Journal of Chromatography B 1076 (2018) 117–129

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Trisaminohexyl isocyanurate, a urinary biomarker of HDI isocyanurate exposure

Zachary Robbins, Wanda Bodnar, Zhenfa Zhang, Avram Gold, Leena A. Nylander-French*

Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, NC 27599, USA

ARTICLE INFO

Keywords:
Biomarker
Exposure
Isocyanate
1,6-Hexamethylene diisocyanate
Isocyanurate
Liquid chromatography–mass spectrometry

ABSTRACT

Biological monitoring of occupational exposure to 1,6-hexamethylene diisocyanate (HDI)-containing spray-paints is limited to analysis of metabolites of HDI monomer although polymeric HDI isocyanurate constitutes the predominant inhalation and skin exposure for workers in the automotive paint industry. A novel method using nanoflow ultra-performance liquid chromatography coupled to nano-electrospray ionization tandem mass spectrometry (nano-UPLC-ESI-MS/MS) was developed to quantify trisaminohexyl isocyanurate (TAHI), a hydrolysis product of HDI isocyanurate, in the urine of spray-painters. Analytical and internal standards were synthesized in-house and weighted linear regression calibration curves were generated using spiked control urine from non-exposed persons (0.06–7.98 μg/L; $N = 13$; $w = x^{-2}$; $r = 0.998$). Urine samples collected from 15 exposed workers ($N = 111$) were subjected to acid hydrolysis and extracted with dichloromethane, then derivatized with acetic anhydride. The derivatized product, trisacetamidohexyl isocyanurate (TAAHI), was analyzed using nano-UPLC-ESI-MS/MS. The protocol was sensitive and specific for analysis of TAHI in the urine of exposed workers with a method detection limit at 0.03 μg/L. TAHI was detected in 33 of 111 urine samples and in 11 of 15 workers. This biomarker for HDI isocyanurate is critical to determine the relative potency and dose-relationships between the monomer and oligomer exposure on the development of diisocyanate induced health effects in future studies.

1. Introduction

Aromatic and aliphatic isocyanates are highly reactive, low-molecular-weight compounds included in the 187 hazardous air pollutants of the Clean Air Act Amendments of 1990 [1]. They are used in the manufacturing of many common products containing polyurethane such as adhesives, spray paints, foams, insulation, resins, sealants, and surface coatings [2,3]. One of the most commonly used isocyanates is 1,6-hexamethylene diisocyanate (HDI), comprised of the monomer and oligomers[1] (Fig. 1) [4]. Occupational exposure occurs during industrial production or during spray-painting operations such as auto-body refinishing or application of marine coatings [2]. Exposures in the general population can occur from contact with isocyanate-containing consumer goods, from slow-curing isocyanate coatings or materials used in housing construction, in outdoor areas near industrial sites where isocyanates are used in manufacturing, or in neighborhoods surrounding auto-refinishing businesses [5–11]. Exposures to aerosols and vapors of HDI monomer and oligomers, including HDI isocyanurate, are associated with a high risk of contact dermatitis and asthma [12–16]. Acute exposure can cause shortness of breath, rhinitis, irritation of the skin, eyes, and mucous membranes, and pulmonary edema [8,9,14,17].

Significant levels of inhalation and skin exposure to HDI monomer and its oligomers have been reported in spray-painters [18–22]. The predominant inhalation and skin exposure in automotive spray-painting is to HDI isocyanurate [20–22], but the relative contributions of exposure to the HDI monomer and isocyanurate in the etiology of immune sensitization and disease is currently unknown. The skin sensitization capacity of HDI isocyanurate has been indicated to be greater than the HDI monomer and HDI biuret in both humans and animals [16,23], and occupational asthma has been linked to HDI oligomer exposure without

Abbreviations: ^{13}C NMR, carbon-13 nuclear magnetic resonance spectroscopy; ESI, electrospray ionization; GC–MS, gas chromatography–mass spectrometry; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; HDA, 1,6-diaminohexane; HDI, 1,6-hexamethylene diisocyanate; HFBA, heptafluorobutyric acid; LC–MS, liquid chromatography-mass spectrometry; LLE, liquid–liquid extraction; MAPE, mean absolute percentage error; MDI, methylene diphenyl diisocyanate; MDL, method detection limit; PFPA, perfluoropentanoic acid; SPE, solid-phase extraction; SRM, selected reaction monitoring; TAAHI, trisacetamidohexyl isocyanurate; TAAHpI, trisacetamidoheptyl isocyanurate; TAHI, trisaminohexyl isocyanurate; TAHpI, trisaminoheptyl isocyanurate; TDA, toluenediamine; TDI, toluene diisocyanate; UPLC, ultra-performance liquid chromatography

* Corresponding author
E-mail address: leena_french@unc.edu (L.A. Nylander-French).

[1] Oligomers of isocyanates, which are indicated with different terms (prepolymers, polyisocyanates, adducts) in the literature, will be referred to as oligomers in this article.

https://doi.org/10.1016/j.jchromb.2018.01.021
Received 8 November 2017; Received in revised form 12 January 2018; Accepted 15 January 2018
Available online 31 January 2018

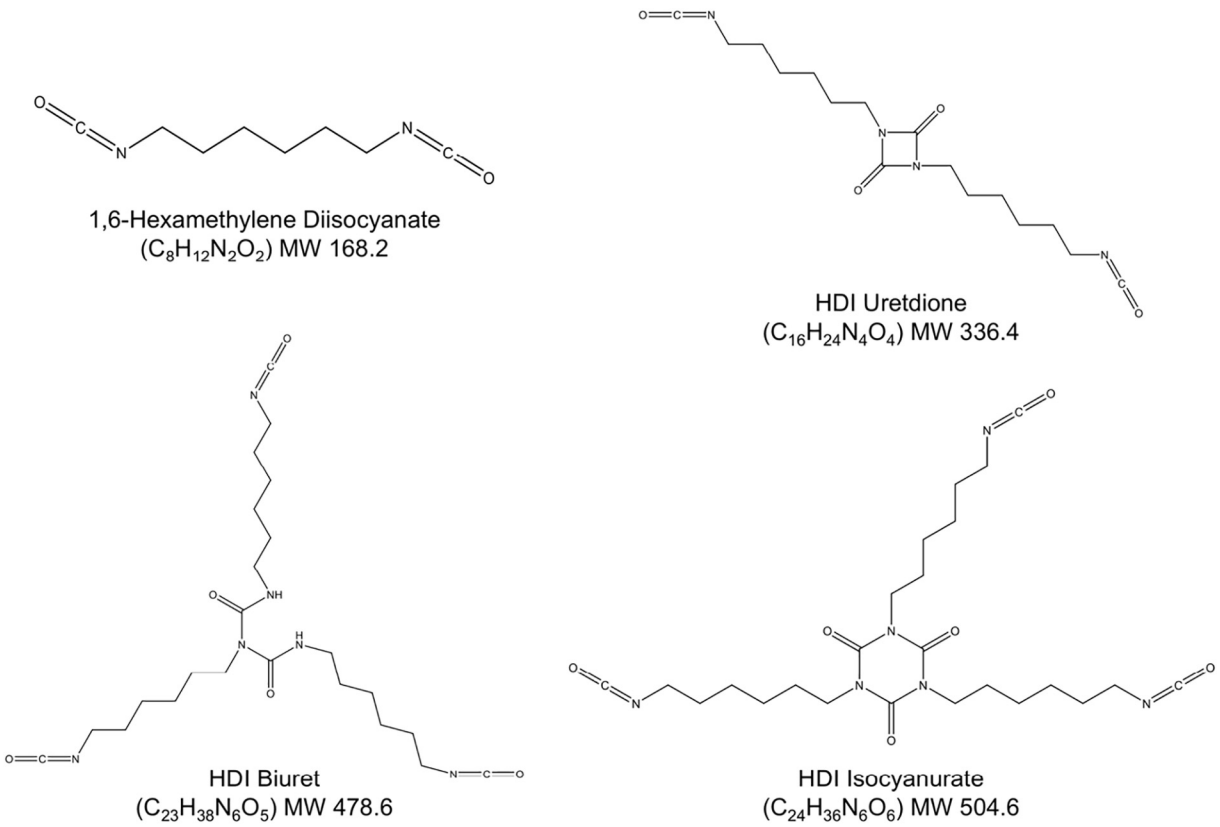

Fig. 1. Molecular structures of 1,6-hexamethylene diisocyanate monomer and its oligomers uretdione, biuret, and isocyanurate.

an immune response to the monomer [24]. Furthermore, it has been shown that HDI isocyanurate also penetrates skin at much faster rates (approximately 350 to 500 times) than HDI monomer [25]. Biological monitoring to estimate the systemic doses of HDI monomer and oligomers through exposure has been limited primarily to 1,6-diaminohexane (HDA), the hydrolysis product of HDI monomer, in urine and blood [18,19,26–31]. However, it has been shown that measured biomarker levels of HDI monomer exposure do not correlate with HDI oligomer exposure [32]. Until now a method has not existed to detect biomarkers of HDI isocyanurate exposure in urine or blood. Therefore, to investigate the relationship between external exposure, exposure routes, and biomarker levels, it is imperative that a biomarker for HDI isocyanurate exposure be established. This biomarker assay is also critical for investigation of relative potency and dose–response relationships of HDI monomer and oligomer exposures, to establish causality for associated health effects from monomer and/or oligomer exposures, and thus, to improve exposure and risk assessment for isocyanates. Towards this end, our goals were to: (i) design an extraction and derivatization protocol and liquid chromatography-mass spectrometry (LC–MS) method for analysis of trisaminohexyl isocyanurate (TAHI), a hydrolysis product and novel urine biomarker of HDI isocyanurate, and (ii) apply this method to quantify TAHI in urine collected from workers exposed to HDI isocyanurate during automotive spray-painting operations.

2. Experimental

2.1. Instrumentation

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were acquired on a Varian INOVA 400 (Palo Alto, CA) at 400 MHz for ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra. Mass spectra were acquired on a TSQ Quantum Ultra triple-quadrupole mass spectrometer with an electrospray ionization (ESI) source (Thermo Scientific, Waltham, MA) coupled to an Acquity ultra-performance liquid chromatography (UPLC) system (UPLC-ESI-MS/MS) (Waters Corp., Milford, MA), and a TSQ Quantum Ultra triple-quadrupole mass spectrometer with a nano-electrospray ionization source coupled to a NanoAcquity UPLC system (nano-UPLC-ESI-MS/MS) (Waters Corp.).

2.2. Synthesis of standards

The analytical standards required for sample processing and quantitative analysis were not available commercially; therefore, they were synthesized in-house. The synthesis and purification was a labor-intensive process and yielded limited quantities of the following four standards: 1,3,5-tris(6-aminohexyl)-1,3,5-triazinane-2,4,6-trione (trisaminohexyl isocyanurate; TAHI), *N*,*N*′,*N*″-((2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(hexane-6,1-diyl))triacetamide (trisacetamidohexyl isocyanurate; TAAHI), 1,3,5-tris(7-aminoheptyl)-1,3,5-triazinane-2,4,6-trione (trisaminoheptyl isocyanurate; TAHpI), and *N*,*N*′,*N*″-((2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(heptane-7,1-diyl))triacetamide (trisacetamidoheptyl isocyanurate; TAAHpI). The chemical structures are shown in Fig. 2. Composition and purity of the four standards were confirmed by NMR and LC–MS/MS in-house (see below).