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# AN APPROACH FOR ESTIMATING WORKPLACE EXPOSURE TO O-TOLUIDINE, ANILINE, AND NITROBENZENE

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*A comprehensive approach to estimating worker exposure to o-toluidine, aniline, and nitrobenzene using a combination of surface wipe, dermal badge, and air samples is described. Desorption of each sample was accomplished with ethanol followed by analyses using capillary gas chromatography with flame ionization detection. Analyte recovery was maximized when the gauze wipes and dermal badges were immediately desorbed in ethanol after sample collection. Sample collection of the airborne analytes was improved over previous solid sorbent samples by using a sampling train consisting of an acid-treated glass fiber filter in series with a large capacity silica gel tube (520/260 mg). The greatest recoveries of aniline and o-toluidine were from the acid-treated glass fiber filters and nitrobenzene from the large capacity silica gel sorbent tubes. The limit of detection for each analyte (1 µg) was approximately 10 times more sensitive than reported in previous National Institute for Occupational Safety and Health methods. Analyte recoveries for air samples were greatest under conditions of moderate relative humidity (53%), moderate sample volumes (< 50 L), and low flow rates (0.2 L/min). The overall relative standard deviation of the analytical method was 4.3%.*

Within the last two years, the National Institute for Occupational Safety and Health (NIOSH) has received requests for health hazard evaluations (HHE) at two chemical manufacturing plants where o-toluidine and aniline were used. o-Toluidine and aniline, aromatic amine intermediates used in the manufacture of dyes, pharmaceuticals, pesticides, antioxidants, and rubber tires, previously have been implicated in the occurrence of bladder cancer.<sup>(1)</sup> These requests were based on concern generated by epidemiological studies indicating a higher-than-expected incidence of bladder cancer among workers exposed to these chemicals during the manufacturing process.<sup>(2)</sup>

Initial walk-through observations of the two plants and an evaluation of the manufacturing process by NIOSH investigators confirmed that workers were potentially exposed to o-toluidine and aniline. At a later date nitrobenzene was included

in the HHE request because it may synergistically enhance the carcinogenic effects of o-toluidine and aniline.<sup>(1)</sup> NIOSH has classified o-toluidine and aniline as potential occupational carcinogens and recommends that exposure be reduced to the lowest feasible concentration.<sup>(1)</sup> A recommended exposure limit REL of 1 ppm (5 mg/m<sup>3</sup>) has been established for nitrobenzene.<sup>(3)</sup> The American Conference of Governmental Industrial Hygienists (ACGIH) has established a Threshold Limit Value (TLV®) of 2 ppm for o-toluidine (suspected human carcinogen); 2 ppm for aniline; and 1 ppm for nitrobenzene.<sup>(4)</sup> The Occupational Safety and Health Administration (OSHA) has established a permissible exposure limit (PEL) of 5 ppm for o-toluidine (22 mg/m<sup>3</sup>) and aniline (19 mg/m<sup>3</sup>), and 1 ppm for nitrobenzene.<sup>(5)</sup> NIOSH, ACGIH, and OSHA recommend that the potential for contact with workers' skin should be minimized.

While worker exposures to o-toluidine, aniline, and nitrobenzene were confirmed during the initial walk-through HHE, the quantitative determination of each dermal and inhalation exposure remained undetermined. A combination of several sampling techniques was required to determine possible routes of exposure and an overall estimation of worker exposure to the chemicals.

A survey of the existing literature produced no suitable protocol for the simultaneous collection and analysis of these three compounds. However, several references served as focal points for the development of a suitable protocol: NIOSH Method 2002 (aromatic amines),<sup>(6)</sup> NIOSH Method 2005 (nitrobenzenes),<sup>(7)</sup> and OSHA Method 73 (toluidine isomers).<sup>(8)</sup> While the literature contained several specific methodologies for o-toluidine, aniline, and nitrobenzene, it did not provide the comprehensive sampling and analytical approach required for an HHE.

In an effort to ascertain and determine all potential routes of worker exposure to o-toluidine, aniline, and nitrobenzene, a combination of new and established sample collection and analytical techniques was utilized. Surface wipes and dermal badges were used as indicators of potential dermal exposures. For airborne exposures, sulfuric acid-impregnated filters in series with various sizes of silica gel tubes, and the use of silica gel tubes alone, were employed as quantitative indicators. The use of surface wipes provided an indirect means of measuring

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worker dermal exposure while the other techniques provided a direct measurement of worker dermal and air exposures.

## METHODS

Initial research efforts focused on the determination of a suitable desorption solvent for all three analytes. Then each sample collection method was evaluated to determine effects of sampling media on recovery, variations due to exposure times, storage stability, relative humidity, and flow rates.

The goal of this initial research was to determine the overall effectiveness of this approach in providing an estimation of worker exposure to the three analytes under a variety of conditions. The number of samples initially studied was not amenable to statistical analyses. However, prior to a second HHE a more thorough and detailed approach was incorporated resulting in more conclusive and statistically tested data for analyte recovery, storage stability, and the effect of the environmental conditions.

### *Desorption Solvent*

Reagents used were o-toluidine (99%, Aldrich Chemical Co.); aniline (99.9%, Alpha Products); and nitrobenzene (99%, Aldrich Chemical Co.). Silica gel sorbent tubes (150/75 mg and 520/260 mg) were obtained from SKC, Inc. Neat solvents and solvent mixtures were evaluated for their ability to desorb all three analytes simultaneously from silica gel tubes. These included methanol (spectral grade, Burdick and Jackson); ethanol (absolute, Quantum Chemical Corp.); ethanol (95%, Quantum Chemical Corp.); and 95% ethanol/2% benzene (benzene, 99%, Fisher-Scientific Co.).

Silica gel sorbent tubes (150/75 mg) were spiked with 5 to 15  $\mu$ L aliquots of a solution of o-toluidine (0.030–0.150 mg), aniline (0.036–0.140 mg), and nitrobenzene (0.034–0.115 mg). The spiked samples were allowed to equilibrate overnight before desorption in 1 mL of the specified test solvent. Desorption was determined after placing the spiked samples in an ultrasonic bath for one hour. After desorption, each sample was analyzed by GC-FID and the resultant desorption efficiencies for each solvent were compared to determine the best solvent selection.

### *Surface Wipes*

Surface wipes (Johnson and Johnson gauze pads, 100% cotton, 2 inch  $\times$  2 inch, 100 cm<sup>2</sup>), were evaluated to determine their suitability for use as an indicator of indirect dermal exposure. Each wipe (N = 8) was spiked with 10  $\mu$ L aliquots of a solution of o-toluidine (0.100 mg), aniline, (0.102 mg), and nitrobenzene (0.460 mg), and placed in a 30 mL scintillation vial. Next, 10 mL of ethanol was added to each sample and the vials were immediately sealed.

Eight additional samples were prepared simultaneously. These remained exposed to the atmosphere for eight hours at ambient temperature (25°C), 53% relative humidity, and under fluorescent lighting before desorption. After a one-hour desorption period in an ultrasonic bath all samples were analyzed by GC-FID.

### *Dermal Sampler*

To determine the level of contact exposure, a passive dermal sampler was constructed. Samplers were prepared by filling cotton pouches (1 inch in length by 0.75 inches wide) with 0.75–0.80 grams of 8–20 mesh silica gel. The ends were sealed with staples. Each sampler (N = 8) was spiked with 5 to 25  $\mu$ L aliquots of a solution of o-toluidine (0.030–0.252 mg), aniline (0.031–0.255 mg), and nitrobenzene (0.0273–0.273 mg), and recoveries were determined after 0, 8, and 24 hours. The environmental test conditions were ambient temperature (25°C), 53% relative humidity, and fluorescent lighting. A determination of the relative passive collection efficiency was established by allowing each sampler to be exposed to the atmosphere for 0, 8, and 24 hours before the addition of the desorption solvent. Each sampler was then immediately placed into a 30 mL scintillation vial and desorbed ultrasonically for one hour in ethanol. Media blanks, storage stability samples (7 days and 30 days), and silica gel comparison samples (silica gel was weighed without cotton pouches and placed into scintillation vials) were prepared using the same experimental conditions.

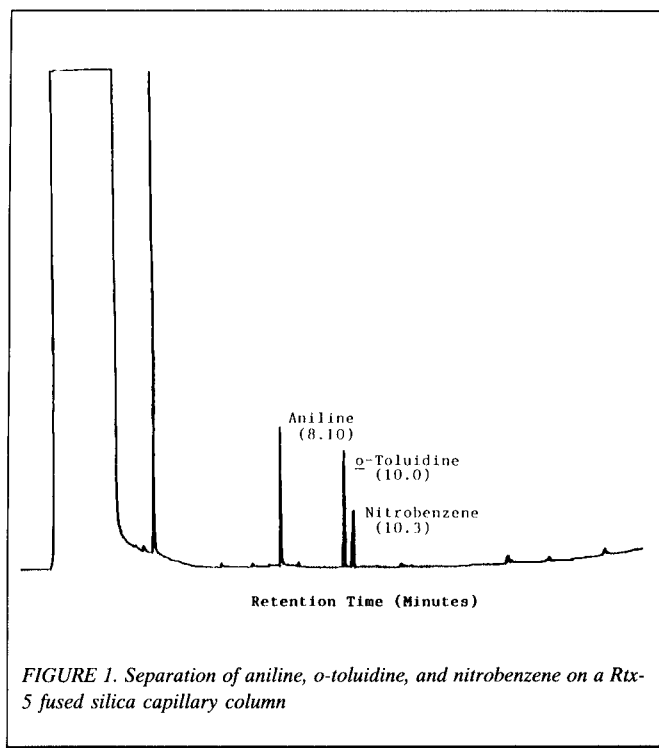
### *Air Samplers*

Two sizes of silica gel (520/260 and 150/75 mg) were evaluated at 53% and 100% relative humidity. Each silica gel sorbent tube was spiked with a 15- $\mu$ L aliquot of the analytes (0.12–0.15 mg). Humidified air, generated by using a vacuum pump to draw room air through a charcoal filter into a midjet impinger filled with deionized water, was pulled through the tubes. Humidity levels, monitored by a hygrometer (Airguide<sup>®</sup>), were regulated by adjusting a needle valve connected to a dry air bypass line.

Various drying agents were placed prior to the tubes to determine their effectiveness in reducing moisture routinely encountered with silica gel tubes in conditions of high relative humidity. Ten- $\mu$ L aliquots of a solution of aniline (0.119 mg), o-toluidine (0.101 mg), and nitrobenzene (0.115 mg) were spiked onto a glass wool plug placed before the drying agent being tested. Humidified air was drawn through the system, carrying the analytes through the drying tubes and onto the silica gel tube.

A sampling train consisting of acid-treated glass fiber filters connected in series with large capacity silica gel sorbent tubes was assembled. The filters were prepared by treating binderless glass fiber filters (Gelman) with 0.5 mL of 0.26 N sulfuric acid and placing them in 37 mm polystyrene cassettes. A solution of the analytes was spiked onto glass wool and humidified air was pulled through the system as previously described. Analysis of the glass fiber filters was performed by DataChem Laboratories (Salt Lake City, Utah) using OSHA Method 73.<sup>(9)</sup>

Long-term storage stability studies were determined for the three analytes on large silica gel tubes (520/260 mg). Ten- $\mu$ L aliquots of a solution of o-toluidine (0.101 mg), aniline (0.119 mg), and nitrobenzene (0.115 mg) were spiked onto silica gel tubes and analyzed after 8, 14, and 21 days. These concentrations represented values approximately 0.5 $\times$  the



NIOSH REL. Lower concentrations of nitrobenzene, representing a range  $3\times$  to  $20\times$  the LOD, were further evaluated after storage for 1, 15, 30, and 60 days at  $5^{\circ}\text{C}$ .

Silica gel sample tubes were desorbed ultrasonically in 2 mL of ethanol for 1 hour, and 1- $\mu\text{L}$  volumes were analyzed by GC-FID.

All sample analyses were performed on a Hewlett-Packard 5890 gas chromatograph with a flame ionization detector. The gas chromatograph was equipped with a 7673A autosampler and a model 3392A integrator system. Separation of the analytes (see Figure 1) was achieved using a 30 m, 0.32 mm ID, Rtx-5 fused silica capillary column (Restek Corp.). A temperature program of  $35\text{--}150^{\circ}\text{C}$  at  $8^{\circ}\text{C}/\text{minute}$  was used. The carrier gas was helium (35 mL/min.). The sample injection volume was 1- $\mu\text{L}$ , splitless mode.

## RESULTS AND DISCUSSION

### Sample Desorption Studies

Sample desorption efficiency studies for o-toluidine, aniline, and nitrobenzene, using silica gel as the collection medium, were determined at levels approximately one-half the REL in various desorption solvents. Evaluation of the recovery data for all three analytes indicated that ethanol was the best choice as a desorption solvent when compared to methanol, 95% ethanol, and 95% ethanol/2% benzene.

### Surface Wipes

The documentation of surface contamination is accepted as an indirect means of determining the potential for skin contact and subsequent dermal absorption. This allows an estimation of worker exposure to contaminants through splashes, spills, and contaminated surfaces.<sup>(10-11)</sup>

**TABLE I. Gauze Surface Wipes Spiked With o-Toluidine, Aniline, and Nitrobenzene**

Analyte	N	Level (mg)	Mean Sample Recovery (mg)	Mean Recovery (%)	RSD
<i>Immediate Desorption in 10 mL Ethanol</i>					
o-Toluidine	8	0.10	0.091	91	5.0
Aniline	8	0.10	0.099	96	7.4
Nitrobenzene	4	0.46	0.41	89	9.2
<i>Equilibration Period of 8 Hours Before Desorption in 10 mL Ethanol</i>					
o-Toluidine	8	0.10	0.088	88	3.9
Aniline	8	0.10	0.085	83	4.8
Nitrobenzene	2	0.46	0.064	(8.1 - 19.1) <sup>A</sup>	

<sup>A</sup> Range of recovery

A summary of the results obtained when measuring the effectiveness of the surface wipes as a sampling medium for o-toluidine and aniline is presented in Table I. Based on these results, surface wipes provide an acceptable quantitative estimate of potential o-toluidine and aniline exposures when the wipes are placed into 30 mL scintillation vials and immediately desorbed in 10 mL of solvent. Mean recoveries for all three analytes averaged 92% with a relative standard deviation of 7.2%.

Surface wipes were spiked with each analyte and exposed to the environment for eight hours before being desorbed in 10 mL of ethanol in an effort to simulate potential worker exposure during an eight-hour workday. o-Toluidine and aniline exhibited a 6% loss in analyte recovery when the results were compared with those achieved from immediate desorption. Nitrobenzene exhibited only a 14% recovery after eight hours' exposure to the environment before desorption. Thus, the immediate addition of the desorption solvent acts to solubilize the analytes present on the surface wipe, reducing sample volatility and preventing sample loss.

The use of surface wipes provides an estimation of surface contamination and potential workplace exposures. The reliability of surface wipes as an indicator of potential worker exposure depends on factors such as the type of sampling media selected, the volatility of the analyte on exposed substrates, the residence time on the substrate, and the type of substrate surface sampled.

### Dermal Badge Samplers

The best recovery of o-toluidine ( $> 95\%$ ) and aniline ( $> 88\%$ ) was obtained when the dermal badge samplers were immediately stabilized in 5 mL of ethanol. Sample recoveries after 8 hours of exposure to the environment were reduced, with further losses in recovery noted after 24 hours (o-toluidine [75% recovery] and aniline [63% recovery]). Because of the sample loss during an eight-hour period, all results obtained are only a minimal estimate of exposure. Nitrobenzene, on the other hand, continued to exhibit a very acceptable sample recovery after 24 hours ( $> 92\%$ ).

**TABLE II. Effect of Humidity and Silica Gel Capacity On Sample Collection of o-Toluidine, Aniline, and Nitrobenzene**

Analyte	N	Level (mg)	Silica Gel Loading (mg)	Relative Humidity (%)	Range of Recovery
o-Toluidine	2	0.15	150/75	53	(86–92)
o-Toluidine	2	0.15	150/75	100	(83–85)
o-Toluidine	2	0.15	520/260	53	(92–100)
o-Toluidine	2	0.15	520/260	100	(62–76)
Aniline	2	0.14	150/75	53	(81–86)
Aniline	2	0.14	150/75	100	(79–80)
Aniline	2	0.14	520/260	53	(70–86)
Aniline	2	0.14	520/260	100	(57–75)
Nitrobenzene	2	0.12	150/75	53	(76–76)
Nitrobenzene	2	0.12	150/75	100	(9.4–11)
Nitrobenzene	2	0.12	520/260	53	(85–101)
Nitrobenzene	2	0.12	520/260	100	(24–58)

**TABLE III. Effects of the Use of Various Drying Agents on Aerosol Sample Recovery of o-Toluidine, Aniline, and Nitrobenzene (N = 1 for each analyte effect)**

Drying Agent	Recovery (%)		
	o-Toluidine	Aniline	Nitrobenzene
None	86	87	95
Activated Alumina	7	11	44
Humid Glass Wool <sup>a</sup>	98	99	28
Humid Dri-rite®	7	8	86
Molecular Sieves	76	80	38

<sup>a</sup> Although humid glass wool is not a drying agent, its effect on sample recovery was pertinent to this study.

The effect of the outer cotton layer of the dermal badge samplers on sample recovery was investigated. Spiked amounts of o-toluidine and aniline on silica gel sorbent placed in 30 mL scintillation vials (no cotton pouch) were compared with those spiked directly onto the dermal badge samplers. Both analytes, when spiked onto the dermal badge samplers, were recovered at levels 16–18% (o-toluidine) and 8.1–20% (aniline) greater than recoveries achieved when silica gel was placed into 30 mL scintillation vials and spiked. One possible explanation is that the cotton layer surrounding the silica gel sorbent medium traps the analyte vapors on the silica gel until equilibration occurs, reducing sample loss due to volatilization.

### Air Monitoring

The results of this study, summarized in Table II, suggest that both o-toluidine and aniline, when sampled onto silica gel sorbent tubes, are most effectively collected on loadings of 150/75 mg/tube at moderate relative humidities (53%). One possible explanation for this result is that the small amount of water normally present on the outer surface of the silica gel at moderate humidity levels (53%) acts to solubilize the aniline and o-toluidine. This action helps to trap these analytes on the silica gel in a synergistic manner. Because there is a greater ratio of water to active sites in the smaller silica gel tubes, the

overall recovery of o-toluidine and aniline is improved over recoveries realized from the larger silica gel tubes. It was further noted that analyte recoveries were slightly increased (5–8%) under conditions of moderate relative humidity during sample collection onto 150/75 mg silica gel loadings per tube. However, this slight increase was statistically insignificant.

Nitrobenzene was collected more effectively when silica gel sorbent loadings of 520/260 mg/tube were used. Recoveries were 20% greater when compared to silica gel loadings of 150/75 mg per tube. Like o-toluidine and aniline, sample collection of nitrobenzene was enhanced at moderate humidity levels (53%).

Because of the effect of humidity on analyte recovery, a study was performed to determine the effect of placing drying tubes prior to the silica gel tubes. A number of drying agents were evaluated and the results appear in Table III. All the drying agents had an adverse effect on analyte recovery.

Acceptable results were achieved when no drying agents were used (the minimum recovery for all three analytes was 86%). Aniline and o-toluidine were quantitatively recovered when spiked onto humidified glass wool (> 98%). The low recovery of nitrobenzene (28%) when collected from humidified glass wool is consistent with results obtained earlier in this study under conditions of high relative humidity (see Table II).

The next phase in this study was a 21-day storage stability evaluation, the results of which are summarized in Table IV. While nitrobenzene stability on the silica gel tubes was > 87%, the storage stabilities of o-toluidine and aniline were reduced after only eight days, with recoveries at 76% and 64%, respectively. The recoveries were reduced to 59% and 50%, respectively, after 14 days. Corrections for analyte recovery would be required when recovery falls below 95%. Recovery below 75% is considered unacceptable for reporting as quantitative data.<sup>(12)</sup>

Because of moderate recoveries on silica gel tubes, the use of acid-treated Gelman glass fiber filters as a collection medium for o-toluidine and aniline was investigated. A sampling train was assembled containing a Gelman acid-treated glass fiber filter in series with a high capacity silica gel tube (520/260 mg). Sample recovery data was obtained for o-toluidine, aniline, and nitrobenzene under varying conditions of relative humidity, sample concentration, and flow rate. The results,

**TABLE IV. Storage Stability Results Obtained for o-Toluidine, Aniline, and Nitrobenzene on High Capacity Silica Gel Tubes (520/260 mg)**

Analyte	N	Storage Period (Days)	Level (mg)	Mean Recovery (%)	RSD
o-Toluidine	4	8	0.10	76	2.1
o-Toluidine	4	14	0.10	59	1.0
o-Toluidine	4	21	0.10	55	1.3
Aniline	4	8	0.12	64	2.9
Aniline	4	14	0.12	50	1.3
Aniline	4	21	0.12	47	1.3
Nitrobenzene	4	8	0.12	110	1.5
Nitrobenzene	4	14	0.12	99	2.5
Nitrobenzene	4	21	0.12	88	5.9

**TABLE V. Comparative Effects of Humidity, Sampling Volume, and Flow Rate on Nitrobenzene (N.B.) Recovery From High Capacity Silica Gel Tubes (520/260 mg) and o-Toluidine and Aniline From Acid Treated Glass Fiber Filters Connected Together as a Sampling Train**

Average Sample Volume (L)	Flow Rate (L/min)	(%) Relative Humidity	N	Recovery (%)		
				o-Toluidine	Aniline	N.B.
<i>Gelman Acid-Treated Filter Recovery Results</i>						
33	0.2	28	4	100	100	N.D.
32	0.2	80+	4	86	99	N.D.
74	0.49	28	2	100	110	N.D.
92	0.49	80+	3	63	74	N.D.
<i>Silica Gel Tube Recovery Results</i>						
33	0.2	28	4	4.5	N.D.	98
32	0.2	80+	4	N.D.	N.D.	33
74	0.49	28	2	N.D.	N.D.	72
92	0.49	80+	3	N.D.	N.D.	17

N.D. = values less than 1%

summarized in Table V, indicate that o-toluidine and aniline are successfully collected on the Gelman acid-treated filters.

Nitrobenzene, which passes through the acid-treated Gelman filters unaffected, was successfully recovered from the silica gel tubes under conditions of low humidity (28%) and at a sampling rate of 0.2 L/minute. High humidity levels (> 80%), coupled with higher sampling rates, effectively reduced the sample collection efficiency of the Gelman acid-treated filters for o-toluidine (63%) and aniline (74%). The collection of nitrobenzene on silica gel tubes was adversely affected by high humidity levels, regardless of sampling rate.

An expanded long-term storage stability study (5× LOD) was performed on nitrobenzene collected on large capacity silica gel tubes. Spiked samples were analyzed after 1, 15, 30, and 60 days. The results indicated that nitrobenzene was effectively recovered from the large silica gel tubes after storage for 60 days (95% recovery).

## CONCLUSION

A qualitative estimate of potential workplace dermal exposure to o-toluidine, aniline, and nitrobenzene may be obtained using gauze surface wipes. These wipe samples provide a means of measuring current levels of potential exposures at any time.

A dermal badge sampler was developed to estimate potential worker dermal exposure to o-toluidine, aniline, and nitrobenzene via splashes, spills, and aerosol vapors. This allows a determination of exposures from high-risk activities during an industrial process.

An air sampling train, consisting of a Gelman acid-treated glass fiber filter in series with a large silica gel tube (520/260 mg), allows airborne workplace exposures to o-toluidine, aniline, and nitrobenzene to be quantitated. Analyte recovery was optimized under conditions of moderate relative humidities (< 53%), low sampling rates (0.2 L/min), and moderate sample volumes (< 50 L). Nitrobenzene collection on silica gel tubes was significantly reduced under conditions of high relative humidity.

Overall, the combined use of surface wipes, dermal badges, and the sampling train provides a comprehensive means for monitoring potential dermal and actual airborne workplace exposure to o-toluidine, aniline, and nitrobenzene.

A number of areas need to be researched in greater detail for use in future applications. These investigations should aim to determine the specific relationship between wipe samples and dermal badge samplers and actual worker exposure as well as determining ways to minimize sample bias. Exposures to o-toluidine, aniline, and nitrobenzene, regardless of the length or type of exposure, would best be determined by measuring the metabolic by-products of these analytes in urine.

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