed 02/05/2023).

ISO. (2012) ISO 14382:2012 - Workplace atmospheres — Determination of toluene diisocyanate vapours using 1-(2-pyridyl)piperazine-coated glass fibre filters and analysis by high performance liquid chromatography with ultraviolet and fluorescence detectors. Available at: <https://www.iso.org/standard/54616.html> (accessed 02/05/2023).

ISO. (2013) ISO 17734-1:2013 Determination of organonitrogen compounds in air using liquid chromatography and mass spectrometry — Part 1: Isocyanates using dibutylamine derivatives. Available at: <https://www.iso.org/standard/58006.html> (accessed 02/05/2023).

Jones K, Galea KS, Scholten B. (2022) HBM4EU Diisocyanates Study-Research Protocol for a Collaborative European Human Biological Monitoring Study on Occupational Exposure. *Int J Environ Res Public Health* 19.

Jones K, Johnson PD, Baldwin PEJ. (2017) Exposure to Diisocyanates and Their Corresponding Diamines in Seven Different Workplaces. *Ann Work Expo Health* 61: 383-393.

Kenny, L. (2016). Isocyanate Dry Sampler Method Validation (CBRU/2016/074). Available on request from HSE.

Klusáčková P, Dušková Š, Mráz J. (2022) Health effects of exposure to isocyanates in a car factory. *Central European journal of public health* 30: 32-36.

Kraft M. (2011) Asthma phenotypes and interleukin-13--moving closer to personalized medicine. *N Engl J Med* 365: 1141-1144.

Lee JH, Kim SH, Choi Y. (2018) Serum Periostin Levels: A Potential Serologic Marker for Toluene Diisocyanate-Induced Occupational Asthma. *Yonsei Med J* 59: 1214-1221.

Maestrelli P, Henneberger PK, Tarlo S. (2020) Causes and Phenotypes of Work-Related Asthma. *Int J Environ Res Public Health* 17.

Margel D, Pevsner-Fischer M, Baniel J. (2011) Stress proteins and cytokines are urinary biomarkers for diagnosis and staging of bladder cancer. *Eur Urol* 59: 113-119.

Matsumoto H. (2014) Serum Periostin: A Novel Biomarker for Asthma Management. *Allergology International* 63: 153-160.

McConnachie G, Johnson P. (2023) . Total Reactive Isocyanate Group (TRIG) Measurement: A Commentary. *Annals of Work Exposures and Health*, wxad007, <https://doi.org/10.1093/annweh/wxad007>

Merget R, Sander I, van Kampen V. (2015) Serial measurements of exhaled nitric oxide at work and at home: a new tool for the diagnosis of occupational asthma. *Adv Exp Med Biol* 834: 49-52.

Nobles C, Bertone-Johnson ER, Ronnenberg AG. (2015) Correlation of urine and plasma cytokine levels among reproductive-aged women. *Eur J Clin Invest* 45: 460-465.

Nwoko KC, Kenny L, Jones K. (2022) Methylendiphenyl diisocyanate lysine conjugates in the urine of workers exposed to methylendiphenyl diisocyanate. *Toxicology and Industrial Health* 38: 636-642.

Oțelea MR, Fell AKM, Handra CM. (2022) The value of fractional exhaled nitric oxide in occupational diseases - a systematic review. *J Occup Med Toxicol* 17: 14.

Puscasu S, Aubin S, Sarazin P. (2017) Use of the Novel Derivatizing Agent 1,8-Diaminonaphthalene With the CIP10 Sampler to Measure 4,4'-Methylene Diphenyl Diisocyanate Atmospheres. *Ann Work Expo Health* 61: 566-574.

Sabbioni G, Dongari N, Sepai O. (2016) Determination of albumin adducts of 4,4'-methylendiphenyl diisocyanate in workers of a 4,4'-methylenedianiline factory. *Biomarkers* 21: 731-738.

Steinke JW, Borish L. (2001) Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. *Respir Res* 2: 66-70.

Sugama K, Suzuki K, Yoshitani K. (2013) Urinary excretion of cytokines versus their plasma levels after endurance exercise. *Exerc Immunol Rev* 19: 29-48.

Suojalehto H, Ndika J, Lindström I. (2021) Endotyping asthma related to 3 different work exposures. *Journal of Allergy and Clinical Immunology* 148: 1072-1080.

Takayama G, Arima K, Kanaji T. (2006) Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 118: 98-104.

Tarlo SM, Lemiere C. (2014) Occupational asthma. *N Engl J Med* 370: 640-649.

Vandenplas O, Godet J, Hurdubaea L. (2019) Are high- and low-molecular-weight sensitizing agents associated with different clinical phenotypes of occupational asthma? *Allergy* 74: 261-272.

Wisnewski AV, Cooney R, Hodgson M. (2022) Severe asthma and death in a worker using methylene diphenyl diisocyanate MDI asthma death. *Am J Ind Med* 65: 166-172.

Wisnewski AV, Liu J, Redlich CA. (2020) Analysis of Lung Gene Expression Reveals a Role for Cl(-) Channels in Diisocyanate-induced Airway Eosinophilia in a Mouse Model of Asthma Pathology. *Am J Respir Cell Mol Biol* 63: 25-35.

Wisnewski AV, Nassar AF, Liu J. (2019) Dilysine-Methylene Diphenyl Diisocyanate (MDI), a Urine Biomarker of MDI Exposure? *Chem Res Toxicol* 32: 557-565.

Isocyanate exposure is one of the leading causes of occupational asthma in Great Britain (GB). Exposure can also cause dermatitis and irritation of the eyes, nose and throat. Isocyanates are widely used in industry, particularly in spray painting, adhesives, flexible foam and polyurethane resin production. Dutyholders must ensure that effective control measures are in place to protect workers. Dutyholders may use air monitoring to demonstrate that airborne isocyanate levels are below the workplace exposure limit (WEL). Dutyholders may use biomonitoring of workers to ensure that exposure control measures are effective. These established HSE monitoring methods have some drawbacks for users, for example, a license is needed to use the air monitoring method, MDHS 25/4, because it uses a restricted chemical. Also, the HSE urine biomonitoring method does not distinguish between exposure to isocyanates and some other chemicals.

This report describes research to evaluate the suitability of potential alternative monitoring methods. It will be of interest to technical specialists undertaking isocyanate sampling and/or analysis. The research included a literature review, laboratory studies, and tests at three volunteer sites (3D printing, a foundry, and aerospace). The researchers' conclusions are: (1) MDHS 25/4 continues to be the most suitable approach to demonstrate compliance with the WEL. (2) HSE's methods for air monitoring and biomonitoring continue to be the most suitable for use in GB and may be adaptable as alternative isocyanates come to market. (3) Other monitoring methods may be appropriate in certain circumstances but are unlikely to be universally applicable for the measurement and analysis of all isocyanates.

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