K. DETERMINATION OF AIRBORNE ISOCYANATE EXPOSURE

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1. INTRODUCTION

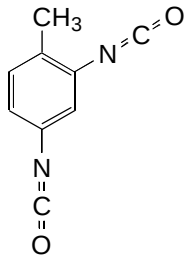
A variety of air sampling and analysis methods for determining workers' exposures to isocyanate-containing compounds have been published or are under development by NIOSH, OSHA, and others. The following chapter provides information on the health effects, exposure criteria, sampling considerations, and analytical considerations used at NIOSH to select isocyanate methods. The purpose is to provide information to the industrial hygienist, chemist, and client of laboratory services to make an informed decision on which isocyanate method is appropriate for a given exposure scenario. Summary tables of isocyanate exposure standards (Table I) and NIOSH and OSHA analytical methods for isocyanates (Table II) are included. Other discussions of air sampling methods and direct reading instruments have been published.[1,2,3,4]

Many material safety data sheets (MSDS) use isocyanate-related terms interchangeably. For the purpose of this discussion, terms are defined as follows.

Diisocyanates (Monomers): The difunctional isocyanate species from which polyisocyanates and polyurethanes are derived (Figure 1a). Common examples of monomeric isocyanates include 1,6-hexamethylene diisocyanate (HDI), 2,4- and/or 2,6-toluene diisocyanate (TDI), 4,4'-diphenylmethane diisocyanate (MDI), methylene bis(4-cyclohexylisocyanate (HMDI), isophorone diisocyanate (IPDI), and 1,5-naphthalene diisocyanate (NDI). Commercial-grade TDI is an 80:20 mixture of the 2,4- and 2,6- isomers of TDI, respectively.

Polyisocyanates: Species possessing free isocyanate groups and derived from monomeric isocyanates either by directly linking these monomeric units (a homopolymer) or by reacting these monomers with

di- or polyfunctional alcohols or amines (a copolymer). Figure 1b shows the structure of a TDI-based polyisocyanate.



Prepolymers: Species possessing free isocyanate groups, prepared from the reaction of a polyol with an excess of di- or polyisocyanate (Figure 1c).[5] Commercially available isocyanate products frequently contain prepolymers in lieu of more volatile isocyanate monomers.

Oligomeric Isocyanates (Oligomers): Relatively low molecular weight polyisocyanates.

Intermediates: Species possessing free isocyanate groups, formed during use of an isocyanate product by partial reaction of the isocyanate species with a polyol.

This discussion covers isocyanate-containing compounds, except monofunctional isocyanates, because monofunctional isocyanates have different industrial applications, such as the manufacture of pesticides, and have very different toxicities.

2. BACKGROUND

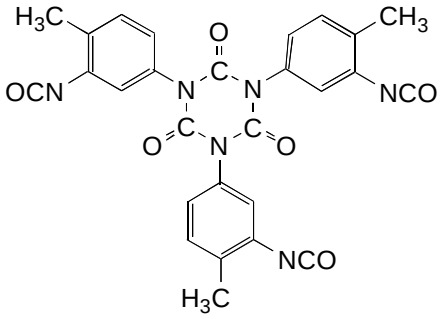
The feature common to all diisocyanates (monomers) is the presence of two -N=C=O (isocyanate) functional groups attached to an aromatic or aliphatic parent compound. These compounds are widely used in surface coatings, polyurethane foams, adhesives, resins, elastomers, binders, and sealants.

In general, the types of exposures encountered during the use of isocyanates (*i.e.*, monomers, prepolymers, polyisocyanates, and oligomers) in the workplace are related to the vapor pressures of the individual compounds. The lower molecular weight isocyanates tend to volatilize at room temperature, creating a vapor inhalation hazard. Conversely, the higher molecular weight isocyanates do not readily volatilize at ambient temperatures, but are still an inhalation hazard if aerosolized or heated in the work environment. The latter is important since many reactions involving isocyanates are exothermic in nature, thus providing the heat for volatilization. As exposure limits decrease, the volatility of solid materials becomes an issue. To reduce the vapor hazards associated with the lower molecular weight diisocyanates, prepolymer and polyisocyanate forms of these diisocyanates were developed and have replaced the monomers in many product formulations. An example is the biuret of HDI, which consists of three molecules of HDI monomer joined together to form a higher molecular weight oligomer having similar characteristics to those found in the monomer. Also, many MDI product formulations consist of a combination of MDI monomer and a MDI-based polyisocyanate (such as polymethylene polyphenyl isocyanate). Many prepolymer and polyisocyanate formulations contain a small fraction (usually less than 1%) of unreacted monomer.

Isocyanates exist in many different physical forms in the workplace. Not only are workers potentially exposed to the unreacted monomer, prepolymer, polyisocyanate, and/or oligomer species found in a given product formulation, they can also be exposed to partially reacted

a. 2,4-TDI monomer.

b. Polyisocyanate of TDI.



c. Prepolymer adduct of TDI and trimethylol propane.

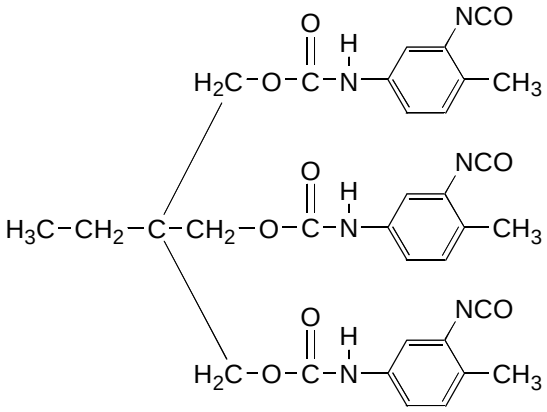


Figure 1. Examples of isocyanate structures.

isocyanate-containing intermediates formed during polyurethane production. In addition, isocyanate-containing mixtures of vapors and aerosols can be generated during the thermal degradation of polyurethane coatings and plastics. The capability to measure all isocyanate-containing substances in air, whether they are in monomer, prepolymer, polyisocyanate, oligomer, and/or intermediate forms, is important when assessing a worker's total airborne isocyanate exposure.

3. ISOCYANATE EXPOSURE RELATED HEALTH EFFECTS

Exposure to isocyanates is irritating to the skin, mucous membranes, eyes, and respiratory tract.[6,7] The most common adverse health outcome associated with isocyanate exposure is asthma due to sensitization; less prevalent are contact dermatitis (both irritant and allergic forms) and hypersensitivity pneumonitis (HP).[7,8,9] Contact dermatitis can result in symptoms such as rash, itching, hives, and swelling of the extremities.[6,9] A worker suspected of having isocyanate-induced asthma/sensitization will exhibit the traditional symptoms of acute airway obstruction, *e.g.*, coughing, wheezing, shortness of breath, tightness in the chest, and nocturnal awakening.[6,8] An isocyanate-exposed worker may first develop an asthmatic condition (*i.e.*, become sensitized) after a single (acute) exposure, but sensitization usually takes a few months to several years of exposure.[6,8,10,11,12] The asthmatic reaction may occur minutes after exposure (immediate), several hours after exposure (late), or a combination of both immediate and late components after exposure (dual).[8,11] The late asthmatic reaction is the most common, occurring in approximately 40% of isocyanate sensitized workers.[13] After sensitization, any exposure, even to levels below an occupational exposure limit or standard, can produce an asthmatic response which may be life threatening. Experience with isocyanates has shown that monomeric, prepolymeric and polyisocyanate species are capable of producing respiratory sensitization in exposed workers.[14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30] Since the intermediates may be chemically similar to these compounds, it is reasonable to assume that they may also produce this condition. Prevalence estimates for isocyanate-induced asthma in exposed worker populations vary considerably: from 5% to 10% in diisocyanate production facilities[10,31] to 25% in polyurethane production plants[31,32] and 30% in polyurethane seatcover operations.[33] The scientific literature contains a limited amount of animal data suggesting that dermal exposure to diisocyanates may produce respiratory sensitization.[34,35,36,37] This finding has not been tested in dermally exposed workers.

Hypersensitivity pneumonitis (HP) also has been described in workers exposed to isocyanates.[38,39,40,41] Currently, the prevalence of isocyanate-induced HP in the worker population is unknown, and is considered to be rare when compared to the prevalence rates for isocyanate-induced asthma.[9] Whereas asthma is an obstructive respiratory disease usually affecting the bronchi, HP is a restrictive respiratory disease affecting the lung parenchyma (bronchioles and alveoli). The initial symptoms associated with isocyanate-induced HP are flu-like, including shortness of breath, non-productive cough, fever, chills, sweats, malaise, and nausea.[8,9] After the onset of HP, prolonged and/or repeated exposures may lead to an irreversible decline in pulmonary function and lung compliance, and to the development of diffuse interstitial fibrosis.[8,9] Early diagnosis is difficult since many aspects of HP, *i.e.*, the flu-like symptoms and the changes in pulmonary function, are manifestations common to many other respiratory diseases and conditions.

The only effective intervention for workers with isocyanate-induced sensitization (asthma) or HP is cessation of all isocyanate exposure. This can be accomplished by removing the worker from the work environment where isocyanate exposure occurs, or by providing the worker with supplied-air respiratory protection and preventing any dermal exposures.

4. EXPOSURE CRITERIA FOR DIISOCYANATES

The primary sources of exposure criteria for workplace inhalation exposures are the following: (1) NIOSH recommended exposure limits (RELs),[42] (2) the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs®),[43] and (3) the U.S. Department of Labor, OSHA permissible exposure limits (PELs).[44] These exposure criteria are for diisocyanate monomers. In July 1992, the 11th Circuit Court of Appeals vacated the 1989 OSHA PEL Air Contaminants Standard. OSHA is currently enforcing the 1971 standards which are listed as transitional values in the current Code of Federal Regulations; however, some states operating their own OSHA approved job safety and health programs can have lower limits. Table 1 contains a comparison of the respective NIOSH RELs, ACGIH TLVs, OSHA PELs, and United Kingdom Health and Safety Executive (UK-HSE) exposure criteria for the isocyanates. The UK-HSE has taken a different approach, *i.e.*, developing a non-specific standard based on the total number of reactive isocyanate groups (TRIG) in a volume of air.[45] The standards and limits listed in Table 1 are based on a general standard that isocyanate exposures should not exceed an 8-hour time-weighted average (TWA) exposure of 5 parts per billion (ppb), or a short term or ceiling exposure of 20 ppb.

Table 1: NIOSH, ACGIH, OSHA, and UK-HSE Exposure Criteria for Isocyanates

Isocyanate Species	***Exposure Criteria - Full-shift TWAs Micrograms per cubic meter of air***			***Exposure Criteria - Short Term or Ceiling Limits Micrograms per cubic meter of air***			
	NIOSH REL	**ACGIH TLV**	**UK-HSE**	**NIOSH REL Ceiling**	**ACGIH TLV-STEL**	**UK-HSE Ceiling**	**OSHA PEL Ceiling**
TDI	CA-LFC[1]	36	None	None	140	None	140
MDI	50	51	None	200	None	None	200
HDI	35	34	None	140	None	None	None
HMDI	None	54	None	210	None	None	None
IPDI	45	45	None	180	None	None	None
NDI	40	None	None	170	None	None	None
TRIG[2]	None	None	20	None	None	70	None

[1] NIOSH considers TDI to be an occupational carcinogen (CA) and recommends that exposures be reduced to the lowest feasible concentration (LFC).
[2] TRIG - total reactive isocyanate group.

Table II. Comparison of NIOSH and OSHA Isocyanate Methods

	NIOSH 5521	**NIOSH 5522**	**NIOSH 2535**	**OSHA 42/47**	**PROPOSED NEW NIOSH[1]**
Isocyanate					
a) Monomers	TDI, MDI, HDI, NDI, HMDI[2]	TDI, MDI, HDI, NDI, HMDI,[2] IPDI [2]	TDI, HDI	42 TDI, HDI 47 MDI	TDI, HDI, MDI, NDI,[2] HMDI,[2] IPDI [2]
b) Oligomers	HDI	TDI, MDI, HDI	None	None	HDI, MDI,[2] TDI[2]
Sampler	impinger	impinger	coated glass wool/opaque tube	coated GFF	impinger; GFF; impinger+GFF

	NIOSH 5521	NIOSH 5522	NIOSH 2535	OSHA 42/47	PROPOSED NEW NIOSH[1]
Reagent	MOPP in toluene	tryptamine in DMSO	nitro reagent	1-2PP	MAP in butyl benzoate
Shelf Life	7d 0 C	6 mo 25 C in dark	7d 25 C in dark	42 0.1 mg; 47 1 mg 6 mo 0 C sealed	unknown
Sampling Rate **Volume**	1 L/min 5-500 L	1-2 L/min 15-360 L	0.2-1 L/min 2-170 L	1 L/min 15 L	1-2 L/min 1-500 L
Personal	No	No	Yes	Yes	Yes
Vapor	Yes	Yes	Yes	Yes	Yes
Particles 2 µm	No	No	No	Yes	impinger: No filter: Yes
Particles 2 µm					
a) Slow-cure	Yes	Yes	No	No[3]	impinger: Yes filter: No[3]
b) Fast-cure[4]	Yes	Yes	No	No	impinger: Yes filter: No
Sample Stability	7d 25 C: 78% 7d 4 C: 88%	28d 25 C in dark: 95-104%	14d 25 C: 91%	15d 22 C: 42 80-86%; 47 94.8%	unknown
Laboratory Sample Preparation	impinger: evap/ redissolve in methanol	none	ultrasonic extraction in methanol	extraction in ACN/ DMSO, 9 /1	impinger: SPE filter: extract or SPE
Technique	HPLC/RP, isocratic	HPLC/RP, gradient	HPLC/RP, isocratic	HPLC/RP, isocratic	HPLC/RP, gradient
Detector 1 **LOD[5]:** **a) amount injected** **b) 15 L air conc.**	UV @ 242 nm/ PDA 14 pmol 1.2 ppb	FL ex 275 nm em 320 nm 0.7 pmol 0.9 ppb	UV @ 254 nm 14 pmol 0.9 ppb	FL ex 240 nm em 370 nm 0.2 - 1 pmol 0.06 - 0.1 ppb	UV @ 253 nm 0.5 pmol 0.08 ppb
Detector 2 **LOD[5]:** **a) amount injected** **b) 15 L air conc**	EC (+ 0.8V) 0.5 pmol 0.04 ppb	EC (+ 0.8V)	None	UV @ 254 nm	FL ex 250 nm em 409 nm est. ~ 5 fmol est. ~ 0.8 ppt
Identification	Monomer: Retention Time Aliphatic oligomers: PDA	Monomer: FL Retention Time Other isocyanate: EC confirmation	Retention Time	Retention Time	Monomer: Retention Time Other isocyanate: UV/FL ratio

[1] This method is under development; procedures may change somewhat pending validation.
[2] Determination possible; lacks validation data.
[3] Usually underestimates concentration.
[4] Half-life under several minutes.
[5] Instrumental limit of detection

Abbreviations: ACN = acetonitrile; conc = concentration; d = days; DMSO = dimethyl sulfoxide; EC = electrochemical detector; em = emission; evap = evaporate; ex = excitation; FL = fluorescence detector; GFF = glass fiber filter; HPLC = high performance liquid chromatography; LOD = limit of detection; MAP = 1-(9-anthracenylmethyl)piperazine; mo = months; MOPP = 1-(2-methoxyphenyl)piperazine; nitro reagent = *N*-[(4-nitrophenyl) methyl] propylamine; PDA = photodiode array detector; 1-2PP = 1-(2-pyridyl)piperazine; RP = reversed phase; SPE = solid phase extraction; UV = ultraviolet detector

The NIOSH REL is for TWA diisocyanate exposures up to 10-hours per workday, and the ACGIH TLV is an 8-hour TWA exposure. The NIOSH RELs-ceiling limits and ACGIH short term exposure limits (STELs) are based on 10- and 15-minute TWA exposures, respectively; and should not be exceeded during the work shift. The OSHA ceiling limit is a concentration that should never be exceeded during a workday. OSHA does not have a full shift, TWA PEL for any of the diisocyanate species. The State of Oregon OSHA has promulgated occupational exposure standards for HDI-based polyisocyanates of 0.5 mg/m^3 as an 8-hour TWA, and a ceiling limit of 1.0 mg/m.[46]

5. AIR SAMPLING AND ANALYTICAL ISSUES

The measurement of isocyanates in air is a challenging sampling and analytical problem for several reasons. Isocyanates can exist in air as vapor, or as aerosol having a wide range of particle sizes. Isocyanates are very reactive, hence, unstable. There are many different chemical species, even in the same air sample, that need to be quantified. Pure analytical standards are not available for the vast majority of isocyanate species, and qualitative standards (bulk products) do not account for isocyanate species generated during polyurethane formation or breakdown. Finally, to measure isocyanates at levels corresponding to current monomer exposure limits, analytical methods must be very sensitive.

Because of the complex problems associated with accurate sampling and analysis of total isocyanate group in air, existing methods have limitations. To assess these limitations and to make rational decisions in choosing methodologies or making improvements to existing methodologies, it is useful to break down the sampling and analysis process, chronologically, into discrete steps. Each of these steps can be examined individually for the likelihood of isocyanate losses or the introduction of errors.

The sampling and analysis of isocyanates can be logically divided into six steps: collection, derivatization, sample preparation, separation, identification, and quantification. Each of these can result in losses of isocyanate or the introduction of other errors. In addition to the issue of accuracy, options for these steps differ in terms of convenience, simplicity, and speed. All of these have to be considered in choosing sampling and analytical methodology. Table II summarizes current OSHA and NIOSH sampling and analytical methods and lists the factors involved in selecting the most appropriate method for a given workplace environment.

a. Collection

Collection is the removal of the isocyanate species from the air sample into a portion of the sampler amenable to subsequent analysis. A generalized isocyanate sampler would have to be able to collect both vapor and aerosol of widely varying particle sizes. Mechanisms of collection of isocyanate vapors include dissolution into a solvent (*e.g.*, impingers) or adsorption onto a sorbent. In some cases, successful collection of isocyanate vapors may depend on reaction with a derivatizing reagent to create a nonvolatile derivative. The aspects of the sampler governing collection efficiency of particulate isocyanates are, of course, independent of the fact that these particles contain isocyanate groups.

Collection errors can be divided into three types:

(1) Aspiration errors: The efficiency with which particles enter the sampler inlet is the aspiration efficiency. The concentration of particles entering the sampler may be biased relative to the concentration outside the sampler or relative to human inhalation efficiency.

(2) Internal wall losses: There can be deposition of the isocyanate species on the internal walls of the sampler where it is not available for subsequent analysis.

(3) Transmission losses: Losses can occur from isocyanate species passing completely through the sampler.

The relative importance of these problems depends on the particle size, the sampler and inlet geometries, the sampling rate, and the collection mechanism of the sampler.

Samplers have been evaluated for aspiration efficiencies and internal losses.[47] A number of samplers have been designed with the intent to collect particles with efficiencies that match human inhalation efficiency.[48] One of these, the UK Institute of Occupational Medicine (IOM) personal inhalable sampler, has been recommended for isocyanate sampling.[49] Problems with poor aspiration efficiency and internal losses may be very important factors in the overall accuracy of the method. A recent study by the International Isocyanate Institute (III) on collection of MDI aerosol by filters and impingers found high variability in the aspiration efficiencies of particles with diameters in the 5 to 30 micrometers (μm) range.[50] This high variability is not inconsistent with what is often found in the sampling of large particles.[48] The III study also found that for large particles, a substantial percentage of the aerosol collected by filter samplers was deposited on the filter holder. It has also been shown that losses of relatively large particles can occur on the walls of both the inlet and the nozzle of the impingers.[47,51]

Assessments of collection efficiencies in isocyanate sampling have often been limited to measuring the relative amount of isocyanate species passing through the sampler. Based on this criterion, reagent-coated glass fiber filters (GFFs) appear to prevent the passage of isocyanate vapors and particles of widely varying sizes.[52,53] Recent studies have investigated the mechanisms by which particles pass through impingers.[54,55] Impingers have been found to prevent passage of vapors and particles greater than 2 μm in diameter, but allow substantial penetration of particles smaller than 2 μm.[50,56,57] Particles smaller than 2 μm include condensation aerosol (*e.g.*, environments where MDI is heated) and aerosol generated from combustion processes.

Two methods have been developed that segregate isocyanate species on collection according to their physical states. Being able to differentiate vapor and aerosol exposures is desirable because vapor and aerosol differ in their extent of penetration and deposition in the respiratory tract. These different types of exposures can result in different health consequences. One method uses a dual filter system, where aerosols are collected on a reagentless front filter and vapors collected on a reagent-coated back filter.[58,59] One potential problem with this system is the loss of isocyanate species in the aerosol fraction due to curing reactions occurring between the times of collection and post-sampling derivatization. This problem would be expected to be greater the longer the sampling time and the more reactive the isocyanate system. Another potential problem is the misclassification of semivolatile species, such as monomers, either by adsorption of vapor on the front filter or volatilization of species originally collected as aerosol. Another sampler used for isocyanates that separates vapor and aerosol consists of an annular denuder for vapor collection, followed by a reagent-coated GFF for aerosol collection.[60] A limitation of this system is that it is too large for personal sampling.

b. Derivatization

Once the isocyanate species have been collected, they must be efficiently derivatized. Derivatization of isocyanate species accomplishes two things. First, it stabilizes the isocyanate, which would otherwise be lost

to reaction with polyols or water. Second, it improves detection of the isocyanate by increasing sensitivity and selectivity.

The two most important factors in achieving efficient derivatization are the inherent reactivity of the reagent and the ability of the collection medium to dissolve or disperse collected particles or droplets and/or make derivatizing reagent accessible to the isocyanate groups. Derivatizing reagents in use today are most commonly primary or secondary aliphatic amines and their inherent reactivities with isocyanates typically differ by less than a factor of five.[61,62] This difference is probably not very important. Probably of greater importance is the efficiency of mixing the collected particles and derivatizing reagent.

Aerosols generated from spray applications of isocyanate products typically contain a mixture of isocyanate and polyol. If the polyol and isocyanate are not separated and the derivatizing reagent is not accessible to the isocyanate group at the time of collection, the isocyanate will be lost to reaction with the polyols within the droplet.[2,63] This would appear to be a significant problem for collection of droplets on reagent-coated GFFs. Micrographs of spray paint droplets on GFFs show that droplets typically make only minimal contact with the fibers.[51] This is not conducive to dispersal of the droplet or mixing of the reagent coated on the fiber with the isocyanate species. The larger the droplet, the greater the problem is likely to be because of the greater potential deficiency of reagent at or near the sites of contact with the fibers. Derivatization of isocyanate species present in particles or droplets is expected to be more efficient using impinger collection. The solvent not only serves to disperse or dissolve the droplet or particle, thereby interfering with the curing reaction but also provides a means for bringing the reagent and the isocyanate species together.

Two practices have been investigated that appear to improve the performance of reagent-coated GFFs when sampling aerosols. One is the presence of a small amount of nonvolatile solvent, such as diethyl phthalate or diphenylmethane, on the filter.[64,65,66,67] Another is the desorption of the filter in a solution of derivatizing reagent immediately after sampling.[66]

c. Sampling: Collection plus Derivatization

Reagent-coated GFFs and sorbents, impingers, and bubblers have all been investigated as samplers for isocyanates. Some sampler comparisons have been conducted under laboratory conditions and others in the field. The majority of comparisons have found that solventless samplers give higher results than impingers or bubblers in laboratory evaluations and impingers and bubblers give higher results in the field.[2] A likely explanation is that collection efficiency is most important in laboratory evaluations, under which conditions the polyols that compete with derivatizing reagent are generally not present. Under conditions where derivatization kinetics are relatively unimportant, reagent-coated GFFs may give higher results than impingers because the GFFs collect small particles more efficiently. In the field, where derivatization rate is clearly important, impingers and bubblers tend to give higher results than solventless samplers. Evidence for this is especially strong in environments where rapidly curing MDI-based products are sprayed.[67,68,69,70] The literature is more ambiguous concerning environments where less reactive HDI-based products are used.[71,72,73,74]

Table II gives recommendations for sampler use based on the size and cure rate of the isocyanate aerosol. The cure rate of isocyanate systems depends on the nature of the isocyanate, the nature of the co-reactant (typically polyols), the type of catalysis (if any), and the temperature. The half-lives of isocyanate species in reactive systems (*i.e.*, the time it takes for half of the isocyanate groups to undergo a curing reaction) can vary from a few seconds to many hours. For the purposes of this document, isocyanate systems having half lives of a few minutes or less are considered fast cure. The faster the product cures, the more strongly an impinger is

recommended for sampling. Also, the larger the particle size, the more strongly an impinger is recommended. An impinger is recommended for all aerosols having particle diameters greater than 2 µm because it is believed that the poor mixing on filters results in poor derivatization efficiency. Based on the thin-walled sampler model of Vincent,[75] estimation of the aspiration efficiency of the impinger indicates reasonably close agreement with the inhalability convention for particles smaller than 20 µm when ambient wind speed is low (as is typical for indoor workplace environments[76]). For larger particle sizes and relatively high wind speeds, the impinger is expected to undersample relative to the inhalability convention. Filters are recommended for sampling particles smaller than 2 µm because these particles are known to be collected inefficiently by impingers and because smaller particles, by requiring much less reagent than large particles, are less susceptible to local depletion of reagent. For environments that are likely to have isocyanate species present as both large and small particles, a sampling train consisting of an impinger followed by a reagent-coated filter is recommended.[2,49]

There are other factors in addition to accuracy that must be considered in choosing a sampler. The use of an impinger is considerably less convenient than use of a filter. Impingers may even be deemed inappropriate for personal sampling. NIOSH Method 5521[77] (which is adapted from United Kingdom Health and Safety Executive Method MDHS 25[78]) uses an impinger containing a solution of reagent in toluene. Because the toluene vaporizes extensively during sampling, NIOSH does not recommend this method for personal sampling. NIOSH Method 5522[79] uses an impinger containing a solution of dimethyl sulfoxide (DMSO). Because DMSO is readily absorbed through the skin, NIOSH recommends that DMSO impingers be used for area air sampling only. The new NIOSH method under development uses impingers containing a solution of reagent in butyl benzoate. Since butyl benzoate is a non-volatile solvent, sampling with it generates minimal vapor. It does not elicit the same concern as DMSO in terms of dermal absorption.

d. Sample Handling and Preparation

Sample handling and preparation include those steps taken to stabilize the sample or make the sample more compatible with the analytical procedure. Sample handling considerations actually begin before sample collection. Some reagents and sampling media have limited shelf lives and require special storage conditions. For example, the nitro reagent and 9-(methylaminomethyl)-anthracene (MAMA) are known to be light sensitive.[4,80] It has also been found that filters coated with 1-(2-pyridyl)piperazine show substantial reagent loss to the back-up pad during storage.[81] This loss is greatly reduced by storing the reagent-coated filters in the freezer before use. Users of methods need to be aware of such problems and follow the method's guidelines for storage of sampling media.

All filter methods require extraction of the filter with a suitable solvent prior to analysis by high performance liquid chromatography (HPLC). The Iso-Chek™ method,[58,59] which utilizes a reagentless filter, requires that extraction with a derivatization solution be done immediately after sampling. It has been reported that extraction in the field, preferably with a reagent solution, is beneficial for reagent-coated filters as well.[66] Typically, reagent-coated filters are transported to the laboratory, where extraction takes place prior to analysis. It may be desirable to filter the extraction solution prior to analysis by HPLC.[49]

Impinger methods frequently require the impinger solvent to be exchanged to a more HPLC-compatible solvent prior to analysis. This is generally achieved by evaporation of the impinger solvent to dryness and the redissolution of the sample in a solvent more compatible with HPLC analysis. Toluene impingers are typically treated in this manner.[77,78] If the volume of solvent used for redissolution is relatively small (or the redissolution solvent is easily concentrated), this step can also serve to concentrate the sample and improve the LOD of the method. Losses can conceivably occur because of incomplete redissolution of the sample components. Ultrasonication of the reconstituted solution may be done to facilitate redissolution.[77] Evaporation/redissolution requires that the impinger solvent be somewhat volatile. Unfortunately, a volatile solvent is undesirable during sampling because of the potential for vapor exposures as well as fire hazards.

If a method uses a non-volatile, HPLC-incompatible impinger solvent, solvent exchange can be accomplished by solid-phase extraction (SPE). This is the procedure used to remove the butyl benzoate in the NIOSH method currently under development. SPE has the advantage of being readily automated. Also, it may enable removal of excess reagent and impurities prior to HPLC analysis. A drawback is the potential for incomplete elution of isocyanate species.

If the derivatizing reagent is not removed prior to HPLC analysis (*e.g.*, by SPE), methods frequently recommend acetylation of the excess reagent with acetic anhydride prior to sample injection.[49,77,82] Excess reagent typically gives a large, tailing peak near the beginning of the HPLC chromatogram. This tail may interfere with the quantification of analytes. Also, repeated injection of large amounts of amine reagent can degrade the analytical column. The acetylated reagent does not tail appreciably and its presence in the sample does not degrade the analytical column.

In cases where a method uses an impinger solvent compatible with HPLC analysis, pre-analysis sample preparation may not be necessary. In NIOSH Method 5522,[79] an aliquot of the DMSO impinger solution is injected directly into the HPLC. This is simple, saves time, and avoids losses of isocyanate that can result from sample manipulation.

e. Separation

The first step in the analysis of a solution containing derivatized isocyanates is the separation. The separation technique isolates individual compounds from a complex mixture to enable correct identification and accurate quantification of each component. A total isocyanate method will be biased low if the separation technique does not deliver all the derivatized isocyanate species to the detector(s) as identifiable and quantifiable peaks.

Reversed-phase HPLC has been the dominant separation technique in isocyanate analysis.[1,3,4] A reversed-phase HPLC analysis can be isocratic, *i.e.*, having a constant mobile phase strength, or gradient, *i.e.*, having the mobile phase strength increasing during the course of the analysis. Isocyanate samples frequently contain compounds of greatly varied molecular weight, which translates to greatly varied retention in an isocratic analysis. Such an analysis is necessarily long, with late eluting peaks that are broad and difficult to detect and quantify accurately.

Gradient elution is frequently used when compounds of widely differing retention need to be determined in the same analysis. Weakly retained compounds are eluted early in the chromatogram with a relatively weak mobile phase. Then the mobile phase is strengthened to accelerate the elution of more highly retained compounds. Not only do these compounds elute faster than in an isocratic analysis, resulting in shorter analysis times, but also the peaks are taller and narrower, improving the LOD and facilitating peak integration.

Several total isocyanate methods have been evaluated for recovery of derivatized oligomeric isocyanate species. An evaluation of MDHS 25 found an average recovery of 105% for three isocyanate prepolymers.[83] In contrast, two separate investigations of prepolymers using NIOSH 5521 (which is very similar to MDHS 25) found 58% and 60% recoveries, respectively.[84,85] An evaluation of NIOSH 5522 found average recoveries for five prepolymer products of 62%.[85] MDHS 25 and NIOSH 5521 are isocratic methods, whereas NIOSH 5522 increases the strength of the mobile phase slightly during the analysis. These evaluations did not distinguish between actual physical losses of isocyanate and apparent losses resulting from prepolymers having lower detector response factors than the monomers used for calibration. However, the similarities in recoveries, comparing NIOSH 5521 and NIOSH 5522, suggest that physical losses are likely. If this is the case, a method using a stronger gradient may improve recoveries. This contrasts with the experience of the developers of MDHS 25/2 who have found no additional isocyanate-derived peaks even after using a gradient ending and holding at 100% acetonitrile.[86]

Gradient elution most frequently involves increasing the percent of organic modifier in the mobile phase. There are several disadvantages to this approach relative to isocratic analyses. It requires preparation of at least two mobile phases. Although the analytes elute faster, there may be considerable time required between analyses to allow for the system to reequilibrate to the initial conditions.[87] The baseline of the chromatogram is likely to change during the gradient, making it more difficult to integrate small peaks. Finally, artifact peaks can elute as the mobile phase is strengthened.[88] There are also some disadvantages of gradient elution of particular importance in isocyanate analysis. Several methods for total isocyanate (MDHS 25,[78] NIOSH 5522,[79] and the Ontario Ministry of Labour tryptamine method[82]) use electrochemical (EC) detectors. Unfortunately, EC detectors are somewhat incompatible with gradient elution because they are especially sensitive to changes in mobile phase. Also, HPLC-based total isocyanate methods quantify isocyanate species for which analytical standards are not available by assuming the detector response per isocyanate group is the same as that of a derivatized monomer standard. Even if the responses are the same in the same mobile phase, the response of a late-eluting isocyanate species may be quite different than that of the derivatized monomer if the two are eluting in substantially different mobile phases. Therefore, there is a potential for quantification errors.

The method under development at NIOSH uses a pH gradient to accelerate the elution of highly retained compounds, rather than the more common organic modifier gradient. This is made possible because isocyanate derivatives of 1-(9-anthracenylmethyl)piperazine[62,89,90] (MAP) contain a highly basic tertiary amine group that is easily protonated. The degree of protonation, which is controlled by the mobile phase pH, has a very large effect on the retention of MAP derivatives, especially those containing multiple derivatized isocyanate groups. Several disadvantages associated with organic modifier gradients are minimized or eliminated with the pH gradient. Reequilibration time between runs is very short, baseline changes during the gradient are relatively small, and elution of artifacts originating from the mobile phase solvents is less likely because the gradient selectively accelerates compounds with amine functionalities. Similarly, if a MAP-isocyanate derivative should co-elute with a non-amine interferant, a small change in pH gradient will move the MAP derivative away from the interferant. With a nonselective organic modifier gradient, the separation of an analyte from a co-eluting interferant is not so straightforward since both compounds will respond to a change in the gradient. The UV response of MAP-derivatized isocyanates is only minimally affected by the changing pH. The fluorescence (FL) response of MAP derivatives is greatly affected by mobile phase pH, but this is readily corrected by lowering the pH with post column addition of acid. Disadvantages of pH gradients are that they are limited to ionizable compounds and these ionizable compounds are more prone to problems associated with adsorption in the chromatographic system. These problems may include tailing peaks, reduced peak heights and areas, and carry-over. Special procedures or equipment (such as inert fluid paths in the HPLC or base-deactivated analytical columns) are necessary to avoid adsorption problems.

Another type of gradient that has been used for total isocyanate analysis is a mobile phase flow gradient.[91] Instead of changing mobile phase composition, the flow rate is increased to reduce the retention time of late-eluting compounds. The advantages of this procedure are that only one mobile phase is required, there is minimal disturbance of the baseline, and no mobile-phase related artifacts are eluted. One of the disadvantages is that, although peaks are narrower, UV or FL detector responses decrease with increasing flow rate. Also, since the acceleration of the analytes is directly proportional to the flow rate increase, pressure limitations make the accelerating power of this gradient modest compared to organic modifier or pH gradients.

Capillary-zone electrophoresis (CZE) has been investigated for isocyanate analysis.[92] It has several advantages relative to HPLC — low solvent consumption, relatively short analysis time, higher resolution, and a greater certainty that all analytes will reach the detector. Moreover, there is a degree of selectivity associated with the technique because only charged analytes migrate to the detector. The major disadvantage of CZE is the relatively poor concentration sensitivity owing to the extremely small injection volumes used. Both 1-(2-methoxyphenyl)piperazine (MOPP) and MAP have been found to be useful derivatizing reagents for CZE analysis.[93] One advantage that MAP derivatives possess is that they are more basic than MOPP derivatives. As a result, they are protonated in the solutions of relatively high organic content needed for dissolving oligomeric isocyanate species.

f. Identification

In typical chromatographic analyses of environmental contaminants, analytical standards exist for the analyte of interest. The analyte is identified as such in a real sample if its chromatographic retention time matches that of the analytical standard. However, for isocyanate species, pure analytical standards generally exist only for derivatized monomers. Moreover, in many environments, monomers contribute very little to the total isocyanate group present. The analysis of a derivatized bulk of prepolymeric isocyanate product can be very useful in identifying non-monomeric isocyanate species in real samples collected during use of that product. There are limitations to using such products as analytical standards for identification and quantification. Not

all isocyanate species to which a worker may be exposed are present in the product. When isocyanate products are being used, the components are typically undergoing curing reactions with polyols, so new species containing isocyanate groups are being generated. Isocyanate-containing species are also generated during thermal breakdown of polyurethane. Chromatographic retention times are not available to identify these new species. In order to identify all isocyanate species — monomers or oligomers, those present in the bulk product and those newly generated — a means of identification other than chromatographic retention time is necessary.

Correct identification of unknown isocyanate species requires that the detection scheme be selective or provide some qualitative information about the species in question. Isocyanate methods generally utilize derivatizing reagents that are responsible for the detectability of the reagent/isocyanate derivative. Total isocyanate methods generally seek to identify all compounds labeled with the derivatizing reagent. Knowledge of the work environment is required to discount any nonisocyanate species that may react with the derivatizing reagent and give a signal in the sample chromatogram. Once these compounds are accounted for, it is assumed that all other compounds in the chromatogram that contain the reagent label are derivatized isocyanates.

A single non-selective detector (such as a UV detector) is insufficient for total isocyanate analysis because it provides little qualitative information about the chromatographic peaks. However, two detectors in series provide a considerable amount of qualitative information. The reason for this is that when the responses for each detector are in the detectors' linear operating range, the ratio of detector responses is a constant (*i.e.*, independent of concentration) for any given compound. Therefore, a compound can be identified by that ratio. If the detector responses of derivatized isocyanates are derived primarily from the reagent label, then it is conceivable that all derivatized isocyanates would have similar detector response ratios and could be identified on the basis of those ratios. Several total isocyanate methods operate by this strategy.[78,79,82,84]

A widely used method for total isocyanate group is MDHS 25.[78] It identifies isocyanates derivatized with MOPP based on the ratio of EC and UV detector (242 nm) responses. The EC detector is sensitive and fairly selective,[94] but it also has been found to be relatively unstable.[82,95] UV absorbance at 242 nm is not selective and not especially strong for MOPP-derivatized isocyanates.[62] As a result, a substantial portion of the absorbance of MOPP-derivatized aromatic isocyanates comes from the aromatic portion of the isocyanates rather than from the MOPP-derivatized isocyanate group. Under these circumstances, an oligomeric aromatic isocyanate compound containing more aromatic rings per isocyanate group than the derivatized monomer will have a substantially higher UV response per isocyanate group than the derivatized monomer. This could adversely affect the identification of unknown isocyanate species. This problem was demonstrated in a study involving 2,4-TDI-based urethane oligomers.[96] This same study found that the EC detector response for these compounds is not directly proportional to the number of derivatized isocyanate groups. It was concluded that MOPP-derivatized oligomeric isocyanates, particularly oligomeric aromatic isocyanates, are likely to give EC/UV ratios substantially different from the EC/UV ratio of the MOPP-derivatized monomer and therefore not recognizable as isocyanates.

Methods based on tryptamine derivatization, including NIOSH 5522[79] and the method developed by the Ontario Ministry of Labour,[82] use FL and EC detection in series for identification. Estimations of the compound-to-compound variabilities of detector responses, as indicated by their relative standard deviations (S_r), vary in different studies (FL S_r 13-26% and EC S_r 18-71%).[62,82,97] Not only is the compound-to-compound response variability relatively small for FL detection of tryptamine derivatives, but the selectivities of FL and EC detection are much greater than that of UV detection. Overall, FL/EC detection of tryptamine derivatives would appear to give more reliable identification than EC/UV of MOPP derivatives.

A total isocyanate method based on derivatization with MAMA and either FL/UV or UV/UV detection has been developed.[91,98] MAMA absorbs very strongly in the UV at 256 nm. Even though this is not a particularly selective wavelength, the intensity of the absorbance is so great that nearly all the absorbance of a MAMA-derivatized isocyanate at 256 nm comes from the MAMA group. This results in very small compound-to-compound variability of UV responses (S_r 7-14%)[62,98], which facilitates identification. Secondary absorbances (such as 366 nm) are relatively weak but highly selective, so that UV/UV ratios are very constant and diagnostic (S_r 3-8%).[62,98] FL compound-to-compound variability is relatively high (S_r 55-59%).[62,98] But, as in the case of tryptamine, the inherent selectivity of FL detection makes FL/UV identification attractive, especially given the substantially better sensitivity than a method relying on UV/UV identification.

The NIOSH method under development using the MAP reagent relies on FL/UV detection for identification. Like MAMA, MAP contains an anthracene group as the chromophore/fluorophore so that the absorbance and fluorescence characteristics of the two reagents are very similar. MAP derivatives appear to have somewhat lower compound-to-compound variabilities in both UV (S_r 3.5%) and FL (S_r 33%) responses than the corresponding MAMA derivatives.[62]

Instead of using two one-dimensional detectors in series, some methods utilize multidimensional detectors for identification. It has been found that photodiode array (PDA) detection, which provides an entire UV spectrum of a chromatographic peak, is useful in identifying MOPP-derivatized isocyanates.[95] The developers of MDHS 25/2 now advocate the use of the PDA detector to strengthen identification.[86] The disadvantage of the PDA is that it is somewhat less sensitive than standard UV detectors. Several researchers have investigated the mass spectrometer (MS) as a detector for derivatized isocyanates.[99,100,101,102] The MS has the potential to go beyond simply identifying a compound as a derivatized isocyanate. It can provide considerable information about the structure of the compound and serves as an important research tool. The primary disadvantages of using HPLC/MS for typical sample sets are the expense of the instrument and the expense and nonroutine nature of its operation.

g. Quantification

Once a chromatographic peak has been correctly identified as being isocyanate-derived, it must be quantified. For methods used to determine monomeric isocyanates only, identification is generally based on retention time and analytical standards exist that enable direct construction of a calibration curve for quantification. The major factor in choosing a derivatizing reagent/detector combination is LOD. The LODs for reagent/detector combinations used in NIOSH and OSHA methods are given in Table II. With regard to NIOSH and OSHA methods limited to measuring monomer, NIOSH 2535[103] uses the nitro reagent with UV detection to determine TDI and HDI. This reagent/detector combination provides relatively poor sensitivity. OSHA 42[104] and OSHA 47[105] use 1-(2-pyridyl)piperazine (PP) to derivatize TDI, HDI, and MDI with detection by either FL or UV. FL detection of PP derivatives provides fairly good sensitivity.

Methods for quantifying non-monomeric isocyanate species can be divided into those that use the bulk product for calibration, such as Miles (Bayer) Method 1.4.3,[106] and those that quantify every isocyanate derivative in the chromatogram based on the response of the derivatized monomer (*e.g.*, MDHS 25/2[49] and NIOSH Method 5522[79]). Miles Method 1.4.3 uses known amounts of derivatized bulk isocyanate product to construct a calibration curve.[106] The areas of the largest peak or several largest peaks in the chromatogram are plotted against the concentration of the product injected. Using this calibration curve, the peak areas in real samples can be correlated with a quantity of bulk isocyanate product. Taking this a step further, the total isocyanate concentration in the sample can be calculated using the known or measured isocyanate content of the bulk

product. There are several advantages to this approach. It requires the integration of at most a few peaks in the sample chromatogram. Since identification is based on retention time, only a single detector is needed. Finally, it does not depend on all isocyanate species in the sample eluting from the HPLC as identifiable peaks. The disadvantage of this approach is that it does not enable accurate quantification of total isocyanate group in all environments. For example, this approach cannot be used to quantify the isocyanate species produced by thermal decomposition of polyurethane. Also, even when an isocyanate product is being used, this approach cannot take into account newly formed isocyanate species that are not present in the product. The approach assumes that the major components in the bulk are the major components to which exposure occurs. This is probably a reasonable assumption for spraying relatively slow-curing isocyanate products. The more reactive the product and the greater the time between initiation of curing reactions and exposure, the more likely that the composition of the isocyanate species to which exposure occurs will differ significantly from the composition in the bulk product.

Total isocyanate methods that attempt to quantify every isocyanate species in a chromatogram typically use two detectors for identification; only one detector is required for quantification. Nevertheless, the method LOD is generally limited by the LOD of the less sensitive detector, since below that LOD a compound cannot be properly identified. A possible exception to this is for isocyanate compounds that are present in the bulk product. Analysis of a relatively high concentration of the derivatized bulk product enables evaluation of each chromatographic peak using both detectors. Derivatized isocyanates can then be identified in real samples if their retention times match those of compounds identified as isocyanates in the bulk product, even when the levels are below the LOD of the less sensitive detector.

In addition to LOD considerations, an important aspect of quantification of total isocyanates is accuracy. To quantify compounds for which analytical standards are not available, the detector response factor for the unknown derivatized isocyanate species must be the same as that of the derivatized monomer. This is achieved by choosing a derivatizing reagent/detector combination such that nearly all the detector response is attributable to the derivatization reagent label and that response does not change from compound to compound. Whereas a certain amount of compound-to-compound variability in detector response is tolerable for compound identification, especially when dealing with very selective detectors, compound-to-compound response variability in the detector used for quantification translates directly to errors in quantification.

For MDHS 25,[78] which quantifies MOPP-derivatized isocyanates by EC, two studies have found compound-to-compound variability of the EC response for model compounds to be S_r of 26%[97] and 28%[62], respectively. Also, a study of MOPP-derivatized urethane oligomers found that the EC response is not proportional to the number of derivatized isocyanate groups. Instead, it was found that the EC response increased as the size of the oligomer increased.[96] Methods employing the tryptamine reagent use FL and EC detection; FL is recommended for quantification. Studies have found the compound-to-compound variabilities by FL in the range of S_r 13-26%.[62,82,97] The sensitivity for detection of tryptamine-derivatized isocyanates by FL is fairly good.

Methods employing the MAMA and MAP reagents use UV and FL detection. The very small compound-to-compound variability in UV response for MAMA- and MAP-derivatized isocyanates, coupled with the fairly good sensitivity for UV detection around 256 nm, makes quantification of MAMA and MAP derivatives by UV arguably superior to other reagent/detector combinations. The sensitivities of detection for MAMA and MAP derivatives by FL are better than any other reagent/detector combination in common use for isocyanate determination. Unfortunately, the compound-to-compound variability is unacceptably high for quantification of derivatized isocyanates for which standards are unavailable. Fluorescence detection can be used to quantify

monomers. It may also be suitable for quantification of other isocyanate species present below the UV LOD if their UV/FL ratios have been previously determined in a sample containing levels above the UV LOD.

Excellent limits of detection have been reported for HPLC/MS, comparable to or better than those of MAMA and MAP derivatives by FL.[99,100] A limitation of MS in quantifying isocyanate species for which analytical standards are not available is that compound-to-compound variability in response is likely to be very high. This is because the kinetics of the processes that produce ions for quantification in the MS are greatly influenced by the molecular structures of the compounds. As a result, quantification of non-monomeric isocyanate species based on the monomer response is likely to be very inaccurate.

6. CONCLUSIONS

The ability to measure isocyanate-containing substances in air, whether they are in monomer, prepolymer, polyisocyanate, and/or oligomer forms, is important when assessing a worker's isocyanate exposure. Adverse health outcomes from isocyanate exposure include irritation to the skin, mucous membranes, eyes, and respiratory tract; contact and allergic dermatitis; hypersensitivity pneumonitis; and respiratory sensitization (asthma). Respiratory sensitization (an asthma-like response) is the most common of these health outcomes with a prevalence ranging from 5 to 30% of workers in a variety of industrial processes. Experience has shown that monomeric, prepolymer, polyisocyanate, and oligomeric isocyanate species are capable of producing respiratory sensitization in exposed workers. After sensitization, any exposure, even to levels below existing occupational exposure limits or standards, can produce an asthma-like response that may be life threatening.

Accurate and sensitive determination of isocyanates is complex and difficult. The advantages and disadvantages of the various methods must be understood, in order to choose the most appropriate sampling and analytical method for a particular workplace environment. Isocyanates may be in the form of vapors or aerosols of various particle size; the species of interest are reactive and unstable; few pure analytical standards exist; and high analytical sensitivity is needed. In addition, there are numerous points in the sampling and analytical procedures where errors can be introduced. The selection of the most appropriate isocyanate method for a given workplace environment is based upon an evaluation of measurement accuracy, specificity, sensitivity, convenience, simplicity, and speed. These factors must be considered for the entire analytical measurement process including collection, derivatization, sample preparation, separation, identification, and quantification. Unfortunately, the need to measure highly reactive isocyanate species at low levels is many times in conflict with the desire of industrial hygienists and chemists to choose methods that are convenient to use in the field and are easy to run in the laboratory.

Table I summarizes NIOSH, OSHA, ACGIH, and United Kingdom HSE isocyanate exposure standards. Table II summarizes NIOSH and OSHA isocyanate methods and method selection for a given workplace environment. The selection of the most appropriate isocyanate method depends upon the isocyanate species, its physical state, its cure rate, the sensitivity required and other factors shown in Table II. This information, which is used to select methods for NIOSH research studies and health hazard evaluations, is provided when employers, industrial hygienists, or laboratories request NIOSH technical assistance on isocyanate methods.

In closing, more research is needed to resolve the limitations of current sampling and analytical methods. Such research is ongoing at NIOSH and elsewhere in government, in academia, and in the private sector. Therefore,

this guidance is subject to revision as isocyanate exposure standards change and as new or improved isocyanate analytical methods are published.

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