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Evaluation of Tryptamine in an Impinger and on XAD-2 for the Determination of Hexamethylene-based Isocyanates in Spray-painting Operations

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Tryptamine was evaluated as a reagent for derivatizing hexamethylene diisocyanate (HDI) monomer and oligomers during actual spray-painting operations. In one side-by-side sampling study, an impinger filled with 1-(2-methoxyphenyl)piperazine in toluene was compared with a second impinger filled with tryptamine in dimethyl sulfoxide (DMSO). The amount of HDI monomer obtained was below the limit of quantification for both impingers. The amount of HDI oligomer obtained when using 1-(2-methoxyphenyl)piperazine in toluene was comparable to the amount obtained when using an impinger filled with tryptamine in DMSO. In a second side-by-side sampling study, a tryptamine-coated XAD-2 resin was used as a sorbent. The relative collection efficiency of the tryptamine-coated XAD-2 resin was on average 60% of the value obtained using an impinger filled with tryptamine in DMSO. The results indicate that using an impinger filled with tryptamine in DMSO gives higher concentrations of isocyanate than a tryptamine-coated XAD-2 sorbent for HDI monomer and oligomer.

Keywords: *Isocyanates; tryptamine; 1-(2-methoxyphenyl)piperazine; XAD-2 resin; impinger sampling*

National Institute for Occupational Safety and Health (NIOSH) Method 5522, which uses 3-(2-aminoethyl)indole (tryptamine) as a derivatizing reagent,[1] has been issued as a method for monomeric and oligomeric isocyanates. The method has been developed and promulgated as an alternative procedure to NIOSH Method 5521, which uses 1-(2-methoxyphenyl)piperazine as the derivatizing reagent.[2] In order to compare the methods, NIOSH Method 5521 was modified for this study to include the determination of aliphatic isocyanates based on hexamethylene diisocyanate (HDI) in the oligomeric form.

The use of tryptamine is based on the idea that the π system (the indole moiety) of the derivatizing reagent is sufficiently isolated from the isocyanate that different isocyanates do not produce a different response.[3–7] If the response comes only from the tryptamine label, then the analysis procedure can use any tryptamine-based isocyanate urea as a standard for the determination of total isocyanates. Furthermore, since both fluorescence and electrochemical detectors may be used for the determination of tryptamine derivatives, one detector can provide sensitive, confirmatory detection for the other after separation by high-performance liquid chromatography.

In this work, we evaluated tryptamine as a derivatizing reagent when employed in actual field studies. In two separate studies, impinger sampling using 1-(2-methoxyphenyl)piperazine in toluene[2] and solid sorbent sampling with a tryptamine-coated XAD-2 resin[8] were compared with impinger sampling with tryptamine in DMSO.[1]

Experimental†

Reagents

1-(2-Methoxyphenyl)piperazine (MOPIP) was obtained from Fluka (Milwaukee, WI, USA), 3-(2-aminoethyl)indole or tryptamine (TRYP) from Sigma (St. Louis, MO, USA], 98% pure HDI monomer from Aldrich Chemical (Milwaukee, WI, USA), Desmodur N-75, which is a 35–40% biuret trimer of HDI and 35% polyisocyanate in xylene,[9] from Bayer Chemical (Pittsburgh, PA, USA). XAD-2 was obtained from Supelco (Bellefonte, PA, USA), DMSO and HPLC-grade acetonitrile and methanol from Burdick and Jackson (Muskegon, MI, USA), acetic anhydride, glacial acetic acid and toluene from Fisher (Fairlawn, NJ, USA) and sodium acetate trihydrate from Mallinckrodt (Paris, KY, USA).

Sampling

Impingers were filled with 15 ml of a 43 mg l^{-1} solution of MOPIP in toluene or 20 ml of a 450 mg l^{-1} solution of tryptamine in DMSO.

XAD-2 was mixed with a solution of tryptamine dissolved in acetonitrile. The final coating contained 1 mg of tryptamine per gram of XAD-2 resin (0.1% m/m). The acetonitrile was then vacuum evaporated until the solid sorbent was dry.[10] Solid sorbent tubes (5 mm id) were filled to a height of 3 cm with the tryptamine-coated XAD-2 resin (0.1% m/m). After sampling, the sorbent was extracted with acetonitrile as described by Wu and Gaind.[8]

Constant-flow personal air-sampling pumps capable of drawing up to 2.0 l min^{-1} were used to obtain the air samples. The air flow rate was 1.0 l min^{-1} for all the impingers and 0.2 l min^{-1} for the sorbent tubes.

Method Comparison Field Study: Keesler AFB

Four different spray paint operations were evaluated: operation 1 involved spray-painting a tractor and the passenger-side door of a pickup truck in a drive-in paint booth; operation 2 involved spray-painting a munitions handling cart and two small items in a walk-in paint booth; and operations 3 and 4 involved spray-painting an aircraft engine and an aircraft stand, respectively, in a paint booth equipped with a waterfall air-cleansing system.

For all operations, a hardener (Deft, Irvine, CA, USA) containing 30% biuret and 0.15% monomer was mixed with a polyenamel (Deft) in a 1 : 1 ratio. High-volume, low-pressure sprayers were used during operation 1 to apply the paint, while

† Mention of company names and products does not constitute endorsement by the Centers for Disease Control and Prevention.

older style Binks hand sprayers (35 psi) were used during operations 2 and 4. A DevilBiss TYPE-MBC sprayer (50 psi) was used for operation 3.

Area samples and personal samples were collected in the area of overspray and in the breathing zone, respectively. Area samplers were 3–5 ft above the floor and about 4–5 ft downdraft from the equipment being painted.

XAD-2 Sorbent Field Study: Tinker AFB

A B-52 bomber was spray-painted. Six hours were spent painting the aircraft and another 2 h were spent cleaning the Kraco Pro AA 4000 electrostatic paint guns. Four personal samples were collected. Two of these were in the breathing zone of the manlift operator and the other two were in the breathing zone of two spray painters. Four area samplers were positioned on the east and west sides of the hanger dock approximately 15 ft from the wing tips of the aircraft. All sampling was performed using tryptamine-coated XAD-2 sorbent tubes, which were positioned next to impingers filled with tryptamine in DMSO.

For all spray-painting, a hardener (Deft) containing 60% polyisocyanate with an unspecified percent monomer was mixed with a polyenamel (Deft) in a 1 : 3 ratio.

Analytical Methods: MOPIP and Tryptamine Impinger Comparison

For the comparison of NIOSH Method 5522[1] and NIOSH Method 5521,[2] TRYP-derivatized samples were analyzed at the Centers for Disease Control and Prevention, NIOSH (Cincinnati, OH, USA), while MOPIP-derivatized samples were analyzed by DataChem (Salt Lake City, UT, USA).

For the TRYP-derivatized samples, the final impinger volumes of the field samples were measured and a 25 μl aliquot was injected directly into the liquid chromatograph. The HPLC system consisted of a Waters (Milford, MA, USA) Model 710B autosampler, two Waters Model 600A reciprocating pumps and a Waters Model 600-MS controller. The column contained Waters μBondapak C_{18}. A Shimadzu (Kyoto, Japan) RF-535 fluorescence detector set at 275 nm excitation and 320 nm emission interfaced to a Dionex (Sunnyvale, CA, USA) Model 450 data system was used to detect the tryptamine derivatives as they eluted. An ESA (Chelmsford, MA, USA) Model 5100A electrochemical detector operated in the oxidative mode (+0.8 V *versus* Ag/AgCl) was used for confirmation of the isocyanate-identified peaks. The mobile phase was 1 + 1 acetonitrile–buffer (0.6% sodium acetate, adjusted to pH 5.5 with glacial acetic acid). The flow rate was 1.0 ml min^{-1}. A gradient up to 100% acetonitrile was applied after the elution of the HDI monomer peak at 11 min in order to elute the major HDI polyisocyanate peaks within a run time of 1 h.

Working standard solutions were prepared by diluting a stock standard solution of the hexamethylene diisocyanate tryptamine (HDI-TRYP) urea prepared as described in NIOSH Method 5522.[1] In the HPLC trace of the bulk, the major peak was attributed to tryptamine-derivatized biuret (TRYP-biuret), since its retention time matched that of the largest peak generated by Desmodur N-75, which is known to contain 35–40% biuret. Fig. 1 shows the HPLC trace of a sample with the biuret peak indicated at a retention time of 18.52 min. In addition to quantifying the biuret peak, the peak at 19.88 min and the peak groups at 22.25 and 25.18 min were also quantified. For each sample, all peaks were quantified against the derivatized monomer standards both in terms of TRYP-biuret (which is an HDI-based trimer) and total tryptamine-derivatized polyisocyanate, TRYP-poly (which contains not only the trimer but other higher molecular mass HDI-based oligomers).

For the MOPIP-derivatized field samples, 10 μl of acetic anhydride were added to react with any residual derivatizing reagent. A solvent exchange step was then performed by evaporating the samples to dryness under nitrogen and reconstituting the residue in 5 ml of methanol. A 25 μl aliquot was then injected into a Hewlett-Packard (Avondale, PA, USA) Model 1090 HPLC system equipped with a UV detector operated at 242 nm, followed by an ESA Model 5100A electrochemical (EC) detector operated in the oxidative mode (+0.8 V *versus* Ag/AgCl), followed by a Hewlett-Packard photodiode-array (PDA) detector scanned over the range 220–300 nm. To confirm a peak as an isocyanate, it had to be detected by both the EC and UV detectors. Further confirmation was achieved by comparing the UV absorption spectrum of each chromatographic peak in the field samples with a reference spectrum obtained from a stock solution of the hexamethylene diisocyanate 1-(2-methoxyphenyl)piperazine urea (HDI-MOPIP). The mobile phase was 60 + 40 acetonitrile–buffer (0.6% sodium acetate, adjusted to pH 6.0 with glacial acetic acid). The flow rate was 1.5 ml min^{-1}.

Working standard solutions were prepared by diluting a stock standard solution of HDI-MOPIP prepared as described in NIOSH Method 5521.[2] The concentrations of HDI monomer and HDI biuret were determined by comparing the area of the HDI monomer peak and the earliest eluting polyisocyanate peak with those of a series of HDI-MOPIP standards. The earliest eluting peak in the HPLC trace of the bulk was identified as the biuret peak since its retention time matched that of the largest peak in Desmodur N-75. Fig. 2 shows the HPLC trace of a sample with the monomer peak at 3.42 min and the biuret peak at 7.92 min. The UV absorption spectra for the later eluting peaks (retention times over 10 min) were not compared with HDI-MOPIP and these peaks were not quantified. Only the monomer and biuret peaks were quantified.

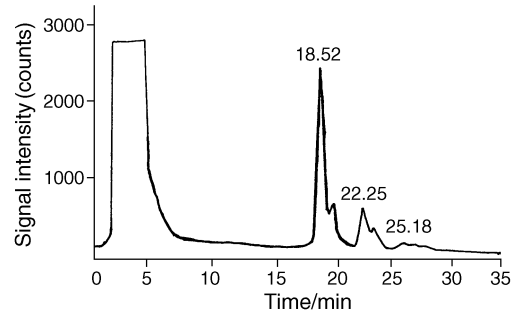

Fig. 1 Fluorescence chromatogram of tryptamine-derivatized polymeric HDI. The peak at a retention time of 18.52 min was identified as the biuret peak and this peak and the later eluting peaks were quantified based on the tryptamine-derivatized monomer standards of HDI.

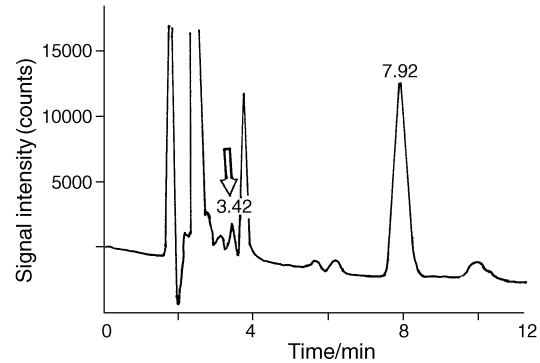

Fig. 2 UV chromatogram of MOPIP-derivatized polymeric HDI. The peak at a retention time of 7.92 min was identified as the biuret peak and was quantified based on the MOPIP-derivatized monomer standards of HDI which appear at 3.42 min. Later eluting peaks were not quantified.

Analytical Methods: XAD-2 versus *DMSO Impinger Sampling*

For the comparison of XAD-2 sorbent and DMSO impinger sampling, all TRYP-derivatized samples were analyzed at Brooks AFB (San Antonio, TX, USA). The HPLC system consisted of a Hewlett-Packard Model 1090 chromatograph equipped with a PDA detector followed by a Hewlett-Packard Model 1049A EC detector operated in the oxidative mode (+0.8 V *versus* Ag/AgCl). An ABI (Foster City, CA, USA) Analytical Spectroflow 90 fluorescence detector operating at an excitation wavelength of 275 nm was attached after the EC detector. The fluorescence detector was not equipped with a monochromator to limit the bandpass of the emission and no filters were used, and therefore the emission intensity was detected over the entire wavelength range of the instrument (190–700 nm). The absence of an emission filter can lead to a poorer limit of detection because of an increase in background noise and can also lead to the improper identification of extra peaks which are not isocyanate-derived. The PDA and EC detectors, however, confirmed all peaks as isocyanate derivatives. Hewlett-Packard 3396 Series II integrators were used to quantify the chromatographic peaks. The column used was a LiChrospher 100 RP 18 (5 μm). The mobile phase was 1 + 1 acetonitrile–buffer (0.6% sodium acetate, adjusted to pH 5.5 with glacial acetic acid). In the determination of polyisocyanate, the mobile phase was adjusted to 60 + 40 acetonitrile–buffer in order to shorten the retention time.

The concentration of HDI monomer was determined from a calibration curve based on HDI TRYP urea standards prepared as described in NIOSH Method 5522.[1] HDI polyisocyanates were quantified by comparing the integrated area of the largest peak in the sample (identified as a biuret trimer derivative) with the corresponding peak of a polyisocyanate standard prepared from the hardener used in the field. The polyisocyanate concentration in the hardener was corrected for dilution since the hardener contained only 60% m/m aliphatic isocyanate[9] and confirmed as 56% in a separate determination.[11]

Results and Discussion

The limit of detection (LOD) for NIOSH Method 5522 was 0.7 μg per sample or 35 μg m^{-3} of air and that for NIOSH Method 5521 was 0.4 μg per sample or 20 μg m^{-3} of air. These LODs are at or slightly below the NIOSH recommended exposure limit (REL) value of 35 μg m^{-3} of air and the ACGIH threshold limit value (TLV) of 0.034 mg m^{-3} of air.[12] HDI monomer was not detected in any of the samples when analyzed using NIOSH Method 5522, whereas the amount of HDI monomer was between 0.5 and 1.0 μg per sample when analyzed using NIOSH Method 5521. Although it appears that NIOSH Method 5521 can detect HDI monomer when NIOSH Method 5522 cannot, the amount of HDI found was below the limit of quantification (LOQ) for both methods.

Table 1 compares the HDI biuret as determined using NIOSH Method 5521 (see MOPIP-biuret) with that determined using NIOSH Method 5522 (see TRYP-biuret). The biuret trimer of HDI in air during spray-painting operations as determined by NIOSH Method 5521 ranged from 0.12 to 1.93 mg m^{-3}, whereas the polyisocyanate in air as determined by NIOSH Method 5522 ranged from 0.18 to 1.89 mg m^{-3}. The manufacturer (Bayer Chemical, Pittsburgh, PA, USA) specifies a 1 mg m^{-3} short-term exposure limit (STEL) and a 0.5 mg m^{-3} 8 h time-weighted average (TWA) permissible exposure limit (PEL) for polyisocyanate exposure to the biuret of HDI.[9] The spray-painting operations at Keesler AFB in this study exceeded the STEL of 1 mg m^{-3} for polyisocyanates about 20% of the time.

In attempting to compare the results from NIOSH Methods 5521 and 5522, the raw data must be interpreted carefully. For NIOSH Method 5521, quantification was performed using a calibration curve based on the UV detector response; late-eluting peaks were not quantified since these represented less than 20% of the area of the peak identified as MOPIP-derivatized biuret (MOPIP-biuret) (see Fig. 2). HDI polyisocyanates were identified as any peaks that gave a response with both the EC and UV detectors and which gave a UV absorption spectrum which was identical with that of the HDI monomer derivative. The results for a number of 1-(2-methoxyphenyl)piperazine-derivatized samples are reported in Table 1 under MOPIP-biuret.

For NIOSH Method 5522, quantification was based on the response of the fluorescence detector. The HDI polyisocyanate results are based on quantifying both the peak attributed to tryptamine-derivatized biuret (see TRYP-biuret) and all the peaks ascribed to tryptamine-derivatized polyisocyanate (see TRYP-poly). The TRYP-biuret/TRYP-poly ratio ranges from a low of 39% to a high of 64% with a mean value of 53% and a standard deviation of 9% based on six samples. The mean value is near the expected range for the mass percentage of biuret in polyisocyanate, which ranges from 46–50%.[9] The results imply that the sampling methodology does not discriminate between the lower molecular mass biuret trimer and the higher molecular mass polyisocyanates.

During operation 3, one of the personal samplers containing TRYP-biuret in DMSO froze in the refrigerator prior to analysis. If this sample is included and a paired *t*-test is used to compare the MOPIP-biuret results with the TRYP-biuret results ($n = 6$), the difference between means is not significant at the 0.05 level, although Pearson's correlation coefficient (r) is low (0.57). Frozen samples consistently produce anomalous results, and the paired *t*-test was used again to evaluate the data pairs, but with the pair containing the results for the frozen sample excluded. The results for $n = 5$ indicate no significant difference at the 0.05 level with $r = 0.99$, which is a very strong correlation.

For the XAD-2 sorbent study, a series of standard solutions prepared from the HDI-tryptamine urea yielded a linear response with $r = 0.99$ within the working range of 0.01–3.0 μg ml^{-1}. Standard solutions prepared from the Deft hardener yielded one large peak at 8.8 min and two late-eluting peaks at 14.6 and 16.8 min. The large peak (attributed to TRYP-biuret) represented 95% of the total polyisocyanate area excluding

Table 1 Field study at Keesler AFB: comparison of polyisocyanate amounts obtained using NIOSH Method 5521 (MOPIP) and NIOSH Method 5522 (TRYP)

		Polyisocyanate†/mg m^{-2}		
Operation	Type*	MOPIP-biuret	TRYP-biuret	TRYP-poly
1	Personal (54)	1.33	—	—
	Area (61)	1.93	—	—
	Personal (53)	0.53	—	—
	Area (75)	0.69	—	—
2	Personal (60)	0.41	0.44	0.80
	Area (70)	0.64	0.64	1.00
3	Personal (27)	0.33	1.11‡	1.89‡
	Area (41)	0.22	0.22	0.49
4	Personal (92)	0.12	0.07	0.18
	Area (94)	0.16	0.18	0.33

* Type denotes whether the sample was collected in the breathing zone (personal) or in an area of overspray (area); the values in parentheses are the sampling times in minutes. † Polyisocyanate concentrations are expressed in terms of mg of HDI per m^3. ‡ Sample in DMSO solidified in the refrigerator prior to analysis.

Table 2 Field study at Tinker AFB: comparison of XAD-2 sorbent and DMSO–impinger collection methods

		HDI[†]/mg m^{-3}		Polyisocyanate[‡]/ mg m^{-3}	
Sample[*]	Type[†]	XAD-2[§]	DMSO[§]	XAD-2[§]	DMSO[§]
P1-1	Painting (240)	0.014	0.026	0.014	0.045
P2-1	Operating manlift (240)	0.005	0.083	0.074	0.160
A1-1	East side (240)	0.021	0.017	0.070	0.085
A2-1	West side (240)	0.014	0.014	0.094	0.164
P1-2	Operating manlift (120)	0.009	0.024	0.023	0.039
P2-2	Painting (120)	0.014	0.039	0.125	0.105
A1-2	East side (120)	0.014	0.028	0.086	0.120
A2-2	West side (120)	0.026	0.039	0.220	0.331

[*] Sample: P denotes personal and A denotes area sampling. The ventilation system was in the exhaust mode for the first four samples and in the recirculation mode for the last four. [†] Type denotes the type of labour being performed by the worker for personal sampling and the location of the sampler for area sampling. The value in parentheses denotes the sampling time in minutes. [‡] HDI and polyisocyanate concentrations are expressed in terms of mg of HDI per m^3. [§] XAD-2 and DMSO denote the sorbent and the solvent used to adsorb and dissolve the tryptamine, respectively.

reagent blank peaks and the peak caused by the HDI monomer.

Table 2 shows the results of spray-painting operations conducted at Tinker AFB. For the determination of the tryptamine derivative of HDI, desorbed from an XAD-2 resin, the HDI concentration ranges between 0.005 and 0.021 mg m^{-3}. For the analysis of HDI collected in the tryptamine DMSO solution, the concentration ranges between 0.014 and 0.083 mg m^{-3}. While the concentration of HDI in air as determined by collection on a solid sorbent never exceeds the ACGIH TLV of 0.034 mg m^{-3}, the results based on impinger collection show that three out of eight samples exceed the TLV. None of the polyisocyanate concentrations exceed the STEL of 1.0 mg m^{-3} established for exposure to the biuret of HDI.

The HDI monomer collection efficiency for the tryptamine-coated XAD-2 sorbent (when compared with collection with a tryptamine DMSO solution) ranges from 6.3% to 126.6%. If the poor collection efficiency of sample P2-1 is excluded, and also the sampling efficiencies associated with samples A1-1 and A2-1, which are well above average, then the average sampling efficiency for the remaining five out of eight samples is 50%, which happens to be close to the overall average for all eight samples (51%). For polyisocyanate collection, the collection efficiency for the XAD-2 sorbent is 66.6% when compared with that of the DMSO solution.[10] The results indicate that in most cases, the results obtained with a sorbent tube are lower than those obtained by collection with an impinger. The average results are lower than those obtained by Wu and Gaind,[8] who found a 90% recovery of phenylisocyanate when using tryptamine-coated XAD-2 as a sorbent. The difference in the results can probably be ascribed to the lower linear velocity used for the capture of aerosol in the XAD-2 resin (the air flow rate was 0.2 ml min^{-1}). It is also possible that the tryptamine-coated XAD-2 sorbent gives lower results because some of the isocyanates can react with polyols and atmospheric moisture before they have a chance to react with the derivatizing reagent,which is relatively immobile on the sorbent surface. Further work is in progress to assess whether sorbents saturated with solvent can facilitate the reaction between polyisocyanate in the aerosol form and the derivatizing reagent.

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