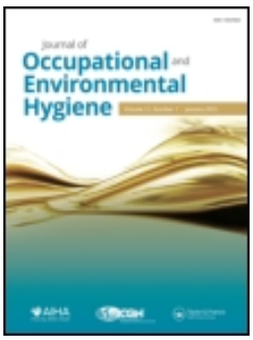

Journal of Occupational and Environmental Hygiene

ISSN: 1545-9624 (Print) 1545-9632 (Online) Journal homepage: http://www.tandfonline.com/loi/uoeh20

Implementation and evaluation of an analytical method for a novel derivatizing agent to measure 4,4'-methylene diphenyl diisocyanate atmospheres

Silvia Puscasu, Simon Aubin, Mark Spence & Sébastien Gagné

To cite this article: Silvia Puscasu, Simon Aubin, Mark Spence & Sébastien Gagné (2016) Implementation and evaluation of an analytical method for a novel derivatizing agent to measure 4,4'-methylene diphenyl diisocyanate atmospheres, Journal of Occupational and Environmental Hygiene, 13:8, 598-603, DOI: 10.1080/15459624.2016.1159691

To link to this article: http://dx.doi.org/10.1080/15459624.2016.1159691

Accepted author version posted online: 08 Mar 2016.
Published online: 21 May 2016.

JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE
2016, VOL. 13, NO. 8, 598–603
http://dx.doi.org/10.1080/15459624.2016.1159691

Taylor & Francis
Taylor & Francis Group

Implementation and evaluation of an analytical method for a novel derivatizing agent to measure 4,4′-methylene diphenyl diisocyanate atmospheres

Silvia Puscasu[a], Simon Aubin[a], Mark Spence[b], and Sébastien Gagné[a]

[a]Institut de recherche Robert-Sauvé en santé et en sécurité du travail, Montréal, Québec, Canada; [b]International Isocyanate Institute, III Scientific Office, Manchester, United Kingdom

ABSTRACT

Accurate measurement of 4,4′-methylene diphenyl diisocyanate (MDI) atmospheres is a challenge since the molecule is both chemically reactive and likely to be present in aerosol form when heated and sprayed because of its low vapor pressure. Meeting this challenge requires optimizing both the sampling device used and the derivatization agent employed to stabilize the isocyanate functional group. This study describes the use of a novel derivatization reagent for isocyanate sampling to address the challenge of MDI aerosol exposure sampling. Like most conventional derivatizing agents for isocyanates, 1,8-diaminonapthalene (DAN) reacts with isocyanate functional groups to form a urea. However, unlike other isocyanate derivatizing agents, the sample workup procedure with DAN includes a second step which yields a single analyte molecule, perimidone, for each isocyanate group. This feature gives DAN the unique ability to assess exposure to "total reactive isocyanate group" (TRIG). The analytical method implemented to quantitate the perimidone uses liquid chromatography coupled with tandem mass spectrometry. Positive mode ionization led to LOD and LOQ of 10 ng/mL and 34 ng/mL, respectively. The dynamic range was from 50–2000 ng/mL (with $R^2 \geq 0.990$), which corresponds to TRIG concentrations in air from 0.07–3.04 μg/m^3, assuming 60 min of sampling at 10 L/min (based on use of the CIP-10M sampler). The intra-day and inter-day analytical precisions were <4% for all of the concentration levels tested, and the accuracy was within an appropriate range of 98 ± 2%. Minimal matrix effect was observed, and a total recovery of 109% was obtained. The approach seems to be promising for TRIG measurements and further work is planned to establish DAN method behavior in samplers used for workplace monitoring.

KEYWORDS

1,8-diaminonaphtalene; 4,4′-methylene diphenyl diisocyanate; isocyanate; perimidone

Introduction

Polymeric MDI (which consists of 4,4′-methylene diphenyl diisocyanate (MDI) and its oligomers) is one of the main components of insulation foam, in which it is reacted with a polyol to form the polyurethane. MDI is known to cause health effects as severe as occupational asthma and respiratory and cutaneous irritations.[1–7] An Occupational Exposure Limit (OEL) of 5 ppb for the monomer is enforced by most countries for this substance in order to protect workers' health.[8-12] Despite these regulations in place, both forms, monomer and oligomers, must be measured and controlled to protect workers and avoid work-related illnesses,[13] as well to comply with other regulations such as the one in place in Australia, Finland, Ireland, and the UK.[14,15] In addition, the sampling strategies must take into account that airborne isocyanate can be found in two different forms: vapor and aerosol. However, fast-curing MDI used in spray applied (heated + pressurized with air) insulation foam is mainly present as a liquid aerosol with minimal vapor.[16] MDI measurements are generally conducted in two steps as this substance is highly reactive: first, chemical structure stabilization occurs in the field using a derivatization agent in a sampling device; next, the derivatized isocyanate in the sample is analyzed in laboratory. Efficient fast-curing MDI aerosol sampling is known to be challenging, as shown in a study comparing filter with impinger[17] and in an analytical method using filter.[18] Strategies for performing effective sampling involve either improving the sampling device used for aerosol collection or refining the derivatization agent used inside the sampling device to stabilize the reactive isocyanate in the field.

CONTACT Sébastien Gagné sebastien.gagne@irsst.qc.ca Institut de recherche Robert-Sauvé en santé et en sécurité du travail, 505 boul. De Maisonneuve Ouest, Montréal, Québec, Canada H3A 3C2.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/uoeh.

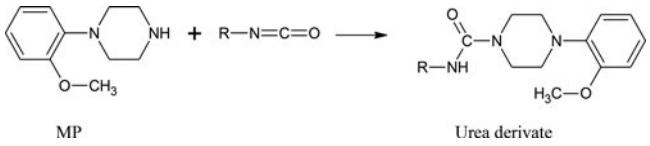

Figure 1. Reaction between MDI and MP to produce the urea derivate.

A liquid-filled impinger is the reference sampling device used for spray applied fast-curing MDI measurements. However, although this device is efficient in terms of sampling, several limitations have been documented regarding the device's fragility, solvent evaporation, flammability, and very cumbersome for the wearer and industrial hygienist. The performance of alternative sampling devices has been characterized for MDI aerosol sampling. Filter-based sampling devices and denuders, commercially known as ASSET EZ4-NCO, are all reported to underestimate MDI aerosol concentrations as compared to impingers.[17,19] Recently, results obtained with a CIP10 in a simulated workplace have been shown to be promising for MDI aerosol sampling,[20] where concentrations in the same range as those provided by the impingers were found. This last approach employed 1,2-(methoxyphenyl)piperazine (MP) in the device in order to stabilize the reactive isocyanate function as a urea derivative.

The chemical reaction seen in Figure 1 is one method currently documented for sampling.[17,18,20-23] Nevertheless, other chemical reactions can be leveraged to stabilize the isocyanate function. Derivatization using 1,8-diaminonaphtalene (DAN) for total reactive isocyanate group (TRIG) has been proposed.[24] This reaction leads to the formation of a urea. During subsequent laboratory workup, an acid-catalyzed cyclization reaction yields one molecule of perimidone for each isocyanate group as shown in the scheme in Figure 2.

This approach provides a method for measuring TRIG and can be a good tool for exposure evaluation targeting both monomer and oligomers.[15] It allows a unique direct evaluation of isocyanate without deriving for individual species, as the health effects are mainly linked to the isocyanate function rather than the individual chemical species. It could be applied to a wide variety of isocyanates without targeting particular isocyanates. TRIG

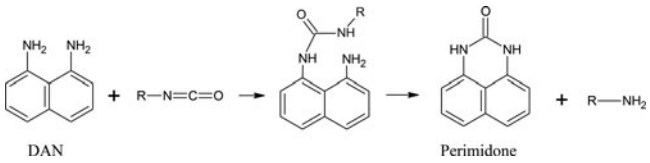

Figure 2. Reaction between MDI and DAN to produce the perimidone.

value is used in OEL in place in Australia, Finland, Ireland, and the UK.[14,15] However, it would have to be used in a prevention context for countries with OEL linked to monomeric species. Work on method development and optimization of the DAN method have been carried out previously.[25] In this article, we report the implementation and evaluation of an analytical method by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) for the analysis of perimidone.

Experimental

Chemicals

4,4'-MDI (98% purity), dimethylsulfoxide (DMSO; >99.9%), acetone (HPLC grade) and formic acid (>95% purity) were obtained from Sigma-Aldrich (Milwaukee, WI) and were used without any further purification. DAN (99% purity), 1,3-dihydrophenalen-2-one (perimidone, 99% purity) and d6-perimidone (99% purity) were obtained from Nuchem Therapeutics (Montréal, Canada). Water (H_2O), methanol (MeOH), formic acid (FA), all optima LC-MS grade, were obtained from Fisher Scientific (St-Laurent, Qc, Canada).

Instruments and analytical conditions

The perimidone samples and standards were analyzed on a UPLC-MS/MS system consisting of a Waters Acquity UPLC coupled with a Waters Xevo TQ triple quadrupole mass spectrometer (Beverly, MA) equipped with an electrospray source. The analytical column used was a Kinetex C18 2.6 µm, 2.1 mm X 100 mm from Phenomenex (Torrance, CA). The software used to operate the system and analyze the data was Masslynx. Peak integration was done using the automatic feature for integrating the peak area. Manual adjustments were done on integrations not covering the entire peak. The regression calibration curve used linear fit.

The mobile phase was composed of MeOH + 0.1% FA (eluent A), and water + 0.1% FA (eluent B). UPLC separation was achieved using a gradient of 20% eluent A held for 3 min, then ramped to 90% eluent A for 2 min, then equilibrated at 20% eluent A for 1 min. The flow rate was 0.5 mL/min and the column was kept at 24°C. The injection volume was 5 µL using the partial loop with needle overfill feature. The samples were kept at 15°C in the autosampler to ensure a stable temperature. The Xevo TQ was operated in positive mode, the capillary voltage was set at 3.5 kV, the source temperature at 150°C, the desolvation temperature at 500°C, the desolvation flow at 1000 L/hr, the collision gas flow at 0.15 mL/min, and the data

Table 1. MRM parameters.

Substances	MRM transition	Cone (V)	Collision energy (eV)
Perimidone	185–115	65	30
	185–130	65	28
d6-perimidone	191–121	65	28
	191–136	65	28

were acquired in multiple reaction monitoring (MRM) mode. The MRM transition and conditions used for perimidone and the internal standard d6-perimidone (ISTD) are listed in Table 1 and were optimized by infusing solutions of 100 µmol/L prepared in 25% water + 0.1% FA / 75% methanol + 0.1% FA at a flow rate of 10 µL/min.

Sample and standard preparation

The samples and standards were prepared as follows. 0.5 mL of DMSO with 50mM of DAN was transferred into a LC-vial. 10 µL of d6-perimidone 10 µg/mL in methanol was spiked in the aliquot as an internal standard (ISTD) for a final concentration of 100 ng/mL. 0.48 mL of FA was added to the LC vial and mixed. After 30 min, 10 µL of acetone was spiked in the LC vial and mixed. The residual solution was injected by UPLC-MS/MS.

The perimidone primary solution was prepared by weighing 10 mg in a 50-mL volumetric flask to which 50 mL of DMSO was added for a final concentration of 200 µg/mL. The calibration standards were prepared from intermediate solutions at 1 µg/mL and 100 µg/mL. Seven perimidone calibration points were used. The standards were prepared in the same matrix as the samples at concentration levels of 0 ng/mL, 50 ng/mL, 100 ng/mL, 500 ng/mL, 1000 ng/mL, 1500 ng/mL, and 2000 ng/mL. The ISTD was added to the calibration standards at the same level as the samples.

Reactivity assessment of MDI with DAN to produce the primidone

To mimic the sampler conditions, 1 mL of 5 mM and 50 mM DAN in DMSO was divided into several test tubes. Then 10 µL of a solution 200 µg MDI/mL ACN was added to the DAN/DMSO solutions and mixed using a vortex mixer. Following the addition of this solution, 20 µL of acetone was added at regular time points to stop the reaction between the MDI monomer and the DAN upon reaction between the acetone and the DAN. Time points of 1, 5, 10, 15, 30, and 60 min were used to document the reaction rate. These time points were easy to conduct in laboratory without overwhelming the person doing the experiment. The perimidone was then produced using the protocol described in the previous section. All determinations were done in duplicate.

Analytical performance evaluation

The reported limit of detection (LOD) and limit of quantitation (LOQ) were based on a signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. The S/N was determined from a spiked concentration which was 4–10 times the estimated LOD. This spiked concentration was analyzed 10 times from 10 different aliquots. All the aliquots followed the same sample preparation procedure as the samples. Recovery and matrix effect were investigated using replicates prepared in DMSO containing DAN and compared with replicates prepared in pure DMSO. The dynamic range, intra-day and inter-day precisions, and accuracy were evaluated using the previously described sample preparation procedure. The intra-day precision was calculated from 6 separate measurements of 3 different concentrations in the desired dynamic range on a single day. The inter-day precision was calculated from 3 different concentrations distributed over the entire dynamic range and repeated 3 times for each measurement by the same person, on the same instrument, but on 6 different days. The accuracy was evaluated by analyzing separate measurements of known concentrations within the dynamic range of the method, and quantified using a standard curve. The solution used in the accuracy measurement came from a different solution preparation than the standard curve to ensure that the calibration solutions were properly prepared and accurate.

Results and discussion

Laboratory method implementation and evaluation

Derivatization using DAN

MDI must be derivatized prior to analysis because this chemical substance is highly reactive. Most methods for isocyanate exposure evaluation use a reaction between an amine and the isocyanate function to form a urea derivate. DAN uses this reaction as well to stabilize the isocyanate function during field sampling. However, the unique aspect of the DAN method is that during subsequent laboratory workup, an acid-catalyzed cyclization reaction yields one molecule of perimidone for each isocyanate group (see Figure 2). This reaction offers several advantages for exposure evaluations targeting TRIG rather than separate monomer and oligomer species. Nevertheless, even if the chemical scheme and rationale are well established, the available analytical performances are limited for this reaction. As a first attempt, the reaction between the DAN (5 mM in DMSO) and the MDI was evaluated, as shown in Figure 3. As can be seen in this figure, an instantaneous reaction with a recovery ~80% is obtained between the DAN and the MDI with maximal

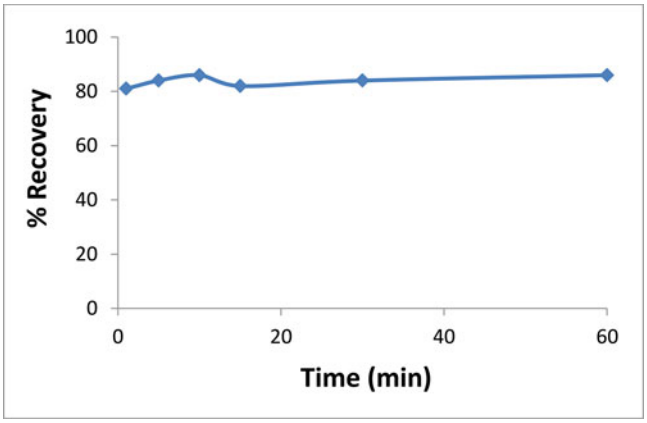

Figure 3. Rate of perimidone formation.

concentration reached after 1 min. Total recovery can be improved by ~10% when 50 mM of DAN is used rather than 5mM (data not shown) probably due to the proximity of the molecules in the solution facilitating the reaction. The chemical behavior has been judged adequate, based on empirical criteria with a recovery >85%, to evaluate formal analytical parameters with the perimidone formed.

Specificity and selectivity

The specificity and selectivity of the analytical method rely on the chromatographic retention time of perimidone and on the MRM transition used. To cause analytical interference, a substance must be present in the sample, have the same retention time under the chromatographic conditions used, and the same mass as the substances of interest. Specificity and selectivity were tested by analyzing several blank solutions. The general chromatogram is crowded at low concentrations in all solutions containing the matrix and chromatographic interferences have been observed for perimidone at concentrations below 25 ng/mL. However, high concentrations are expected for the samples and no further clean-up is conducted as the interference does not influence the expected concentrations in the samples, as seen in Figure 4.

Recovery and matrix effect

Perimidone spiked concentrations in DAN/DMSO were compared to perimidone spike concentrations in pure DMSO and total recovery 109% ± 13% was obtained as shown in Table 2. This minimal matrix effect can be explained by the chromatographic gradient used. The chromatographic gradient minimizes the impact of DAN artifact on the perimidone. Also, the use of a deuterated internal standard helps to minimize the impact of the matrix on the signal of the perimidone by correcting for all variations occurring due to ionization efficiency, injection volume difference and column deviation, to name a few. The standard curves were prepared in DAN/DMSO.

Carryover

A blank injection after the highest concentration of the standard curve was conducted at each run to assess the potential carryover. No detectable carryover was observed throughout the analysis conducted.

Dynamic range, LOD/LOQ, precision, and accuracy

The overall analytical performances of the method are presented in Table 3. The dynamic range was adjusted to

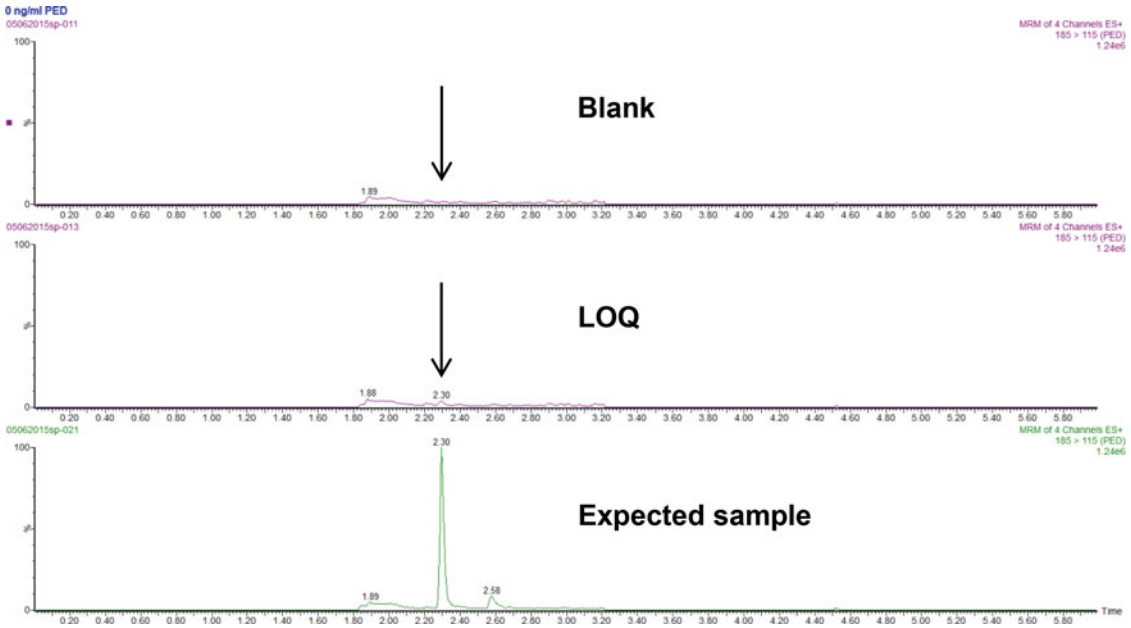

Figure 4. Representative chromatograms.

Table 2. Recovery and matrix effect evaluation.

	Concentration (ng/mL)	100 n = 6	500 n = 6	1000 n = 6	1500 n = 6	2000 n = 6
Spike in DAN/DMSO matrix	Mean	106	491	976	1468	1986
	Standard deviation	18	41	59	23	126
	% CV	17%	8%	6%	2%	6%
Spike in pure DMSO	Mean	81	458	959	1407	1996
	Standard deviation	3	19	18	31	47
	% CV	4%	4%	2%	2%	2%
	% Recovery	131%	107%	102%	104%	99%

cover the range of interest with standards ranging from 50–2000 ng/mL, with $R^2 \geq 0.990$. The estimated LOD and LOQ are sufficient to meet expectations. The intra-day and inter-day precisions are <4% for all the concentration levels tested, showing robustness of the quantitative data. The accuracy was within an appropriate range of 98 ± 2% at a target level of 1000 ng/mL. The accuracy also stayed within appropriate ranges even with concentrations over the calibration curve. All these parameters validated adequate implementation of the method as ready to be applied in the analysis of actual field samples.

Air sampling with DAN

The analytical parameters by UPLC-MS/MS were judged suitable for conducting future field evaluations with the DAN reagent. As the reaction has currently been evaluated in solution, samplers such as an impinger or CIP10 can be considered for field evaluations. More work would be needed to evaluate the DAN reagent for use in solvent-free samplers such as filters. Comparative studies using a sampling jar have been reported[19, 20] between impinger, CIP10 and ASSET EZ4-NCO samplers for MDI sampling using the MP derivatization reagent. This set-up is fully appropriate for evaluating the DAN reagent and will be considered in future evaluations of the DAN reagents in samplers as well as real workplaces using MDI applications such as spray polyurethane foam and wood product binders.

Table 3. Analytical parameters.

Estimated LOD (ng/mL)	10	
Estimated LOQ (ng/mL)	34	
Dynamic range (ng/mL)	50–2000	
R^2	>0.990	
Intra-day precision (n = 6)	100 ng/mL	8%
	500 ng/mL	1%
	1000 ng/mL	3%
	Mean	**4%**
Inter-day precision (n = 6)	100 ng/mL	7%
	500 ng/mL	4%
	1000 ng/mL	2%
	Mean	**4%**
Accuracy (n = 6)	500 ng/mL	98% ± 8%
	1000 ng/mL	98% ± 2%
	2000 ng/mL	99% ± 6%

Conclusion

A novel alternative for measuring TRIG from isocyanate such as MDI has been implemented and evaluated. The approach proposes the use of DAN reagent to stabilize the MDI. Once stabilized in urea form in the sampler, subsequent laboratory sample workup leads to the formation of a single perimidone per NCO function which is analyzed by UPLC-MS/MS in positive ionization mode. The analytical method has been shown to be useful and reliable for the concentrations of interest. The method's performances in terms of dynamic range, intra/inter-day precisions, accuracy, recovery, specificity, and carryover have been fully evaluated and the analytical method was judged ready for analysis of field samples. Current plans are to use the DAN reagent in a sampler such as the CIP10 because the reaction is currently validated in solution. Future comparative evaluations with a reference method in simulated and real workplaces can be initiated to determine if the overall sampling approach is reliable.

Acknowledgments

The authors would like to thank Lucile Richard for her technical involvement; she contributed significantly to this project during the laboratory analysis. The authors also thank Dhimiter Bello, Robert Streicher, and Jacques Lesage, who provided invaluable advice.

Funding

Our thanks also go to IRSST and International Isocyanate Institute for their technical assistance and financial support.

References

[1] **Banks, D.E.**, **B.T. Butcher**, and **J.E. Salvaggio**: Isocyanate-induced respiratory disease. *Ann. Allergy 57(6)*:389–396 (1986).

[2] **Malo, J.L.**, **G. Ouimet**, **A. Cartier**, **D. Levitz**, and **C.R. Zeiss**: Combined alveolitis and asthma due to hexamethylene diisocyanate (HDI), with demonstration of crossed respiratory and immunologic reactivities to diphenylmethane diisocyanate (MDI). *J. Allergy Clin. Immunol. 72(4)*:413–419 (1983).

[3] **Mapp, C.E.**, **B.T. Butcher**, and **L.M. Fabbri**: Polyisocyanates and Their Prepolymers. In *Asthma in the Workplace*, **I.L. Bernstein**, **M. Chan-Yeung**, and **D.I. Bernstein** (eds.). New York: Marcel Dekker, Inc, 1999. pp. 457–478.

[4] **Mapp, C.E.**, **P.C. Corona**, **N. De Marzo**, and **L. Fabbri**: Persistent asthma due to isocyanates. A follow-up study of subjects with occupational asthma due to toluene diisocyanate (TDI). *Am. Rev. Respir. Dis. 137(6)*:1326–1329 (1988).

[5] **Musk, A.W.**, **J.M. Peters**, and **D.H. Wegman**: Isocyanates and respiratory disease: current status. *Am. J. Ind. Med. 13(3)*:331–349 (1988).

[6] **Vandenplas, O.**, **A. Cartier**, **J. Lesage, et al.**: Prepolymers of hexamethylene diisocyanate as a cause of occupational asthma. *J. Allergy Clin. Immunol. 91(4)*:850–861 (1993).

[7] **Vandenplas, O.**, **A. Cartier**, **J. Lesage**, **G. Perrault**, **L.C. Grammer**, and **J.L. Malo**: Occupational asthma caused by a prepolymer but not the monomer of toluene diisocyanate (TDI). *J. Allergy Clin. Immunol. 89(6)*:1183–1188 (1992).

[8] **American Conference on Government Industrial Hygienists**: 2014 *TLVs and BEIs: Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices*. Cincinnati, Ohio: ACGIH, 2014.

[9] **National Institute for Occupational Safety and Health**: *Preventing Asthma and Death from Diisocyanate Eexposure DHHS*. Cincinnati, OH: NIOSH, 1996.

[10] **Occupational Safety & Health Administration**: *Code of Federal Regulations: Air Contaminants*, [29 CFR 1910.1000], 1992.

[11] **Quebec (Province)**: *Regulation Respecting Occupational Health and Safety, [S-2.1, r.19.01]*. Quebec: Publications du Québec, 2014.

[12] **Swedish National Board of Occupational Safety and Health**: *Occupational Exposure Limit Values*. Stockholm, Sweden: Arbetarskyddsstyrelsens Fösfattningssamling, 2000.

[13] **Bello, D.**, **S.R. Woskie**, **R.P. Streicher, et al.**: Polyisocyanates in occupational environments: A critical review of exposure limits and metrics. *Am. J. Ind. Med. 46(5)*:480–491 (2004).

[14] **Allport, D.C.**, **D.S. Gilbert**, and **O.S.M.**: Occupational Exposure Limits, Stack Limits and Community Limits. In *MDI and TDI: Safety, Health and the Environment: A Source Book and Practical Guide*, D.C. Allport, D.S. Gilbert, and S.M. Outterside (eds.). New York: John Wiley & Sons, 2003. pp. 343–358.

[15] **Health and Safety Laboratory**: "Organic Isocyanates In Air." In *Methods for the Determination of Hazardous Substances*, HSE Books (ed.). Sudbury, Suffolk, Health and Safety Laboratory, 2014. p. 16.

[16] **Roberge, B.**, **R. Gravel**, and **D. Drolet**: "4,4'-diphenylmethane Diisocyanate (MDI) Safety Practices and Concentration During Polyurethane Foam Spraying." In *Studies and Research Projects: Chemical Substances and Biological Agents*, IRSST (ed.). Montréal: Institut de Recherche Robert-Sauvé en santé et en sécurité du travail, 2009. p. 66.

[17] **Lesage, J.**, **J. Stanley**, **W.J. Karoly**, and **F.W. Lichtenberg**: Airborne methylene diphenyl diisocyanate (MDI) concentrations associated with the application of polyurethane spray foam in residential construction. *J. Occup. Environ. Hyg. 4(2)*:145–155 (2007).

[18] **Streicher, R.P.**, **C.M. Reh**, **R. Key-Schwartz**, **P.C. Schlecht**, and **M.E. Cassinelli**: Determination of Airborne Isocyanate Exposure. In *NIOSH Manual of Analytical Methods*, NIOSH (ed.). Cincinnati, OH: NIOSH, 1998. pp. 115–140.

[19] **Puscasu, S.**, **S. Aubin**, **Y. Cloutier**, **P. Sarazin**, **H. Van Tra**, and **S. Gagné**: Comparison between the ASSET EZ4 NCO and impinger sampling devices for aerosol sampling of 4,4'-methylene diphenyl diisocyanate in spray foam application. *Ann. Occup. Hyg. 59(7)*:872–881 (2015).

[20] **Puscasu, S.**, **S. Aubin**, **Y. Cloutier**, **P. Sarazin**, **H.V. Tra**, and **S. Gagne**: CIP10 optimization for 4,4-methylene diphenyl diisocyanate aerosol sampling and field comparison with impinger method. *Ann. Occup. Hyg. 59(3)*:347–357 (2015).

[21] **Gylestam, D.**, **J.B. Riddar**, **D. Karlsson**, **J. Dahlin**, **M. Dalene**, and **G. Skarping**: Dry sampling of gas-phase isocyanates and isocyanate aerosols from thermal degradation of polyurethane. *Ann. Occup. Hyg. 58(1)*:28–49 (2014).

[22] **Marand, A.**, **D. Karlsson**, **M. Dalene**, and **G. Skarping**: Solvent-free sampling with di-n-butylamine for monitoring of isocyanates in air. *J. Environ. Monit. 7(4)*:335–343 (2005).

[23] **Nordqvist, Y.**, **U. Nilsson**, **A. Colmsjo**, **A. Dahl**, and **A. Gudmundsson**: A chemosorptive cylindrical denuder designed for personal exposure measurements of isocyanates-evaluation on generated aerosols of 4,4'-methylenediphenyl diisocyanate. *J. Environ. Monit. 7(5)*:469–474 (2005).

[24] **Streicher, R.P.**, **D. Bello**, **S.R. Woskie**, and **A.A. Grote**: Measurement of total reactive isocyanate groups in samples using bifunctional nucleophiles such as 1,8-diaminonaphthalene (dan). Patent #20060130565. 2006.

[25] **Bello, D.**, and **R.P. Streicher**: *Evaluation of the DAN Method for the Determination of Total Reactive Isocyanate Group: Phase III-LC-MS/MS Analytical Finish & Field Testing*. Manchester, UK: International Isocyanate Institute Inc., 2013. p. 48.