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Determination of methyl isopropyl hydantoin from rat erythrocytes by gas-chromatography mass-spectrometry to determine methyl isocyanate dose following inhalation exposure

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ABSTRACT

Methyl isocyanate (MIC) is an important precursor for industrial synthesis, but it is highly toxic. MIC causes irritation and damage to the eyes, respiratory tract, and skin. While current treatment is limited to supportive care and counteracting symptoms, promising countermeasures are being evaluated. Our work focuses on understanding the inhalation toxicity of MIC to develop effective therapeutic interventions. However, in-vivo inhalation exposure studies are limited by challenges in estimating the actual respiratory dose, due to animal-to-animal variability in breathing rate, depth, etc. Therefore, a method was developed to estimate the inhaled MIC dose based on analysis of an N-terminal valine hemoglobin adduct. The method features a simple sample preparation scheme, including rapid isolation of hemoglobin, hydrolysis of the hemoglobin adduct with immediate conversion to methyl isopropyl hydantoin (MIH), rapid liquid-liquid extraction, and gas-chromatography mass-spectrometry analysis. The method produced a limit of detection of 0.05 mg MIH/kg RBC precipitate with a dynamic range from 0.05–25 mg MIH/kg. The precision, as measured by percent relative standard deviation, was < 8.5%, and the accuracy was within 8% of the nominal concentration. The method was used to evaluate a potential correlation between MIH and MIC internal dose and proved promising. If successful, this method may be used to quantify the true internal dose of MIC from inhalation studies to help determine the effectiveness of MIC therapeutics.

1. Introduction

Methyl isocyanate (MIC) is used for carbamylation of amines as an important precursor for the synthesis of carbamate pesticides and diisocyanates (i.e., intermediates in synthesis of plastics) (1–3). Although industrially important, MIC is also highly toxic, as tragically demonstrated by Bhopal disaster in 1984, where it is estimated that 8000 or more people died within minutes of exposure to MIC (4–6). Victims of MIC exposure suffer from severe health effects, both acutely and long-term, with MIC causing irritation and injury to the eyes, respiratory tract, and skin, with most damage occurring to the lungs and airways (7–9). MIC can enter the body via inhalation or skin contact with the toxicity of MIC stemming from its ability to readily react with electronegative groups in biological molecules (e.g., amino groups). Specifically, MIC mainly carbamoylates N-terminal amino acids of tissue proteins and side-chain amino groups of lysine (2,10,11), although about 20 other adducts have also been found (12). MIC-protein and DNA alkylation results in tissue hypoxia and cytotoxicity (10–15), targeting various organs, and leading to ophthalmic, respiratory, reproductive, neuromuscular, psychological, neural-behavioral, and other systematic problems (10,16–24).

While current treatment is limited to supportive care and treatment of symptoms, promising drug treatments are being evaluated. New investigations are underway which focus on understanding the inhalation toxicity of MIC to develop effective therapeutic interventions. However, in-vivo inhalation exposure studies are limited by challenges in determining the actual respiratory dose, because the dose depends on many factors other than vapor concentration and exposure time, including respiratory rate, tidal volume, minute volume, and deposition rate within various regions of the respiratory tract (25). These

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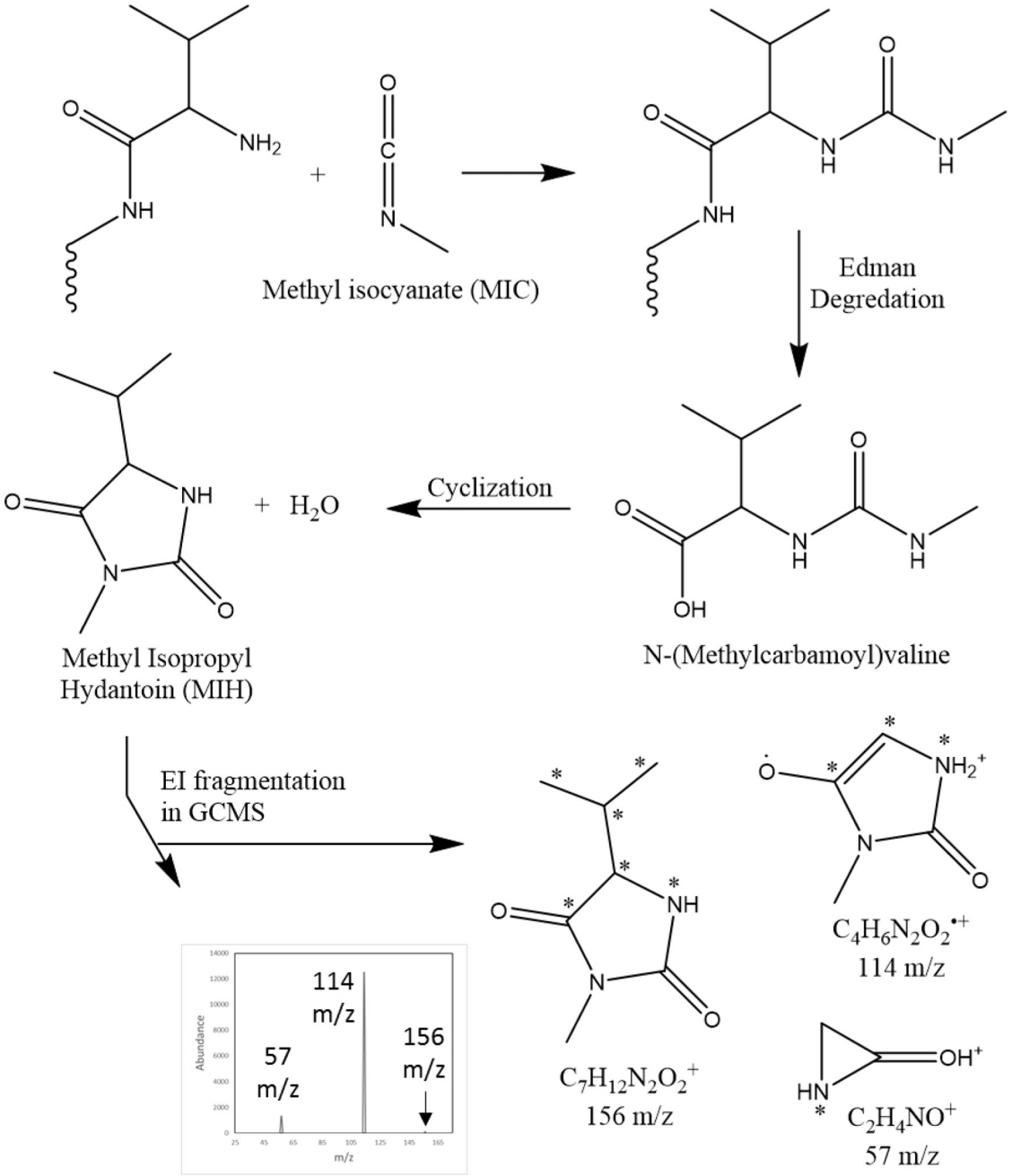

Fig. 1. Reaction of methyl isocyanate with the N-terminal valine of hemoglobin to produce a carbamoylated hemoglobin. The carbamoylated hemoglobin is hydrolyzed by Edman degradation to form N-(methylcarbamoyl)valine which cyclizes under acidic conditions to form MIH for subsequent analysis. Structures from electron ionization fragmentation and the resulting mass spectrum are also shown. Asterisks denote the locations of the stable isotopes in the internal standard for each of the fragments.

parameters are very difficult to estimate in animals, especially during active MIC exposure. Therefore, for inhalation exposures, it is difficult to quantify how much MIC is internalized (i.e., the "internal dose") from external parameters. To address this problem, quantification of a known MIC biomarker may allow better estimation of internal dose.

Because of its reactivity, MIC is quickly eliminated from biological systems, mainly via its reaction with protein-based amino groups. Therefore, biomarkers used to verify exposure to MIC are typically based on protein adducts, with the most common based on the reaction of MIC with the N-terminal valine in hemoglobin (Hb) (2,15,26–29). In fact, the MIC-N-terminal valine adduct has been used to verify exposure of individuals following the Bhopal disaster (15). Detection and quantification of the MIC-N-terminal valine Hb adduct is accomplished by the Scheme shown in Fig. 1, featuring acid hydrolysis, cyclization of the resulting N-(methylcarbamoyl)valine, and subsequent extraction of the methyl isopropyl hydantoin (MIH) using liquid-liquid extraction. This process is performed in each of the methods available for the analysis of the MIC-N-terminal hemoglobin adduct listed in Table 1. Although each of these methods are effective, most of the methods are arduous, lengthy, complex, generate a relatively large amount of organic waste, and consume considerable amounts of energy (Table 1). Mráz et al. (28) simplified the typical sample preparation as compared to the other methods in Table 1. Although the method was simplified to 20 steps and approximately 9 h, it still was lengthy, consumed considerable amounts of energy, and generated a large amount of organic waste. Wang et al. (29) also simplified the sample preparation of MIH from