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Factors affecting variability in the urinary biomarker 1,6-hexamethylene diamine in workers exposed to 1,6-hexamethylene diisocyanate

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Abstract

Although urinary 1,6-hexamethylene diamine (HDA) is a useful biomarker of exposure to 1,6-hexamethylene diisocyanate (HDI), a large degree of unexplained intra- and inter-individual variability exists between estimated HDI exposure and urine HDA levels. We investigated the effect of individual and workplace factors on urine HDA levels using quantitative dermal and inhalation exposure data derived from a survey of automotive spray painters exposed to HDI. Painters' dermal and breathing-zone HDI-exposures were monitored over an entire workday for up to three separate workdays, spaced approximately one month apart. One urine sample was collected before the start of work with HDI-containing paints, and multiple samples were collected throughout the workday. Using mixed effects multiple linear regression modeling, coverall use resulted in significantly lower HDA levels ($p = 0.12$), and weekday contributed to significant variability in HDA levels ($p = 0.056$). We also investigated differences in urine HDA levels stratified by dichotomous and classification covariates using analysis of variance. Use of coveralls ($p = 0.05$), respirator type worn ($p = 0.06$), smoker status ($p = 0.12$), paint-booth type ($p = 0.02$), and more than one painter at the shop ($p = 0.10$) were all found to significantly affect urine HDA levels adjusted for creatinine concentration. Coverall use remained significant ($p = 0.10$), even after adjusting for respirator type. These results indicate that the variation in urine HDA level is mainly due to workplace factors and that appropriate dermal and inhalation protection is required to prevent HDI exposure.

Introduction

Due to their work with HDI-containing products, auto-body painters risk becoming sensitized to HDI and developing occupational asthma.[1,2] The effectiveness of the exposure protection methods used, therefore, are important to understand. The use of urinary 1,6-hexamethylene diamine (HDA) as a biomarker for inhalation exposure to 1,6-hexamthylene

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diisocyanate (HDI) has been established.[3–7] Previously, we demonstrated a quantitative linear relationship between dermal exposure to HDI and urine HDA levels.[3] We further concluded that creatinine should be used as an independent variable in exposure modeling to account for the water content in the urine sample collected from a worker exposed to HDI.[3,8] However, in our exposure models relating inhalation and dermal HDI exposure to urine HDA levels, considerable intra- and inter-person variability was observed,[3] which would compromise the use of urine HDA as a biomarker for occupational exposure to HDI. To effectively use individual urine HDA levels in monitoring exposure, evaluating personal protection, and establishing regulatory compliance, determining the cause(s) of the variability is critical.

HDA levels in hydrolyzed urine may be derived from both the non-enzymatic hydrolysis of HDI as well as monoacetyl-HDA and diacetyl-HDA formed from *N*-acetyl transferase 1 (NAT1) activity. Upon acid hydrolysis of the urine sample during analysis, protein adducts formed due to direct reaction with HDI or as a product of HDI metabolism may be broken down and released in the form of HDA.[9–12] Thus, a person's inherent ability to metabolize HDI (*e.g.*, metabolic rate, genetic polymorphisms) may be only one contributing factor to the elimination rate of HDA after exposure to HDI. Liu *et al.*[7] observed considerable variability between exposure to HDI biuret, a HDI oligomer, with a small amount of the HDI monomer, and urine HDA levels that was attributable to subjects over 32 years old having significantly higher pre-exposure HDA levels but not post-exposure levels than younger subjects. In addition, they observed higher HDA levels both at pre-exposure and immediately post-exposure in non-smokers compared to smokers, but the difference was statistically significant for pre-exposure levels only. Job category, age, and years working in the automotive spray-painting industry did not affect the individual variability in urine HDA levels in their study.

Uncertainty in the relationship between HDI exposure and urine HDA levels is mostly due to the lack of knowledge of the complete physical and biological processes from exposure to elimination. Several researchers have noted that the amount of HDA excreted is only a small amount of the actual exposure dosage.[5,6,13,14] Brorson *et al.*[5] observed that subjects classified as slow-acetylators excreted more of the inhaled HDI dose than subjects classified as rapid-acetylators, while Brorson *et al.*[13] observed the opposite with orally administered HDA. However, this difference may be due to route and type of exposure. In order to investigate the cause(s) for the intra- and inter-person variability observed in urine HDA levels after occupational exposure to HDI,[3] we examined the individual and work environment factors that may affect the relationship between HDI exposure and urine HDA levels.

Materials and methods

Study population

The study population has been described previously.[3,15] Briefly, spray painters in automotive repair shops who worked with HDI-containing paint were recruited for the study in the Raleigh-Durham area of North Carolina and the Puget Sound area of Washington State. Eleven shops in North Carolina with a total of 15 workers and 25 shops in

Washington with a total of 32 workers participated in the study. Each exposed worker was monitored over the workday on up to three separate days over a 12-month period. Due to attrition, five subjects were monitored once and 15 subjects were monitored twice. The subjects were all male, ranging in age from 21 to 59 years with an average age of 34 years. Thirty subjects identified themselves as white, nine as Hispanic, four as African-American, one as Asian, one as Native American, and two as mixed ethnicity. This study was approved by the Institutional Review Board in the Office of Human Research Ethics at the University of North Carolina at Chapel Hill and by the Washington State Institutional Review Board at the Washington State Department of Social and Health Services.

A questionnaire was developed for this study and information on worker age, weight, height, ethnicity, and medical history with regard to susceptibility to occupational asthma (*i.e.*, had allergies, asthma, or medical problems after painting) was collected. No one reported diisocyanate-induced asthma. Information on the type of personal protective equipment (PPE) that they typically wore during painting and the maintenance schedule of their PPE and paint booth was also obtained.

Dermal and breathing-zone air sampling

Personal breathing-zone and dermal tape-strip sampling were performed to estimate inhalation and dermal exposure during every spray-application of HDI-containing paints and coatings.[16] The collection and analyses of the breathing-zone and dermal tape-strip samples have been published previously.[15,17] On each sampling visit, breathing-zone air samples were collected during each HDI-containing painting task, and tape-strip samples were collected immediately following each task. The painter was observed during the paint tasks to note the duration of exposure and the type of respirator worn. The Assigned Protection Factor (APF) designated by the Occupational Safety and Health Administration[18] for the respirator worn by a worker (none, APF = 1; air purifying half-face, APF = 10; air-purifying full-face, APF = 50; supplied air full-face or hood, APF = 1000; powered air-purifying (PAPR), full-face or hood, APF = 1000) was used to adjust the measured breathing-zone concentrations (BZC) in order to account for the respiratory protection in inhalation exposure levels used in the analyses.

Urine sampling

The urine sampling protocol has been published previously.[3] Briefly, during each sampling visit, urine samples were obtained from the worker each time he urinated. At a minimum, one end-of-day sample was collected. An average of 2.5 post-exposure urine samples were obtained per worker per day. The maximum number of samples obtained from a worker on a single day was eight. A total of 282 post-exposure urine samples were collected.

HDA analysis

The analysis of HDA levels in the urine samples collected from the spray painters has been published previously.[3] Briefly, an internal standard 1,7-diaminoheptane (HpDA) was added to the urine sample before hydrolysis at 100 °C with concentrated sulfuric acid. The samples were cooled, neutralized with saturated sodium hydroxide (NaOH), mixed with sodium chloride, and then extracted three times with toluene. The samples were then derivatized

with heptafluorobutyric anhydride at 55 °C, cooled, and potassium phosphate buffer added to remove excess derivatizing agent. The organic layer was retained, and sodium sulfate was added to dry the sample. The organic solution was moved to a clean vial and dried. The samples were then reconstituted with 200 μl ethyl acetate, sonicated, and transferred to GC vial inserts. The samples were again dried to completion and reconstituted with 60 μl ethyl acetate. The samples were analyzed by gas chromatography-mass spectrometry (GC-MS) (Thermo, Austin, TX) in negative chemical ionization mode with methane as the reagent gas. The HDA and HpDA were determined using selective ion monitoring at *m/z* 448 and *m/z* 462, respectively.

Standard curves were prepared by spiking pooled urine from four unexposed individuals with HDA. Each standard curve consisted of a reagent blank (no HDA or HpDA), a negative control (HpDA but no HDA), and nine different HDA concentrations (0.08 to 20 μg/l) with HpDA (1.5 μg/l). Weighted linear regression was used to construct a standard curve using the HDA/HpDA ratio.[19] Different weighting factors ($w = x^{-0.5}$, x^{-1}, x^{-2}, $y^{-0.5}$, y^{-1}, y^{-2}, $y^{-1.5}$; where x = HDA/HpDA instrument response ratio, y = HDA concentration) were evaluated for fitting standard curves. The weighting factor that gave the smallest sum of absolute relative error as a percentage of the nominal concentration was used for fitting the standard curve.[19] The standard curve was linear from 0 to 20 μg/l ($w = y^{-2}$, $R^2 = 0.98$). The method detection limit (MDL) of 0.04 μg/l was calculated using the MDL procedure established by U.S. EPA.[20]

Creatinine analysis

The creatinine concentration in the urine was determined using the Creatinine Companion assay kit (Exocell, Inc., Philadelphia, PA)[21,22] as described previously.[3] Briefly, samples were diluted in distilled water and then aliquoted, in duplicate, into a microtiter plate along with creatinine standards, in duplicate. NaOH was added to alkaline picrate reagent, and this solution was added to each well. The plate was incubated at room temperature for 10 min, and the absorbance determined at 500 nm (Emax, Molecular Devices, Sunnyvale, CA). The acid reagent provided with the kit was then added to each well, and the absorbance at 500 nm determined after a 5-min incubation at room temperature. The difference between the two absorbance values was recorded for each well. A standard curve was calculated based on the standards and their responses. Unknown samples were evaluated by comparing their responses to the standard curve.

Statistical analysis

The data were analyzed using SAS statistical software (SAS 9.1; SAS Institute Cary, NC). BZC and dermal levels of HDI and urine HDA levels were natural log-transformed to satisfy normality assumptions (Shapiro Wilks $W > 0.85$) prior to statistical analysis. Creatinine concentrations were approximately normally distributed ($W = 0.89$). However, natural log-transformation of the creatinine concentrations improved the normality ($W = 0.96$).

Due to the relatively high percentage of non-detectable levels of HDA in the urine samples (38%) as well as HDI in the breathing-zone air (9%) and dermal tape-strip (63%) samples, multiple imputation was used to impute data below the detection limits. For each

observation with a non-detectable level, ten values were imputed. Methods for performing the multiple imputation of the exposure data have been described previously.[3,15,17] Briefly, we applied logarithmic transformation to all exposure variables to make them normally distributed before imputation and imputed from truncated multivariate normal distributions with an upper truncation at the logarithmic transformed limit of detection for HDI or MDL for HDA. PROC MIANALYZE was used to combine the results of the analyses carried out on the 10 imputed datasets and to obtain valid estimates and statistical inferences. Averages were computed where PROC MIANALYZE could not be used (*i.e*. fit statistics, *t*-tests, and analysis of variance).

We investigated the effect of different covariates on the relationship between HDI exposure (inhalation and dermal) and post-exposure urinary HDA levels by including the covariates as independent variables in the mixed effects multiple linear regression models (PROC MIXED). Each urine sample was used as an individual observation. Previously, we reported that the measured personal BZC adjusted by the APF, as designated by the Occupational Safety and Health Administration,[18] for the respirator worn by the worker provided a better model fit when investigating the effect of dermal and inhalation exposure on urine HDA levels.[3] Since the adjusted BZCs provided the best-fit model, we used BZCs adjusted for APF in the models presented here.

The mixed effects multiple linear regression model to investigate the relative influences of different covariates including creatinine concentration to the relationship between urine HDA level (unadjusted for creatinine concentration) and dermal and inhalation exposure is as follows:

$$Y_{ij}=\beta_0+\beta_1 X_{1ij}+\beta_2 X_{2ij}+\beta_3 X_{3ij}+\beta_4 X_{4ij}+\alpha_i+\varepsilon_{ij}, \quad (1)$$

where Y_{ij} represents the natural logarithm of the urinary HDA concentration (the *j*th measurement obtained for the *i*th worker), X_{1ij} represents the natural logarithm of the measured BZC adjusted for the APF (BZC-APF) based on the respirator worn, X_{2ij} represents the natural logarithm of the measured dermal exposure, X_{3ij} represents the natural logarithm of the creatinine concentration of the urine sample, X_{4ij} represents the covariate being tested, and α_i and ε_{ij} represent the random effects associated with worker (α_i for i = 1,2,…,48 workers) and an error term (ε_{ij} for j = 1,2,…,16 measurements per worker). Models were constructed using standard regression techniques, and model fit was examined with regression diagnostics such as residual analysis. The statistical significance was evaluated at α-level 0.10.

Using this model, we assumed that α_i and ε_{ij} are mutually independent and normally distributed with means of zero and respective variances σ_B^2 and σ_W^2 representing the between and within-worker variance components, where total variance $\sigma_Y^2 - \sigma_B^2+\sigma_W^2$. It is also assumed that Y_{ij} is normally distributed with mean $\mu_Y = \beta_0 + \beta_1 X_{1ij} + \beta_2 X_{2ij}$ and variance σ_Y^2. Compound symmetry was used for the covariance structure.

Cumulative exposure measures for BZC-APF and dermal exposure were used in the statistical analyses. Cumulative exposure was calculated by summing all the respective

exposure levels that occurred before a urine sample was obtained, as provided by the following equation,

$$X_{ij}=ln\left(\sum_{0}^{T}C_t\,(if\ t<T)\right) \quad (2)$$

where C_t is the concentration at time t of the measured dermal or BZC-APF exposure, T is the time of the urine sample, and t is the exposure time.

A list of all covariates tested including the descriptions and the ranges for the variables is provided in Table 1. These covariates included dichotomous variables such as the type of PPE worn by the painter (gloves, long sleeves, coveralls, and hat), multiple painters at shop, smoker *vs*. non-smoker, allergies, or asthma. Categorical variables such as ethnicity, the weekday of sampling, and the type of paint booth used (cross-draft, semi-downdraft, or downdraft) were also tested. We also included continuous variables such as worker's age, BMI, and number of clear coats tasks performed in the past week. Candidate covariates for the mixed effects multiple linear regression models were selected by running separate models that considered only the exposure variables, creatinine, the candidate covariate, and the interaction terms between the candidate covariate and dermal or inhalation exposure (*i.e*. the covariates were tested one at a time). From these models, those variables with *p*-values <0.15 were used to build the final models. Final models were built using a backward elimination procedure in which the least significant variables ($p < 0.15$) were eliminated one at a time. Statistically nonsignificant main effects were always retained if their respective interaction terms were significant.

Although we reported that BZC-APF provided the best fit in the mixed effect models,[3] we also chose to investigate multiple linear regression models where X_{1ij} represented BZC unadjusted for APF and X_{4ij} represented the multi-class respirator type where $X_{4ij} = 0$ for half-face cartridge respirator, $X_{4ij} = 1$ for full-face cartridge respirator, and $X_{4ij} = 2$ for air-supply respirator or PAPR. The respirator types were categorized into these four levels because they were categorized in this manner for designating APFs.

In addition, we used a marginal R^2 statistic proposed by Vonesh and Chinchilli[23] to assess the goodness-of-fit of fixed effects in our mixed effects multiple linear regression models. Several R^2 statistics have been proposed for assessing the goodness-of-fit of fixed effects for models.[24,25] However, marginal R^2 statistic is more appropriate than conditional R^2 statistic for estimating explained variability from fixed effects as marginal R^2 statistic does not use random effects in the computation of predicted means that lead to residuals.[24] Orelien and Edwards[24] found this statistic to perform extremely well at differentiating between full and reduced models and not diverging when models were over-fitted.

We also investigated the effect of different covariates on the urine HDA levels using analysis of variance (ANOVA). For this analysis, we used hypothetical, pooled urine samples consisting of all post-exposure urine samples on a given workday. Each worker has n number of post-exposure urine samples, and each urine sample has a volume V_j, HDA level Y_j (μg/l), and creatinine concentration C_j (g/l). If all post-exposure urine samples were

pooled, the pooled urine would have volume V_T, HDA level Y_T (μg/l), and creatinine concentration C_T (g/l) where and

$$V_T - \sum_0^n V_j \quad (3)$$

and

$$Y_T - \frac{\sum_0^n (Y_j \times V_j)}{V_T} \quad (4)$$

C_T is calculated similarly to eqn (4). If creatinine is excreted at a constant rate, then

$$\frac{C_j}{V_j} - \frac{C_{j+1}}{V_{j+1}} - \frac{C_T}{V_T} \quad (5)$$

Then with substitution and rearrangement,

$$\frac{Y_T}{C_T} - \frac{\sum_0^n (Y_j \times C_j)}{\sum_0^n \left(C_j^2\right)} \quad (6)$$

We calculated the geometric mean (GM) and geometric standard deviation (GSD) of the hypothetical pooled HDA levels from the natural log-transformed data (PROC MEANS) stratified by class-type covariates. Statistical analysis of the stratified data was performed using PROC TTEST for dichotomous variables and PROC GLM for covariates with more than two levels (at α-level 0.10) across each imputed data set and *p*-values averaged. All dichotomous and categorical variables listed in Table 1 were tested in this manner. However, the covariates describing the painter's allergies or asthma symptoms were not tested with ANOVA, due to the limited number of workers who reported having allergies (n = 11) or asthma (n = 4), which made accurate testing impossible. In addition, we performed these statistical analyses using only the last urine sample obtained at the end of the day for each worker to be consistent with other published studies in which only urine samples obtained at the end of the workday were examined for exposure-biomarker association in HDI exposed workers.[6,26] These urine samples are hereon specified as end-of-day urine samples.

Results

The mixed effects multiple linear regression models for predicting urinary HDA levels are summarized in Table 2. Models built using either BZC (Models 1 and 3) or BZC-APF (Models 2 and 4) with significant covariates were evaluated. The starting Models 1 and 2 originate from our previously published work[3] and include only exposure variables and creatinine. The final Models 3 and 4 include exposure variables, creatinine, and statistically

significant individual and workplace factors. When tested individually (data not shown), the only covariates with a *p*-value less than 0.15 were weekday ($p = 0.056$) and coveralls ($p = 0.12$).

In the original starting model for BZC (Model 1) containing only exposure variables and creatinine, BZC exposure was not significant ($p = 0.38$). When respirator type was added to the model along with the exposure variables, respirator type was significant ($p = 0.082$, data not shown), but BZC exposure was not ($p = 0.33$). In the final BZC model containing significant covariates (Model 3), respirator type ($p = 0.11$) and weekday ($p = 0.10$) were the only significant covariates, and BZC was removed from the model since it was not significant.

In the original starting model with BZC-APF (Model 2) containing only exposure variables and creatinine, dermal exposure was not significant ($p = 0.24$). In the final model with BZC-APF and covariates (Model 4), dermal exposure became even less significant and was removed from the model. When the two significant covariates were both placed in a single model (Model 4), both weekday ($p = 0.05$) and coveralls ($p = 0.09$) retained significance.

We further investigated the effect of weekday on urine HDA levels using ANOVA. BZC-APF exposure did not significantly differ between the weekdays, but weekday was borderline significant for dermal exposure ($p = 0.11$). The effect of weekday on exposure levels was also evaluated using linear mixed modeling. Weekday was borderline significant for BZC-APF exposure ($p = 0.18$) and significantly affected dermal exposure ($p = 0.05$). Thus, significant variation on dermal exposure levels on different weekdays may have contributed to the observation that weekday affected the levels of HDA in urine. However, weekday was significant in both final linear regression models (Table 2, Models 2 and 4) while dermal exposure was insignificant only in the final model with BZC-APF (Table 2, Model 2). Therefore, since dermal exposure did not significantly affect HDA levels in this model, weekday variation in dermal exposure by itself should not cause weekday to be a significant variable in the model. Furthermore, in the final BZC model where dermal exposure was significant (Table 2, Model 4), any weekday variation in dermal exposure would be accounted for by the dermal exposure variable, and thus, the significance of weekday in this model is independent of dermal exposure variability.

A summary of the hypothetical, pooled post-exposure urine HDA levels adjusted for creatinine concentration and stratified by the different dichotomous and classification covariates as well as the results of the ANOVA is provided in Table 3. Workers who wore coveralls had significantly lower urine HDA levels ($p = 0.05$) than workers who did not wear coveralls. Workers using a PAPR or air supply respirator, as opposed to a cartridge-type respirator, had significantly lower urine HDA levels when the respirator was stratified as a dichotomous covariate of cartridge-type respirator *versus* all others ($p = 0.06$). Painters who worked in semi-downdraft or downdraft booths had significantly lower HDA levels than those who worked in cross-draft booths ($p = 0.02$). Painters who worked at shops with more than one painter had significantly higher levels of HDA than those who worked at a shop where there was only one painter ($p = 0.10$). Smokers had borderline significantly higher HDA levels than non-smokers ($p = 0.12$).

No significant variables were observed to affect the end-of-day urine HDA levels adjusted for creatinine concentration when stratified by the different dichotomous and classification covariates (Table 3). Since we previously observed that smoking affected creatinine levels,[8] we examined the urine HDA levels unadjusted for creatinine. Smokers had significantly higher unadjusted HDA levels than non-smokers ($p = 0.07$; results not shown).

Stratification of the HDA levels twice allowed for analysis of the effect of dermal protection within groups of similar respirator types (Table 4). The HDA levels were first stratified by the use of cartridge-type respirators *versus* PAPR or air-supply respirators. Then, they were stratified again by use of coveralls or gloves. Among workers who wore cartridge-type respirators, those who wore coveralls had significantly lower post-exposure urine HDA levels than those who did not wear coveralls ($p = 0.10$). No significant difference was observed in post-exposure urine HDA levels between coverall users and non-users among workers who used a PAPR or air-supply respirator. There was also no significant difference in post-exposure urine HDA levels between workers wearing and not wearing gloves among any respirator group. In addition, no significant differences were observed in HDA levels in the end-of-day urine samples with any of these stratification methods.

The data was also stratified twice to determine if the reason for the differences between HDA levels for smokers and non-smokers was due to differences in PPE use (Table 4). The HDA levels were first stratified by the use of cartridge-type respirators *versus* PAPR or air-supply respirators. Then, they were stratified again by the smoker status (yes, no). Among workers using a PAPR or air-supply respirator, smoker status remained significant ($p = 0.03$). This procedure was repeated for coverall use. The HDA levels were stratified first by coveralls use (yes, no). The data were then stratified by the smoker status (yes, no). Among workers wearing coveralls, smoker status remained significant ($p = 0.06$). For the end-of-day urine HDA levels, smoker status was significant, but only for workers wearing coveralls ($p = 0.05$ for unadjusted HDA levels, and $p = 0.10$ for adjusted HDA levels).

Discussion

Previously, we reported that inhalation and dermal HDI exposure significantly affected the urine HDA levels in workers occupationally exposed to HDI.[3] We also determined that the water content of urine should be accounted for by using creatinine concentration as an independent variable in a linear regression model.[3,8] Here, we examined individual and workplace factors that may modify urine HDA levels in occupationally exposed workers and how those factors affect exposure models.

We observed that painters who used the more protective PAPR or air-supply respirators had significantly lower urine HDA levels than painters who wore cartridge-type respirators. Respirator type was a significant variable in the linear regression model that used BZC unadjusted for APF. BZC was not a statistically significant variable in the model when used along with dermal exposure level and creatinine concentration. When respirator type was added to the model, BZC retained its insignificance. Consequently, this lends support for the use of BZC modified for APF to account for the inhalation exposure in the linear regression models.

Workers who wore coveralls and, thus, received some protection from dermal exposure, had significantly lower urine HDA levels than those who did not wear coveralls (Table 3). Austin reported similar results for workers dermally exposed to TDI.[27] This significance remained when the workers were stratified by respirator type to control for the effect of differences in inhalation protection. Coverall use significantly lowered urine HDA levels among painters wearing cartridge-type respirators, but not among painters wearing PAPR or air-supply respirators. However, this result may be biased due to the small number of urine samples obtained from workers wearing PAPR or air-supply respirator but not wearing coveralls (N = 4).

Coverall use was also a significant predictor of urine HDA levels in the linear regression models with BZC-APF exposure (Table 2). No other categorical variables that describe the dermal PPE worn by the worker improved model fit. However, dermal exposure as measured by the tape-strip method reflected the effect of PPE protection since the tape-strip samples were taken from the areas that had been covered during painting.[17] Thus, the dermal exposure measured accounted for the protectiveness of the gloves or coverall. However, the measured dermal exposure may have been underestimated due to the possible rapid absorption of HDI through the stratum corneum and/or conjugation of HDI to macromolecules in the skin. This may have contributed to the large number of non-detectable samples collected from the skin (63%) even though the tape-strips were collected immediately after each paint task. Further, although both final models included a dermal exposure variable (coverall in Model 2 and dermal in Model 4), the high percentage of non-detectable tape-strips may have led to an underestimation of dermal exposure's true significance.

Painters in the multi-painter shops had significantly higher urine HDA levels than painters in the single-painter shops. This indicates a bystander effect where painters are being exposed to HDI through sources other than their painting tasks. Shops with more than one painter may be busier and potentially have higher HDI-exposures, thus, increasing urine HDA levels. However, no significant difference was observed in the cumulative daily BZC-APF or dermal exposure between one- and multi-painter shops. Booth type was also found to significantly affect urine HDA levels. Because Fent *et al*.[15] previously observed that booth type significantly affected BZCs in the same study population, this result was expected. Booth type was not observed to be a significant variable in the linear regression models most likely because booth type modified the exposure levels and was, therefore, accounted for in the dermal and BZC-APF exposure variables.

Our ANOVA results showed smokers to have significantly higher HDA levels than non-smokers. After stratification by cartridge-type respirator and coverall use to control for the effect of different inhalation or dermal protection, the significance of smoking was retained but only for the more protected workers (*i.e*., those wearing coveralls or PAPR or air-supply respirators). In contrast, Liu *et al*.[7] observed smokers to have lower urine HDA levels in a group of healthy auto-body workers previously exposed to HDI. Neither current smoker status or smoking history were observed to significantly affect urine HDA levels in the linear regression models. This indicates that while smoking predicts urine HDA levels, it does so to a much lesser degree than HDI-exposure and creatinine concentration. The reason

why smoker status affected urine HDA levels in workers wearing more protective PPE is unclear. It is plausible that smokers were exposed to HDI through ingestion by transferring HDI from contaminated hands to cigarettes and mouth. The reason may also be biological. It is plausible that HDI is eliminated faster in smokers due to tobacco smoke related induction of metabolic enzymes.[28] It is also plausible that HDA and toxins in tobacco smoke compete for the same binding sites in macromolecules, and thus, due to potentially greater affinity of tobacco toxins to macromolecules compared to HDA, HDA may be cleared faster from the body. For example, benzo[*a*]pyrene, which is found in cigarette smoke, is known to bind tightly to albumin.[29] Perez-Reyes *et al*. proposed a similar reason to explain the rate of increase in THC plasma concentrations in marijuana smokers.[30]

No other demographic variables improved the predictability in the linear regression models, most likely due to the demographic similarities between the individuals in this small study population. Further, the linear regression models were not affected by worker health conditions (*e.g*., allergies, asthma). Weekday was found to significantly affect urine HDA levels in the linear regression models. This finding supports our previous observation that HDA has a biphasic elimination[3] and may reflect the fact that HDA binds to macromolecules in the body[9,11,12] and, consequently, is slowly released or broken down over time.

Finally, when we examined only the end-of-day urine samples, smoker status was the only factor that significantly affected the urine HDA levels according to ANOVA. However, we demonstrated previously that HDA levels can vary greatly throughout the day,[3] and that the short-term half-life of HDA is about 1.2–2.9 h.[3,5,7,13,14] Therefore, the end-of-day sample may not reflect the exposure dose received during the morning hours. Thus, the results indicate the importance of collecting multiple urine samples throughout the day instead of just one end-of-day spot sample.

Conclusion

This study provides further evidence that dermal exposure to HDI along with inhalation exposure contribute to urine HDA levels. Further, this study demonstrates the importance of proper dermal and inhalation protection during automotive spray painting with HDI-containing paints. The results indicate differences in the effectiveness of the respiratory protections used. Individual factors, with the exception of smoking status, were not observed to significantly affect HDA levels, thus confirming the need to focus our efforts on exposure reduction in work places. Also, evidence was provided for a bi-phasic elimination pattern of HDA and the importance of collecting multiple urine samples throughout the workday to reliably assess the contribution of the workday exposure to the HDA level.

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Environmental impact

Urinary HDA can be used as a biomarker of exposure to HDI. However, a large variation in the levels of HDA exists in occupationally exposed workers. Our goal was to determine the individual and workplace factors that affect urine HDA levels after accounting for HDI exposure and urine water content. The use of dermal and respiratory protection was significantly associated with lower urine HDA levels. The presented information will aid occupational and environmental health professionals and researchers to determine methods to improve worker protection.

Table 1

Summary of variables tested in linear regression models to predict urine HDA levels

Type	Name	Description	Range of Values	Mean[a]	Median[a]	Variable tested with interactions[b]
Dichotomous	Gloves	Use of gloves	0: no, 1: yes	0.78	1	no
	Long sleeves	Use of long sleeves	0: no, 1: yes	0.05	0	no
	Coverall	Use of coveralls	0: no, 1: yes	0.67	1	no
	Hat	Use of hat	0: no, 1: yes	0.38	0	no
	Goggles	Use of goggles	0: no, 1: yes	0.17	1	no
	Multi-painter shop	Shop has more than one painter	0: no, 1: yes	0.40	0	no
	State	Location of worker	0: NC, 1: WA	0.71	1	no
	Smoker	Current Smoker	0: no, 1: yes	0.32	0	no
	Skin allergies	Has skin allergies	0: no, 1: yes	0.02	0	no
	Seasonal allergies	Has season allergies	0: no, 1: yes	0.19	0	no
	Year-round allergies	Has year-round allergies	0: no, 1: yes	0.07	0	no
	Allergy medicine	Uses allergy medicine	0: no, 1: yes	0.12	0	no
	Asthma	Has asthma	0: no, 1: yes	0.09	0	no
Classification	Ethnic group	Worker's ethnicity	0: White, 1: Black, 2: Hispanic, 3: Asian, 4: Native American, 5: mixed	0.83	0	no
	Smoker History	Historical smoking	0: never, 1: past, 2: current	0.89	1	no
	Weekday	Day of week	0: Monday, 1: Tuesday, 2: Wednesday, 3: Thursday, 4: Friday	1.97	2	yes
	Booth type	Type of ventilated paint booth	0: cross draft, 1: semi-down draft, 2: down draft	1.5	2	no
	Respirator type[c]	Type of respirator worn	0: half-face, 1: full-face, 2: air supplied hose or PAPR	0.48	0	yes
Continuous	Age	Worker's age	21–59	34.2	34	yes
	BMI	Worker's BMI	18.7–38.8	28.3	27.4	yes
	Years experience	Worker's numbered years of painting experience	0.25–40	12.6	11	no
	Times painting in the past week	Number of clear coat tasks performed in past seven days	0–45	14.0	12	yes

[a] Mean and median values are based on worker-visits. A worker who was visited three times, would have his response used in the calculation three times. Mean and median for dichotomous and categorical variables, are based on the numeric value of each response as indicated in range of values.

[b] Variables, designated as tested with interaction, were tested with interaction with dermal and BZC-APF exposure.

[c] Variable, respirator type, was only used in model with BZC, not in the models with BZC-APF.

Table 2

Summary of the linear mixed models for predicting natural log-transformed, post-exposure urinary HDA concentrations in the spray-painters using unadjusted (BZC, Models 1 and 3) or APF adjusted BZC levels (BZC-APF, Models 2 and 4). The starting Models 1 and 2 originate from our previously published work[3] and include only exposure variables and creatinine. The final Models 3 and 4 include exposure variables, creatinine, and statistically significant individual and workplace factors.[a]

Model	Variable	Estimate	Standard error	*p*-value	AIC	R^2
1. Starting model with BZC	InBZC	0.08	0.09	0.38	1038	0.25
	Indermal	0.11	0.07	0.12		
	Increatinine	1.25	0.17	<0.0001		
	worker var	0.99	0.07			
	residual var	1.78	0.15			
2. Starting model with BZC-APF	InBZC-APF	0.14	0.06	0.03	1035	0.28
	Indermal	0.08	0.07	0.24		
	Increatinine	1.28	0.17	<0.0001		
	worker var	0.93	0.07			
	residual var	1.76	0.15			
3. Final model with BZC[b]	Indermal	0.16	0.06	0.01	1025	0.29
	Increatinine	1.35	0.17	<0.0001		
	Half-face	0.62	0.39	0.11		
	Full-face	1.33	0.70			
	Monday	−0.16	0.26	0.10		
	Tuesday	0.57	0.20			
	Wednesday	−0.06	0.20			
	Thursday	−0.02	0.24			
	worker var	1.00	0.10			
	residual var	1.11	0.15			
4. Final model with BZC-APF	InBZC-APF	0.19	0.06	0.001	1025	0.29
	Increatinine	1.34	0.17	<0.0001		
	Monday	−0.20	0.25	0.05		
	Tuesday	0.64	0.20			
	Wednesday	−0.03	0.19			
	Thursday	−0.11	0.24			
	coverall	−0.48	0.29	0.09		
	worker var	1.01	0.09			
	residual var	1.68	0.15			

[a] InBZC = natural log-transformed breathing zone concentration; InBZC-APF = natural log-transformed respirator adjusted breathing zone concentration; Indermal = natural log-transformed dermal exposure; Increatinine = natural log-transformed creatinine concentration; AIC = Akaike's Information Criterion; var = variance.

[b] BZC was removed from the final model since it was not statistically significant.

Table 3

Post-exposure and the end-of-day urine HDA levels[a] adjusted for creatinine concentration and stratified by workplace and individual factors.[b]

				Pooled post-exposure urine (μg HDA/g creatinine)			End-of-day urine (μg HDA/g creatinine)		
Variable	**Level**	**n**	**N**	**GM**	**GSD**	***p*-value**	**GM**	**GSD**	***p*-value**
Coverall	no	14	38	0.16	3.48	0.05	0.13	4.95	0.21
	yes	33	77	0.09	4.94		0.08	5.60	
Gloves	no	8	25	0.14	3.51	0.29	0.12	5.07	0.41
	yes	39	90	0.10	4.80		0.09	5.52	
Long sleeves	no	41	109	0.11	4.65	0.89	0.10	5.62	0.47
	yes	6	6	0.11	2.65		0.13	2.54	
Hat	no	26	71	0.13	4.56	0.22	0.10	5.76	0.56
	yes	21	44	0.09	4.39		0.09	4.92	
Googles	no	37	95	0.12	4.49	0.15	0.10	5.76	0.56
	yes	10	20	0.07	4.37		0.09	4.92	
Respirator	PAPR/air supply	10	26	0.07	3.92	0.06	0.07	4.72	0.23
	cartridge	38	88	0.13	4.63		0.11	5.65	
APF respirator type	1	1	1	0.09	1.00	0.23	0.09	1.00	0.44
	10	37	85	0.12	4.70		0.07	5.73	
	50	1	3	0.30	1.76		0.32	1.91	
	1000	10	26	0.07	3.92		0.07	4.72	
Booth type	cross	9	20	0.25	2.60	0.02	0.18	3.53	0.20
	semi	7	17	0.09	4.33		0.09	5.59	
	down	31	78	0.09	4.79		0.08	5.76	
Multipainter shop	no	28[c]	68	0.09	4.48	0.10	0.08	5.09	0.28
	yes	19	47	0.15	4.38		0.12	5.86	
Location	North Carolina	14	33	0.11	4.57	0.91	0.10	5.26	0.75
	Washington	33	82	0.11	4.53		0.09	5.52	
Weekday	Monday	18	18	0.08	3.77	0.47	0.07	4.76	0.47
	Tuesday	23	23	0.15	5.21		0.13	6.39	
	Wednesday	34	34	0.10	4.59		0.11	5.75	
	Thursday	24	24	0.15	4.49		0.12	4.56	
	Friday	16	16	0.08	3.78		0.05	4.80	
Current smoker	no	33	78	0.09	4.88	0.12	0.08	5.66	0.16
	yes	16	37	0.15	3.67		0.13	4.78	
Ethnicity	Caucasian	31	78	0.11	4.51	0.33	0.10	5.63	0.58
	African-American	4	6	0.06	6.57		0.06	5.89	
	Hispanic	9	19	0.15	3.22		0.13	4.00	
	Asian	1	3	0.03	3.12		0.04	3.60	
	Native American	1	3	0.23	1.55		0.25	1.32	
	mixed	2	6	0.07	6.81		0.06	8.67	

[a] Pooled, post-exposure urine level includes all urine samples collected from a painter after at least one exposure had occurred while the end-of-day urine level was measured in the last urine sample of the workday.

[b] n = number of workers (may not always total 47, since workers would change PPE use or other factors between visits; two workers quit smoking between visits); N = number of worker-days; GM = geometric mean; GSD = geometric standard deviation.

[c] Number is based on painters participating in this study. Some shops may have had another painter who did not participate in the study.

Table 4

Summary of all post-exposure and only end-of-day urine HDA levels[a], adjusted for creatinine concentration, stratified twice by workplace and individual factors. The HDA levels were first stratified into the two levels of variable A. Then, they were stratified again into the two levels of variable B.[b]

					Post-exposure urine (μg HDA/g creatinine)			End-of-day urine (μg HDA/g creatinine		
Variable A	**Level A**	**Variable B**	**Level B**	**N**	**GM**	**GSD**	***p*-value**	**GM**	**GSD**	***p*-value**
Respirator	PAPR/air supply	Coverall	no	4	0.08	5.56	0.77	0.08	5.59	0.78
			yes	22	0.07	3.65		0.07	4.57	
	Cartridge	Coverall	no	33	0.17	3.22	0.10	0.14	4.95	0.31
			yes	55	0.10	5.40		0.09	5.99	
Respirator	PAPR/air supply	Gloves	no	3	0.09	3.46	0.74	0.09	4.34	0.67
			yes	23	0.07	3.98		0.07	4.77	
	Cartridge	Gloves	no	21	0.16	3.56	0.44	0.13	5.35	0.52
			yes	67	0.12	4.96		0.10	5.72	
Respirator	PAPR/air supply	Current	no	20	0.05	4.12	0.03	0.05	4.71	0.16
		smoker	yes	6	0.16	2.08		0.15	3.55	
	Cartridge	Current	no	57	0.12	4.93	0.47	0.10	5.95	0.44
		smoker	yes	31	0.15	4.05		0.13	5.04	
Coverall	no	Current	no	21	0.17	3.17	0.65	0.14	4.30	0.74
		smoker	yes	17	0.14	3.84		0.15	4.19	
	yes	Current	no	57	0.08	5.23	0.06	0.06	5.90	0.10
		smoker	yes	20	0.16	3.62		6.41	6.46	

[a] Post-exposure urine level includes all urine samples collected from a painter after at least one exposure had occurred while the end-of-day urine level was measured in the last urine sample of the workday.

[b] N = number of urine samples; GM = geometric mean; GSD = geometric standard deviation.