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Urinary biomonitoring of occupational exposures to Bisphenol A Diglycidyl Ether (BADGE) – based epoxy resins among construction painters in metal structure coating

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ABSTRACT

Epoxy resin systems based on Bisphenol A Diglycidyl Ether (BADGE) monomer and its higher oligomers are important commercial formulations used widely in construction for protective coating of steel structures, such as bridges. The literature on occupational exposures and biomonitoring of BADGE-based epoxies among construction painters is remarkably limited. In this first occupational biomonitoring study of epoxies, 44 construction painters performing mid- and top-coating were recruited from 12 metal structure coating sites in New England. Cross-shift changes in the urinary levels of total BADGE and its three major hydrolysis derivatives - BADGE·$2H_2O$, BADGE·H_2O, BADGE·HCl·H_2O – were assessed. Results for 81 urine samples collected from coating workers were compared with 28 urine samples of a reference group of 14 spray polyurethane foam (SPF) insulation workers.

The highest concentrations of all biomarkers were found in the urine samples of mid-coat applicators. The major urinary biomarker of BADGE in this cohort of workers, BADGE·$2H_2O$, was detected in 100% of urine samples. The post-shift BADGE·$2H_2O$ (specific gravity normalized data) in mid-coat applicators had a geometric mean (GM) of 1.46 ng/mL and a geometric standard deviation (GSD) of 3.6 (range, 0.2–18.7 ng/mL). The second most abundant biomarker in urine, BADGE·HCl·H_2O, was measured in 84% of samples, and had a post-shift GM (GSD) of 0.17 (2.3) ng/mL (range, <0.025–0.59 ng/mL). BADGE·$2H_2O$ was 8.6 times more abundant than BADGE·HCl·H_2O. BADGE·H_2O was quantified only in 10% of the samples (range, 0.11–0.41 ng/mL). Free BADGE in post-shift urine, corrected for background, had GM (GSD) of 0.04 (2.5) ng/mL (range, <0.025–0.16 ng/mL). Urinary BADGE·$2H_2O$ were significantly higher ($p = 0.01$) in mid-coat applicators compared to top-coat and SPF workers. Post-shift urinary BADGE·$2H_2O$ in mid-coat applicators increased by ~2.9× ($p = 0.02$) and 1.36× in top-coat applicators ($p = 0.18$) compared to pre-shift values, but not in SPF workers (0.95×; $p = 0.40$).

In conclusion, we demonstrate that (i) significant BADGE uptake occurs via inhalation and skin exposures during application of epoxy-containing paintings (mid-coat), suggesting the need for improvements in hygiene practices and personal protective measures; (ii) BADGE·$2H_2O$ is a robust and sensitive biomarker for biomonitoring of exposures to BADGE-based epoxies in occupational settings; and (iii) widespread occurrence of BADGE and BADGE·$2H_2O$ in the urine of all workers, including SPF workers, suggest common exposures from non-occupational sources, such as ingestion or do-it-yourself consumer applications of epoxy resins. In light of this observation, establishing a reliable biological monitoring guidance value (BMGV) for BADGE·$2H_2O$ will require more background biomonitoring and health effect data. An initial reference value for BADGE·$2H_2O$ of 0.5 ng/mL (SG-normalized) or 180 nmol/mol creatinine is being proposed as the threshold to discriminate occupational from non-occupational exposures based on the maximum values observed in the reference SPF group.

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1. Introduction

Epoxy resin systems are important commercial formulations that are used widely in construction protective coating of steel structures, such as bridges, storage tanks and wind turbines, for their excellent adhesion to surfaces, durability and anticorrosive properties (Gibson 2017; Spee et al. 2006). These formulations are used as two (sometimes three) component systems comprised of the epoxy component, the cross-linking agents (e.g. amines, phenols, thiols, acid anhydrides, etc.) and other additives and fillers. Epoxy resins used on steel structure coatings derive almost exclusively from Bisphenol A Diglycidyl Ether (BADGE) representing 75–95% of the total epoxide market (Aalto-Korte et al. 2015; Niklasson et al. 2009). Occupational exposures to epoxy resins have been associated with frequent reports of allergic and irritant contact dermatitis (ACD, ICD), as reflected in a large body of literature published in the past three decades (Aalto-Korte et al. 2015; Condé-Salazar et al. 1995; Jolanki et al. 1990; Niklasson et al. 2009). Occupational asthma and other respiratory conditions have also been reported, but to a lesser extent (Hannu et al. 2009; Meadway 1980; Rempel et al. 1991). In vitro toxicity studies have implicated BADGE in possible mutagenic effects (reactive oxirane groups bind to DNA) (Poole et al. 2004; Sueiro et al. 2001), although the evidence for mutagenicity in humans is lacking. The International Agency for Research on Cancer (IARC) has classified BADGE as a Group 3, unclassifiable as to carcinogenicity due to the lack of evidence in humans (IARC 1999). Hanaoka et al. 2002 found higher levels of follicle stimulating hormone (FSH) in plasma of workers exposed to BADGE compared to unexposed controls (Hanaoka et al. 2002). Exposure to BADGE (and its hydrolysis byproducts) has been implicated in human adipogenesis and mouse multipotent mesenchymal stromal stem cells (MSCs) at low nanomolar concentrations (Chamorro-García et al. 2012). Marqueño et al. 2019 highlight 'the need to monitor human exposure to these compounds [BADGE and its byproducts], at least as intensely as BPA [bisphenol A] is monitored' (Marqueño et al. 2019).

Coating of bridges and other metal structures typically requires application of three different layers in order to achieve surface technical specifications for weather, corrosion, and mechanical wear and tear resistance. The base coat is a three-component system of high solids, zinc rich, epoxy rich - polyamide formulation typically applied immediately post-metal surface cleaning with abrasive blasting to ensure maximum adhesion. The intermediate coat (or mid-coat) is a fast cure two-component epoxy resin system, whereas the topcoat is a polyurethane coat based on aliphatic isocyanates. Typically, the same crew of workers applies all three coating layers (base-, mid- and top-coat), a process that can continues through several days or weeks to allow time for adequate product curing and is affected by factors such as outdoor temperature and humidity conditions. Construction painters are directly exposed to BADGE and its oligomers during primer and mid-coat applications thorough product mixing, painting, cleaning and other miscellaneous tasks. Exposures can occur via inhalation of overspray aerosols and vapors generated during spray painting, rolling/brushing, as well as skin contact with the raw uncured epoxy components, contact with contaminated tools, or from deposition of overspray aerosols directly onto surfaces and clothing.

Biotransformation of BADGE in the body follows complex hydrolysis, oxidation, conjugation, and excretion pathways, yielding to multiple biomarkers excreted in urine and feces (Fig. 1). BADGE itself is very stable in water at physiological pH. However, in humans, one of the two oxirane groups of BADGE undergoes enzymatic hydrolysis by epoxide hydrolases (primarily in the liver) to produce the first byproduct, the mono-diol epoxide of BADGE [bisphenol A bis (2,3-dihydroxypropyl) ether or BADGE·H_2O]. Further hydrolysis of the second oxirane ring of BADGE·H_2O by the same enzymatic system (epoxide hydrolase) yields the bis-diol epoxide of BADGE [bisphenol A (2,3-dihydroxypropyl) glycidyl ether or BADGE·$2H_2O$], which is a major oxidation byproduct of BADGE (Boogaard et al. 2000; Climie et al. 1981a; Climie et al. 1981b). 'BADGE·$2H_2O$' can be further broken down by monooxygenases to glyceraldehyde and the bisphenol A (2,3-dihydroxypropyl) ether [BPA diol], but this seems to be a minor pathway. Other known metabolites have been reported in human urine including chlorohydroxy derivatives of BADGE, such as bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether [BADGE·HCl·H_2O] (Satoh et al. 2004; Wang et al. 2012). The exact formation mechanism of this biomarker is not known, especially whether it originates from BADGE·H_2O following enzymatic hydrolysis or directly from non-enzymatic hydrolysis of BADGE in the presence of hydrochloric acid in the stomach. To date, these BADGE derivatives have been measured in a variety of human specimens (adipose tissue, blood, urine) in studies of the general population and reflect ingestion exposures of leachable BADGE and other hydrolysis byproducts of epoxy resin coatings used in canned food. Urinary biomonitoring of BADGE is suitable for documenting short-term exposures to BADGE-based epoxies, such as during cross-shift, because it is noninvasive, convenient, and sensitive to cross-shift changes for chemicals with a short half-life. Estimated plasma clearance half-life of BADGE in humans following inhalation exposure is around 4 h (Boogaard et al 2000). Workplace biomonitoring is important for assessing the relative contribution of occupational exposures to epoxides relative to consumer exposures (such as from canned foods), as well as to elucidate the relative contribution of different exposure pathways (inhalation, skin, and/or ingestion).

The literature on occupational exposures, biomonitoring, and health effects of BADGE and related epoxide exposures among the most exposed workers, such as the construction painters of bridges and steel structures, is remarkably limited. We recently published the results of an exposure assessment study to BADGE-based epoxy resins among construction steel structure painters in New England using a combination of

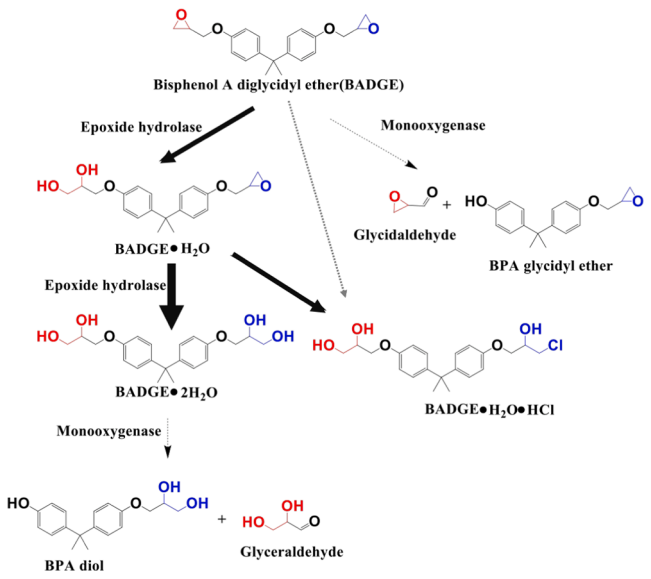

Fig. 1. Main metabolic biotransformation pathways of bisphenol A diglycidyl ether (BADGE) in humans. Solid lines denote established pathways in humans. Line thickness denotes relative predominance of each pathway. Hydrolysis by epoxide hydrolase is the major biotransformation / detoxification pathway of epoxides, including BADGE, in humans, leading to three major metabolic byproducts: Bisphenol A (2,3-dihydroxypropyl) glycidyl ether [BADGE·H_2O], bisphenol A bis (2,3-dihydroxypropyl) ether [BADGE·$2H_2O$], bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether [BADGE·HCl·H_2O]. BADGE·$2H_2O$ is the major product, followed by BADGE·HCl·H_2O and BADGE·H_2O. All three are excreted in urine as free metabolites, as well as conjugates with sulphate and glucuronide. Biotransformation of BADGE·$2H_2O$ to bisphenol A (2,3-dihydroxypropyl) ether ('BPA diol') and glyceraldehyde is expected to be minor. Figure reconstructed from reports in Wang et al 2012, Boogaard 2000, and Climie et al 1981a,b.

novel personal inhalation samplers and analytical methods (mass spectrometry and ion chromatography) and documented high potential for inhalation and skin exposures to BADGE and its higher oligomers (Xue et al., 2021). In this follow up biomonitoring study of the same cohort of construction workers we aimed to: i) determine urinary concentrations of BADGE and its derivatives in construction painters at metal structure coating sites; ii) determine cross-shift changes of BADGE biomarkers in urine to gain insights into BADGE uptake during the shift and assess the adequacy of exposure controls; iii) compare urinary BADGE biomarker concentrations in three distinct groups of coating workers – mid-coat and top-coat applicators (exposed to epoxies) and a reference group of spray polyurethane foam (SPF) sprayers who are not occupationally exposed to epoxy resins. Results of this work are important for developing effective workplace exposure reduction strategies and in subsequent epidemiologic studies to elucidate exposure -internal dose - health effects relationships in these construction workers.

2. Methods

2.1. Study sites and participants

Forty-four painters were recruited from 12 metal structure coating sites in Massachusetts as part of a larger study that focused on occupational exposures to reactive chemical systems based on isocyanates and epoxies in construction. The study sites consisted of 8 bridges, a reactor dome, a wind turbine, a water tank, and an indoor painting shop (Table 1). The basecoat (or primer) used at our study sites was a three-component (A, B, F) formulation (Sherwin-Williams, Zinc CLAD ® 4100), the intermediate mid-coat was a two-component epoxy resin system (Sherwin Williams, Macropoxy® 646), and the top - coat was a two-component polyurethane based on aliphatic isocyanates (Sherwin Williams, Acrolon™ 218 or hi-solids polyurethane). Their chemical composition is presented in the supplementary material (SI), Table S1.

Among the 44 construction workers who agreed to participate in this study, 17 were directly involved in epoxy mid-coat applications and 27 in aliphatic isocyanate top-coat application (Table 1). Workers applying mid-coats were exposed to epoxy paints at the day of urine sample collection, while workers applying the isocyanate-based top - coat were not directly exposed to epoxy-based products at the day of sampling. Workers applying mid-coats were exposed to epoxy paints at the day of urine sample collection, while workers applying the isocyanate-based top - coat were not directly exposed to epoxy-based products on the day of sampling. Exposure of top-coat workers to epoxy resins occurred during mid-coat application, one to several days prior to urine collection. Typically, the same crew of workers applied both mid- and top - coats within a week of each other, depending on the size of the job and environmental conditions. However, urine collection for mid-coat and top-coat applicators in our study was not matched to the same crew of workers. Thirty-two of the 44 participants were involved in painting, of which 14 performed spray painting; 18 performed rolling or brushing; 6 were helpers who performed product mixing and other auxiliary tasks, and 6 were bystanders (managers or site supervisors).

A group of 14 SPF construction workers with no known sources of occupational exposure to epoxy resins were included as a comparative/reference group. This group was selected randomly from a larger set of 47 SPF workers who were studied in a larger investigation of SPF construction workers (Bello et al. 2019). Twenty-eight urine samples (14 pre-shift and 14 post-shift) were analyzed for BADGE biomarkers in the same manner as samples of coating workers.

The study was approved by the UMass Lowell Office of Research Integrity (#14–109-BEL-XPD).

2.2. Work practices and personal protective equipment (PPE) on site

The study team collected relevant contextual information about site characteristics, products in use, worker activities, tasks and duration, number of workers / tasks, PPE types in use at the study sites, engineering controls (e.g. enclosures), and environmental conditions using field observations and activity logs.

2.3. Inhalation and potential skin exposures

Personal inhalation exposure to epoxy paints for mid-coat applicators were collected with a novel CIP-10MI inhalable solvent-based sampler (CIP-Capteur Individuel de Poussiere; M−microbiologic; I-inhalable) (Arelco, Fontenay-Sous-Bios Cedex, France) sampler in 2 mL of N,N-Dimethylformamide (DMF). The cup of CIP-10MI rotates at 7600 rpm (Gorner et al. 2006), and collects aerosol droplets by impaction at a flow rate of 10 L/min. The sampler offers the advantages of an impinger sampler for direct sampling of reactive aerosols in a liquid, and the convenience of a passive sampler-like system that has no tubing or external pumps. This sampler has been used successfully for sampling aromatic isocyanates in SPF applications (Bello et al 2019), and aliphatic isocyanates in top-coating applications (Bello et al. 2020). At the end of sampling, the liquid was transferred in a glass vial and stored in a cooler until it was transported in the lab for analysis.

Gloves used by workers were collected at the end of the task and submerged in the field in a jar with 50 mL DMF to inhibit polymerization reactions. Air and glove samples were analyzed with liquid chromatography-tandem mass spectrometry for individual species (BADGE and higher oligomers), and ion chromatography for the total epoxide group, using new methods developed in our lab. Details of these sampling and analytical methods, and sampling results are presented in detail in (Xue et al., 2021). Sampling and analysis methods for inhalation and potential skin exposures to isocyanates in top-coat (aliphatic isocyanates) and SPF applicators (aromatic methylene diphenyl diisocyanate) have been reported in earlier publications (Bello et al. 2019, 2020).

Table 1
Characteristics of metal structure coating sites investigated in this work, site activities, products and tasks, number of workers and urine samples collected.

Sites	Nr. of sampling sites	Activity	Tasks performed by workers at the day of sampling (n)	Product used onsite at the day of urine sampling	Urine samples, N	
					Pre-shift	Post-shift
Mid-coat application	4	Bridge and indoor shop painting	Spraying (7) Rolling and brushing (4) Helping (3) Bystanders (3) *Total = 17*	Zinc CLAD ® 4100 and Macropoxy® 646	14	17
Top-coat application	8	Bridges, reactor dome, wind turbine, water tank and indoor shop painting	Spraying (7) Rolling and brushing (14) Helping (3) Bystanders (3) *Total = 27*	ACROLON™ 218 HS Hi-solids Polyurethane Semi-Gloss (Part S)	24	26
Total	12 coating sites		44 participants		38	43

2.4. Urine sample collection

Study participants provided spot urine samples at the beginning of the work shift and at the end of their daily tasks or end of shift, depending on worker schedules and their availability. A total of 81 urine samples were collected among coating workers: 38 pre-shift and 43 post-shift (Table 1). The time interval between pre- and post-shift urine collection had a median of 300 min (range, 185–525 min). For the SPF workers, the median interval between the pre- and post-shift urine collection was 190 min (range 70–330 min).

All urine samples were collected in sterile polyethylene plastic cups, sealed and immediately stored inside coolers with dry ice and subsequently transported to the laboratory where they were stored at −80 °C until further processing and analysis as described in later sections.

2.5. Chemical analysis of urine samples

2.5.1. Chemicals and supplies

Bisphenol A diglycidyl ether (BADGE), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O), bisphenol A bis (2,3-dihydroxypropyl) glycidyl ether (BADGE·$2H_2O$), bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BADGE·H_2OHCl) were purchased from Sigma-Aldrich (St. Louis, MO). D6-bisphenol A diglycidyl ether (D6-BADGE, catalog # B519502; six Ds on the two methyl groups of the bisphenol A moiety), was used as internal standard (IS) for BADGE and was purchased from Toronto Research Chemicals (Toronto, CAN).

2.5.2. Urine sample processing

Urine samples were thawed in a temperature-controlled water bath at 37 °C. Ten milliliter (mL) urine were aliquoted and centrifuged at 1000× rpm to remove any cellular debris. Specific gravity was measured with a handheld digital pocket refractometer (PAL −10S Atago, Japan). Urine creatinine concentration was measured with LC-MS/MS according to the method of (Hou et al. 2012) as previously described (Bello et al. 2019) and briefly summarized in the chemical analysis section.

For the urine analysis of BADGE biomarkers, we used a modification of the total BADGE biomarker method of Wang et al. 2012, which measures the total of the free and the conjugated forms. Two mL urine (instead of 0.5 mL in Wang et al. 2012) was transferred to a 15-mL sterile polypropylene (PP) tube and spiked with internal standards (IS, Section 2.5.3) of D6-BADGE and D6-BADGE·$2H_2O$. Samples were buffered with 1 mL of 1.0 M ammonium acetate containing 22 units of β-glucuronidase/arylsulfatase (derived from *Helix pomatia*; Millipore Sigma, SKU# 10127698001) and digested at 37 °C for 12 h, followed by three 2 mL liquid–liquid extraction (LLE) steps with ethyl acetate. For each LLE step, samples were shaken using an oscillator shaker for half an hour, then centrifuged for 15 min at 4500*g*. The three organic fractions were mixed together in a 20 mL glass vial, washed with 1 mL of water, followed by concentration to near-dryness under a gentle stream of nitrogen. The extract was reconstituted with 200 Âµl (µL) of methanol and vortex mixed for 1 min prior to analysis by LC-MS/MS.

2.5.3. Preparation of calibration standards and quality control samples

Stock solutions of BADGE, D6-BADGE (IS), BADGE·H_2O, BADGE·$2H_2O$ and BADGE·H_2OHCl were prepared in methanol and stored at −20 °C until use. Since only deuterated commercial products of BADGE are commercially available, we used D6-BADGE and its hydrolysis byproducts as internal standard. D6-BADGE was hydrolyzed to D6-BADGE·$2H_2O$ to be used as the corresponding IS for BADGE·$2H_2O$ by dissolving 10 mg of D6-BADGE in 1 mL of water/acetone solution (5/1) and hydrolyzed at 100 °C for 24 h (hrs). Calibration standards in methanol were prepared by spiking relevant amounts of stock solutions (10 µg/mL) or their sequential dilutions in D.I. water to produce a final analyte concentration in the range of 0.05–100 ng/mL (twelve points). Standards and samples were spiked with D6-BADGE and D6-BADGE·$2H_2O$ IS (from a 100 ng/mL stock in methanol, 10 µL/mL spike) to give a final concentration of 1 ng/mL each.

For quality control purposes, urine from two healthy subjects with no known occupational exposure to epoxides was pooled together, centrifuged as samples, and aliquoted into 2 mL vials. Aliquots (2 mL each) of this pooled urine were spiked with standards and IS to produce a final concentration of 0.0 (no spike), 1.0, 10 and 50 ng/mL (IS, 1 ng/mL each), and were included with each fresh batch of urine samples as quality control samples. These spiked urine samples underwent the same sample preparation procedure as regular urine samples. In addition, mixed standards, spiked in D.I. water (in triplicates) to give a final concentration of 1, 10, and 50 ng/mL (IS, 1 ng/mL), were used to investigate recovery rates of analytes. These spikes were processed as urine samples, starting with LLE extraction steps. The calibration standards did not undergo sample preparation.

2.5.4. LC-MS/MS analysis and quantitation

The LC-MS/MS system was an Applied Biosystems API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) in positive electrospray ionization mode coupled to a Shimadzu LC20 series UHPLC system. The system was controlled through the Analyst v1.7 software. Chromatographic separation was carried out in on a Kinetex polar C18 column 100 × 4.6 mm I.D., 2.6 µm particle size column (Phenomenex, CA, USA) preceded by a matching phase guard column (Phenomenex, CA, USA). Sample injection volume was 10 µL. The mobile phases were 0.15% (w/v or 20 mM) ammonium acetate in D.I. water (A) and 0.15% (w/v) ammonium acetate in methanol (B). Analytes were separated by gradient elution at a flow rate of 600 µL/min as follows: isocratic 20% (v/v) A for 2.5 min, linear gradient from 20% to 70% B for 1.5 min, followed by a second linear gradient from 70% to 95% B for another 4 min, and another isocratic run at 95% B for another 6 min (total time 14 min), followed by 3 min of post-column equilibration at 20% B. Injection-to-injection time was ~17 min. The BADGE and its derivatives were analyzed in the positive electrospray ionization mode of multiple reaction monitoring (MRM). MS source parameters, MRM transitions and compound specific settings are presented in Table S2. Two MRM transitions were monitored for each analyte. The second transition was used for quality control/assurance purposes, such as to assess urine matrix effects and for confirmation purposes (Figure S1).

Quantification of the three BADGE derivatives - BADGE·$2H_2O$, BADGE·H_2O, and BADGE·HCl·H_2O - was performed using the isotope dilution method and a 10-point standard calibration curve over the 0.05 to 10 ng/mL calibration range (excluding 50 and 100 ng/mL points), since all analytes but one (BADGE·$2H_2O$ maximum, 18.7 ng/mL) were below 10 ng/mL, by plotting the ratio of peak areas of the analyte/internal standard of each target analyte vs. the ratios of concentrations of analyte/internal standard. D6-BADGE·$2H_2O$ was used as the IS for BADGE·$2H_2O$, BADGE·H_2O, and BADGE·HCl·H_2O, whereas D6-BADGE was used as the IS BADGE. The regression coefficient (r) for all calibration curves was always greater than 0.997. The limit of quantitation (LOQ) was determined based on the lowest quantifiable calibration standard with a signal-to-noise ratio ≥10. The limit of detection (LOD) for each analyte, was calculated as 3× the standard deviation of signal noise around the region of analyte retention time and was compared to the LOQ/3 values. The highest of these two values was assigned as the LOD for each analyte. The LODs in ng/mL urine were: free BADGE, 0.025; BADGE·$2H_2O$, 0.025; BADGE·HCl·H_2O, 0.08; and BADGE·H_2O, 0.08.

A typical extracted ion chromatogram for the primary MRM transitions of a standard at 2.5 ng/mL concentration and a typical urine sample are presented in Fig. 2. Total BADGE biomarker (nmol/mL urine) was calculated as the sum of all biomarkers, including the free and conjugated fractions: BADGE, BADGE·$2H_2O$, BADGE·HCl·H_2O and BADGE·H_2O.

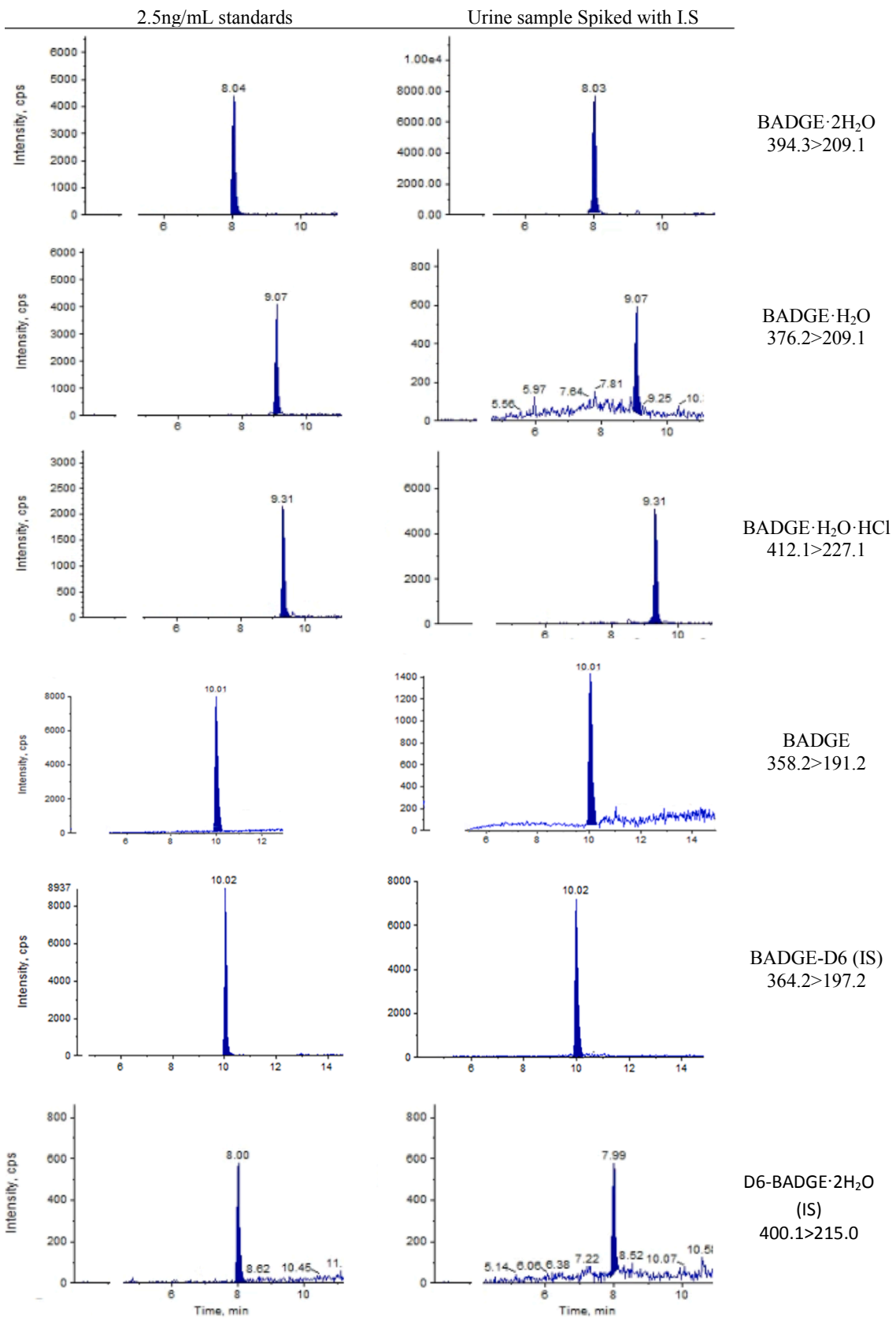

Fig. 2. Example of extracted ion chromatograms of a standard (2.5 ng/mL or 25 pg on column) and a typical urine sample spiked with the internal standards BADGE-D6 and D6-BADGE·2H_2O. The MRM transitions are provided in SI Table S2. Analyte concentrations in urine sample (ng/mL) are as follows: BADGE·2H_2O, 7.11; BADGE·H_2O, 0.81; BADGE·HCl·H_2O, 1.08; BADGE, 0.42; BADGE-D6 and D6-BADGE·2H_2O, 1 ng/mL.

2.5.5. Quality assurance and quality control

To check the instrument's drift in the response factor, a midpoint calibration standard was injected after every 10 samples. Pure solvent (methanol) was injected for every 10 samples to check for instrument carryover. Several laboratory and solvent blanks were analyzed with each batch of samples to determine contamination caused by laboratory materials and solvents. Quality control samples (pooled spiked urine) and standard spikes at three concentrations were also included, as described earlier. Absolute and relative recoveries were also calculated for all analytes. Absolute recovery was based on the instrument response of the analyte. The ratio of the analyte signal to that of the internal standard was used for calculation of relative recovery. Relative recoveries of analytes ranged from 96.1% to 99.2%, and the absolute recoveries ranged from 92.9% to 98.3%. No carryover was noted, and this is attributed in part to routine autosampler needle washes, blank injections and instrument cleanups between batch runs. No significant drift in the instrument response was observed. Precision from repeated analysis (including sample preparation) of the same urine spikes was excellent, with <9% coefficient of variation across the tested concentration range. The ratios of peak areas for both MRM transitions of each analyte in pooled urine (with and without spike) and three random urine samples were not different from the same ratios when standards were spiked in water or methanol and processed as regular samples (~2:1 for BADGE·2H_2O). No significant urine matrix effect was observed for these analytes.

2.6. Statistical analysis

Concentrations of free BADGE and its derivatives in urine were examined for underlying distribution using the Shapiro-Wilks statistic and log transformed data were used for subsequent statistical analysis performed with SAS 9.4 (SAS Institute Inc. Cary, NC). Descriptive statistics such as geometric mean (GM), geometric standard deviations (GSD) were calculated separately for pre-and post-shift samples among mid-coat, top-coat workers, and the reference exposure group (SPF workers). Concentrations of biomarkers in urine were normalized to both specific gravity (SG) (ng biomarker/mL urine) and creatinine (nmol biomarker/mol creatinine) to adjust for different hydration rates of individual workers (Barr et al. 2005). BADGE·H_2O was excluded from further statistical analysis due to the low level of detection, since only 10% of samples were above the limit of detection (LOD) of the method. For other biomarkers, namely BADGE·HCl·H_2O, with <20% non-detectable samples, non-detects were substituted with LOD/$\sqrt{2}$ (Croghan and Egeghy 2003). Paired t-tests on log-transformed data were used to test for differences between pre- & post-shift urinary biomarkers normalized to creatinine and specific gravity.

Linear mixed effects models (using site ID as a random effect) were run with the goal of identifying significant external exposure factors that may influence epoxy uptake during the work shift. These analyses where performed for the following variables: activity (mid-coat, top-coat, SPF); task (spraying, rolling, helping); enclosure (yes, no); and PPE type (respirators, gloves, and coveralls). It is acknowledged that the sample size was not sufficiently large to enable detecting modest differences in these comparative analyses. Furthermore, univariate and multivariate linear regression models were utilized to determine the effect of inhalation and potential skin exposures on the total BADGE biomarker levels (dependent variable).

3. Results

3.1. Work practices and PPEs at investigated sites

Field observations related to PPE use at the study sites reveal that 47% of coating workers wore half-face organic cartridge respirators (OVC) with particulate filters, 18% wore full-face OVC, and 3% wore N95 masks, while 32% of workers did not use any respirator (Fig. 3). With regards to the gloves used, polymer coated cotton gloves on the palmar side were the most frequently used type (53%), followed by thin nitrile (18%), construction-type (thick) cotton gloves (13%), and rubber gloves (8%), while 8% (all helpers) did not wear gloves while performing their daily tasks. Coveralls use also varied by site: 29% of workers wore cotton coveralls, 26% polyethylene coveralls, and 45% wore only standard clothing. Overall, painters used PPEs more conservatively when applying isocyanate-based products (top-coat) compared to epoxy-based products (mid-coat). Based on field observations, we noted frequent paint contact with unprotected skin, especially forearms, neck and head/face region, and occasionally hands.

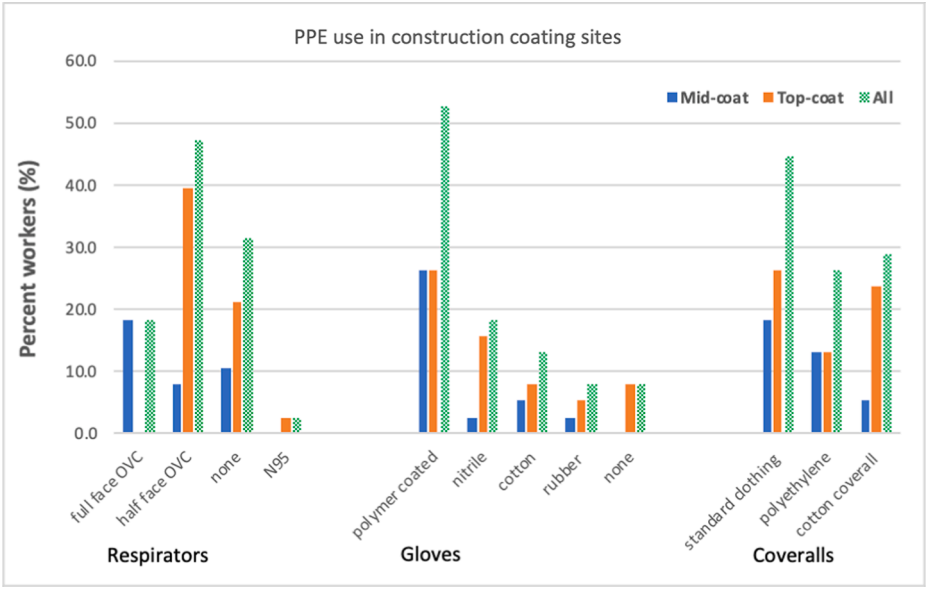

Fig. 3. Personal protective equipment (PPE) used by workers at the study sites. Among all (n = 44) study participants, 38 workers were directly involved with painting and helping tasks, whereas 6 managers or supervisors were not involved in panting and did not wear any PPE. The percentages reported are based on n = 38 painters.

3.2. Inhalation and potential skin exposures to BADGE and other oligomers

Details about analytical methods, processes, and exposure data are provided in our recent published work (Xue et al., 2021). Table 2 provides a succinct summary of inhalation and potential skin exposures to epoxy paints in this group of workers. No BFDGE was found in products and samples. BADGE inhalation exposures had a GM (GSD) of 803 (3.2) μg/m^3 and range of 111–3850 μg/m^3. The BADGE dimer and trimer were found at much lower concentrations and had GM (GSD) of 26.4 (7.2) and 13.1 (10.2) μg/m^3, respectively. The GM (GSD) of total epoxy group (TEG) in air was 276.9 μg TEG/m^3 (3.9). High potential for skin exposure was also documented. The BADGE loading on gloves had a GM (GSD) of 547.2 (2.9) mg/pair and a range of 55–1963 mg/pair. BADGE contributed ~90% of TEG in air samples and 70% of TEG in glove samples and was highly correlated with TEG in air and gloves (R^2 of fitted regression lines were 0.95, and 0.92, respectively).

3.3. Creatine and specific gravity

Creatinine in pre-shift urine ranged from 2.5 to 90 mmol/L for mid-coat workers and 1.3–53.6 mmol/L for top-coat workers (SI, Table S3, Figure S2). In SPF applicators, pre-shift creatinine ranged from 2.5 to 36.8 mmol/L. Creatinine concentrations were significanlty higher ($p = 0.001$) in post-shift urine samples of top-coat workers, but not for mid-coat applicators ($p = 0.24$) (SI, Table S3). Similarly, post-shift urinary creatine values in SPF applicators were higher than in pre-shift ($p = 0.02$). About 40% of pre-shift and 80% of post-shift urine samples among mid- and top-coat applicators had creatinine concentration above the recommended upper normal sampling range of 26.52 mmol/L (300 mg/dL) (Barr et al. 2005; World Health Organization 1996). Similar to creatinine, SG was significantly higher in post-shift urine relative to pre-shift only in top-coat applicators (p-values 0.02 vs. 0.45) (SI Table S3, Figure S2).

3.4. Urinary BADGE biomarkers among construction painters

Table 3 summarizes biomonitoring results normalized to SG and creatinine. BADGE·2H$_2$O was quantified in 100% of the samples, BADGE·HCl·H$_2$O in 90%, free BADGE in 84%, and BADGE·H$_2$O only in 10% of the samples. Free BADGE itself was ubiquitously present in all non-urine blank samples at 0.07–0.15 nM (0.02–0.05 ng/mL), with occasional blank samples having free BADGE at concentrations as high as 0.25 nM (0.085 ng/mL), that was traced to solvents and D.I. water. This observation is consistent with earlier reports of urinary BADGE in the general population (Wang et al. 2012). All free BADGE results in urine were adjusted by subtracting the upper range of blank value of 0.15 nM. After the blank adjustment, ~84% of all urine samples had free BADGE values higher than the laboratory blanks. All other biomarkers of BADGE were non-detectable in solvents, water, and other laboratory blanks.

BADGE·2H$_2$O was the most abundant urinary biomarker of BADGE. SG-normalized BADGE·2H$_2$O concentrations in the post-shift urine of mid-coat applicators ranged from 0.2 to 18.7 ng/mL and had a post-shift GM (GSD) of 1.46 (3.6) ng/mL. Post-shift urinary GM concentrations of BADGE·2H$_2$O were 8.6× higher than those of BADGE·HCl·H$_2$O concentrations for mid-coat applicators (as GM ratios) and over 3× higher among top-coat applicators (Table 3). The first hydrolyzed derivative BADGE·H$_2$O was quantified only in 8 samples (3 pre- and 5 post-shift), with concentration ranges of 0.11–0.41 ng/mL. The majority of these samples corresponded to workers performing mid-coating. Concentrations of free BADGE were the lowest measured, with a post-shift GM of 0.04 (2.5) ng/mL, or 4× lower than BADGE·HCl·H$_2$O (ratio of GMs). Similar overall trends were observed for creatinine normalized biomarkers (Table 3).

The highest BADGE·2H$_2$O value of 18.7 ng/mL belonged to one painter in the indoor painting shop who sprayed epoxy based mid-coat on small bridge parts. The highest urinary BADGE·2H$_2$O concentration of 10.0 ng/mL among the top-coat applicators belonged to one painter at the reactor dome coating site, who had applied epoxy coating by rolling and brushing a day prior to urine sampling, during a very hot summer day (heat wave week with average daily temperatures exceeding 35 °C). Due to the high heat workers removed coveralls and respirators during painting.

In all samples, BADGE·2H$_2$O was highly correlated with total BADGE (Pearson correlation coefficient = 0.98), since it was by far the major contributor to total BADGEs (72% in pre-shift and 88% in post-shift urine in mid-coat applicators) (Table S4; Table S5). BADGE·H$_2$OHCl contributed 24% of total BADGE in pre-shift and 10% in post-shift urine of mid-coat applicators. Free BADGE was not correlated with the total BADGE (Pearson correlation coefficient 0.11) and contributed only 5% and 2% of pre- and post-shift urine of mid-coat applicators, respectively (Table S5).

3.5. Pre- vs. post-shift changes in urinary BADGE biomarkers

The mean post-shift urinary BADGE·2H$_2$O was higher than pre-shift in both mid- and top-coat applicators (SG normalized data), although it was statistically significant only in mid-coat applicators ($p = 0.02$). This outcome in mid-coat applicators did not change, regardless of the normalization procedure (SG $p = 0.02$; creatinine $p = 0.08$, Table 3).

Table 2
Summary of personal breathing zone and potential skin exposures to epoxies measured in construction metal structure coating tasks using recently developed LC-MS/MS for individual epoxy species and ion chromatography (IC) methods for TEG. A novel CIP-10MI personal sampler filled with 2 mL of N,N-dimethylformamide was used for collection of airborne aerosols.

Epoxy Species	**Inhalation exposures**[c] (n = 9)				**Potential skin exposures**[d] (n = 11)			
	Non-detects n[b] (%)	*Breathing zone concentrations (μg/m^3)*			Non-detects n (%)	*Glove loading (mg/pair)*		
		GM	(GSD)	Range		GM	(GSD)	Range
BADGE MW[a] = 340.42	0 (0)	802.7	(3.2)	111–3,850	0 (0)	547.2	(2.9)	55–1,963
Dimer MW = 624.77	0 (0)	26.4	(7.2)	1.6–478	0 (0)	10.7	(4.5)	0.5–70.7
Trimer MW = 909.13	1 (11)	13.1	(10.2)	nd[c]-325.8	1 (9)	8.3	(3.0)	0.6–23.0
TEG, IC[e]	0 (0)	276.9	(3.9)	30–1,551	0 (0)	173.1	(3.0)	18.4–752

[a] MW, Molecular weight (g/mol);
[b] Number of non-detectable samples;
[c] Air sampling, which continued for the duration of the whole task, had a median of 90 min (20–230 min);
[d] Glove sampling duration had a median of 90 min (15–240 min).
[e] TEG, Total Epoxy Group (equivalent weight, 43 g/mol). *Details of analytical methods and results can be found in* Xue et al., 2021. *Table is reproduced in part from* Xue et al., 2021, *with permission.*

Table 3
Descriptive statistics of BADGE biomarkers in urine samples of construction mid-coat and top-coat applicators on steel structure coatings, as well as the reference group of spray polyurethane foam (SPF) workers.

Urinary Biomarkers[a] Activity	SG normalized data (ng/ml)				Creatinine normalized data (nmol biomarker /mol creatinine)			
	Free BADGE [b]	BADGE·2H_2O	BADGE • HCl·H_2O	Total BADGE	Free BADGE	BADGE·2H_2O	BADGE • HCl·H_2O	Total BADGE
Mid-coat								
Pre-Shift GM (GSD)	0.04 (3.0)	0.50 (2.0)	0.17 (3.1)	0.69 (2.2)	6.04 (2.8)	77.2 (2.1)	23.4 (3.1)	117.3 (2.3)
Post-Shift GM (GSD)	0.04 (2.5)	1.46 (3.6)	0.17 (2.3)	1.66 (3.2)	4.4 (2.7)	155.4 (4.4)	16.6 (2.9)	196.2 (3.9)
Range [c]	nd – 0.16	0.20–18.7	nd – 0.59	0.24–17.2	0.9–47.8	12.9–3226	3.3–576.3	43.1–291.0
Ratio of GM Post/Pre	1.00	2.92	1.00	2.41	0.73	2.01	0.71	1.67
P-value (GM pre vs. post)	0.82	0.02	0.90	0.04	0.40	0.08	0.34	0.15
Top-coat								
Pre-Shift GM (GSD)	0.04 (2.4)	0.67 (2.4)	0.21 (2.4)	0.91 (2.2)	7.1 (3.2)	103.8 (3.9)	30.1 (3.1)	154.5 (3.5)
Post-Shift GM (GSD)	0.04 (3.0)	0.91 (3.0)	0.29 (3.0)	1.23 (2.7)	3.5 (3.1)	77.1 (2.8)	22.2 (2.5)	114.3 (2.5)
Range	nd – 0.27	0.18–9.97	0.06–5.18	0.29–3.54	nd – 125.9	17.5–856.0	4.0–837.5	33.7–3214
Ratio GM Post/Pre	1.00	1.36	1.38	1.35	0.49	0.74	0.74	0.74
P-value (GM pre vs. post)	0.49	0.18	0.34	0.18	0.01	0.13	0.15	0.12
SPF (reference group)								
Pre-Shift GM (GSD)	0.04 (1.8)	0.28 (1.3)	0.10 (1.5)	0.39 (1.3)	8.6 (1.8)	50.6 (1.5)	16.3 (1.8)	77.8 (1.6)
Post-Shift GM (GSD)	0.03 (1.8)	0.27 (1.3)	0.09 (1.7)	0.36 (1.3)	6.6 (2.1)	48.5 (1.8)	14.9 (2.3)	72.3 (1.9)
Range	0.02–0.14	0.20–0.46	0.05–0.22	0.25–0.64	2.7–29.2	19.8–176.2	4.9–63.4	28.1–271.7
Ratio of GM Post/Pre	0.75	0.96	0.90	0.92	0.77	0.96	0.91	0.93
P-value (GM pre vs. post)	0.01	0.40	0.46	0.20	0.20	0.77	0.66	0.65

[a] Represents the total amount of that biomarker, which includes the free form and conjugated to glucuronide and sulphates.
[b] Free BADGE in urine was corrected for background BADGE, which appears to be a ubiquitous contaminant in several laboratory solvents and possibly other sources.
[c] nd = sample was below method limit of detection. BADGE·2H_2O was detectable in 100% of the samples, BADGE·HCl·H_2O in 90%, free BADGE in 84%, and BADGE·H_2O only in 10% of the samples (the latter is not included in statistical analysis). LODs as follows: free BADGE, 0.025 ng/mL urine; BADGE·2H_2O, 0.025 ng/mL urine; BADGE·HCl·H_2O, 0.08 ng/mL urine and BADGE·H_2O 0.08 ng/ml.

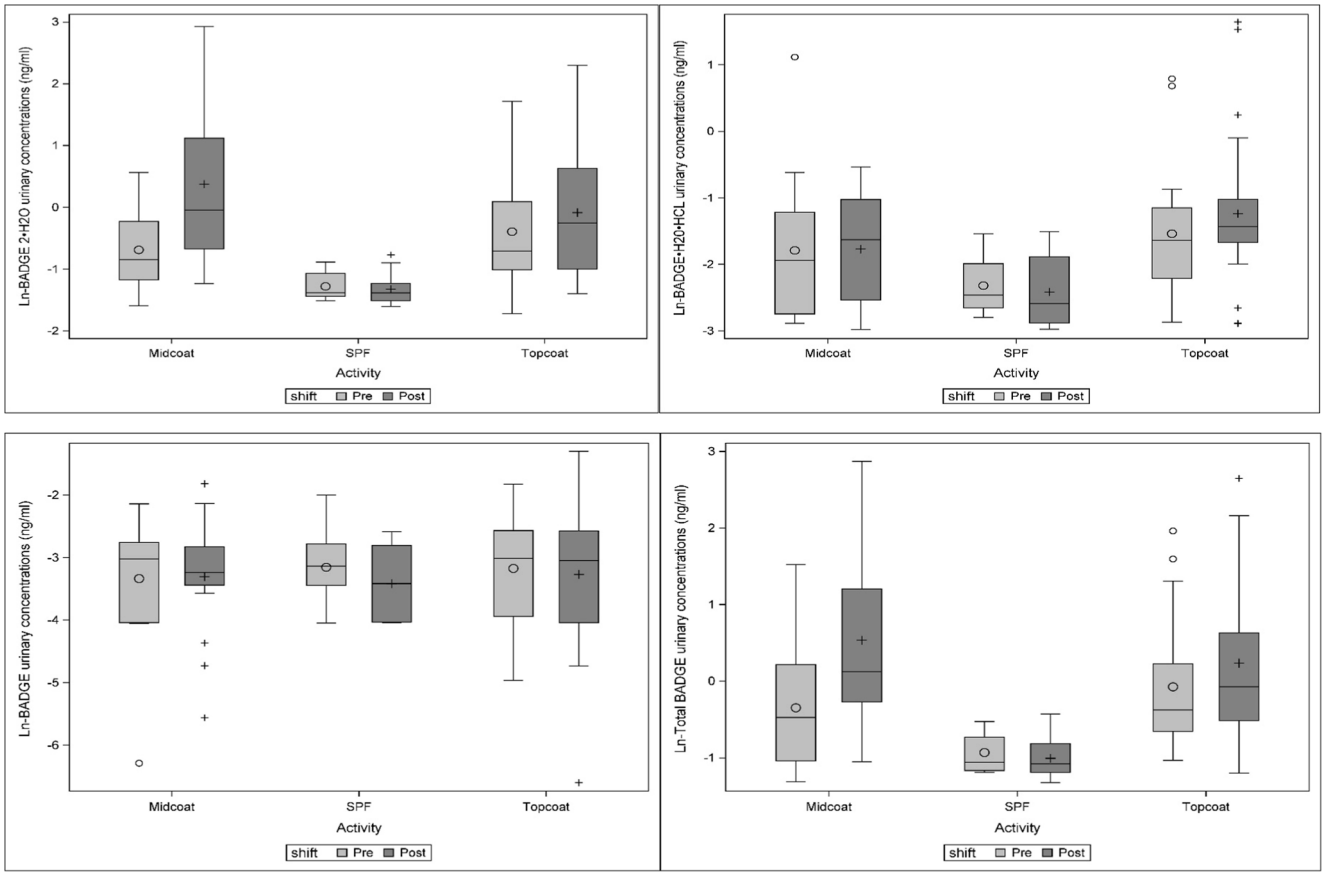

Fig. 4. Comparison of four urinary epoxy BADGE biomarkers as Ln-distributions, normalized to specific gravity, in pre- and post-shift urine samples collected among workers performing mid-coat (n = 17) and top-coat (n = 27) painting, as well as the comparative group of SPF insulation workers (n = 14).

The post-shift BADGE·2H$_2$O GM increased by 2.9× in mid-coat applicators compared to pre-shift GM (1.46 vs. 0.50 ng/mL, SG normalized); 1.36× (p = 0.18) in top-coat applicators; and declined slightly (0.96×) for SPF applicators (p = 0.40) (Table 3 and Fig. 4). These biomarker trends demonstrate significant BADGE uptake during mid-coating tasks that involve active use of epoxy paints. Exposure to BADGE epoxies appears to be occurring to some extent during some top-coating tasks (Figure S3).

The mean BADGE·HCl·H$_2$O was higher in post-shift urine samples of top-coat applicators (post-/pre-shift GM ratio = 1.38, p = 0.34), but not in mid-coat applicators (GM ratio = 1.0, p = 0.90) or SPF applicators (GM ratio = 1.0, p = 0.40) (Table 3).

No significant changes were found in pre-and post-shift concentrations of free BADGE for mid- and top-coat applicators (GM ratios = 1, p > 0.50) and identical GM values (0.04 ng/mL). However, post-shift BADGE was lower than in pre-shift urine of SPF workers (GM ratio = 0.75, p = 0.01). The total BADGE paralleled the BADGE·2H$_2$O behavior. Similar results to the SG-normalized data were found for the creatinine normalized values, except that cross-shift differences were less prominent as a result of similar cross-shift increases in urinary creatinine.

3.6. Important modifying factors of urinary BADGE biomarkers from statistical modeling

Results of univariate modeling for important exposure modifying factors revealed that "activity" was a statistically significant predictor of the main urinary biomarker BADGE·2H$_2$O (Table 4). The results were statistically significant when the difference between post-shift and pre-shift levels was used as a depended variable in the models, but not when the post-shift biomarker values were used. These modeling results provide additional statistical evidence of significantly higher exposures among mid-coat workers who were exposed to epoxy resins during the day of sampling compared to top-coat workers who were exposed one or more days before urine sampling, or non-epoxy exposed SPF group (Table 4). None of the other variables investigated (task performed, enclosure, respirator type, glove type and coverall type) were significant predictors of the urinary biomarkers (data not presented) in models with post-shift values or cross-shift differences.

Eight workers in mid-coat sites provided matched air, glove and urine samples (Table S6). For these eight data points univariate and multivariate modeling did not yield any statistically significant association between inhalation or/and potential skin exposures and urinary biomarkers. This is most likely due to the small number of matched samples available for these analyses.

4. Discussion

In this study we present urinary biomonitoring results for BADGE epoxy paints amongst construction painters in industrial metal structure coatings. We measured free BADGE and its three major hydrolysis byproducts - BADGE·H$_2$O, BADGE·2H$_2$O, BADGE·HCl·H$_2$O – for workers applying epoxy-based mid-coats and isocyanate-based top-coats and assessed cross-shift changes in these biomarkers. SPF workers, who have no known occupational exposures to epoxy, were used as a comparative group. To the best of our knowledge this is the first biomonitoring study of exposures to epoxies in occupational settings, including construction.

We found that BADGE·2H$_2$O biomarker, the most predominant urinary hydrolysis biomarker of BADGE in this cohort of workers, provided high sensitivity (quantified in 100% of urine samples), and specificity (able to discriminate between various exposure scenarios and cross-shift changes). A statistically significant 2.9× increase in the post-shift GM BADGE·2H$_2$O biomarker in mid-coat applicators (active users of epoxy paints) relative to 1.4× (40% increase, not statistically significant) in top-coat applicators, and no increase (~1.0×) in SPF applicators, provides evidence of active epoxy uptake during the work shift. BADGE·2H$_2$O contributed ~80% of the total BADGEs in most scenarios, and is therefore, the preferred biomarker for routine biomonitoring purposes and for establishing future urinary biological monitoring guidance values (BMGV). Oxidation of BADGE to BADGE·2H$_2$O by epoxide hydrolyses appears to be the preferred metabolic pathway in humans and explains why the intermediate oxidation product BADGE·H$_2$O was found only in a small number of samples. BADGE·H$_2$O is hydrolyzed quickly to BADGE·2H$_2$O by epoxide hydrolases, whereas further oxidation of BADGE·2H$_2$O by monooxygenases to other oxidation byproducts proceeds more slowly, leading to accumulation of BADGE·2H$_2$O in urine. BADGE·H$_2$O was measured in the urine of workers with high exposures to epoxies, and it is likely that its concentrations would be much lower, should urine have been collected at later time points. Based on these observations, we conclude that BADGE·H$_2$O is a biomarker of limited value for biomonitoring purposes in occupational cohorts.

The second most abundant biomarker, BADGE·HCl·H$_2$O, was present in urine in much lower concentrations, ~9× lower than BADGE·2H$_2$O. Our biomonitoring data demonstrate convincingly that this particular biomarker is not responsive to short-term occupational exposures to epoxies (Table 3) as its cross-shift concentrations do not change for mid-coat applicators. Although the BADGE·HCl·H$_2$O GM values in mid-coats was slightly higher than in SPF (0.17 vs. 0.1 ng/mL), neither the formation mechanisms nor the half-life of this biomarker are currently understood. The fact that the GM of BADGE·HCl·H$_2$O was higher in top-coat applicators than in mid-coats (0.17 vs. 0.21 ng/mL), and slightly increased cross-shift from 0.21 to 0.29 ng/mL, suggest that this biomarker may reflect ingestion exposures pathways with slower absorption kinetics that favor non-enzymatic conversion of BADGE to BADGE·HCl·H$_2$O in the stomach and/or intestines, or non-occupational exposures. It is interesting to note that the ratios of BADGE·2H$_2$O / BADGE·HCl·H$_2$O in pre- and post-shift samples were ~3 in all comparisons (Table 3, pre- and post-shift top - coat and SPF; pre-shift mid-coat applicators), and much higher (~9) in post-shift urine of mid-coat applicators who are experiencing acute BADGE exposures.

Free BADGE was found at low levels in all three exposure groups and no cross-shift changes were observed for all three. Considering ubiquitous BADGE contamination of laboratory solvents and D.I. water and very low urinary concentrations, routine biomonitoring of free BADGE in occupational cohorts may not be worthwhile.

There is only one peer-reviewed paper on biomonitoring of epoxies in the general population (Wang et al 2012). Our findings are in good agreement with the findings of Wang et al. 2012 in samples of the general populations from the US and China. In Wang et al. 2012, urinary concentrations of BADGE·2H$_2$O in US adults (n = 31) ranged from 0.2 to 4.6 (GM = 1.11) ng/mL and BADGE·HCl·H$_2$O concentrations ranged from < 0.08–3.4 (GM = 0.32) ng/mL. Although the GM of the main biomarkers in Wang et al. 2012 study and ours are comparable, we report here much higher maximum values for all biomarkers in mid- and top-coat applicators. The highest concentrations of BADGE·2H$_2$O were measured in our mid-coat cohort at 4× higher than the highest values measured in US adults (18.7 vs. 4.6 ng/mL). The maximum values for BADGE·HCl·H$_2$O were in our top-coat workers at 1.6x higher (35%; 5.2 vs. 3.4 ng/mL). However, direct comparison between the two studies is

Table 4
Results of PROC MIXED univariate modeling of cross-shift changes in the urinary BADGE·2H$_2$O biomarker by activity (categorial variable with SPF as reference group).

Depended variable	Models with SG normalized data				
	Variable	Parameter estimates	Pr. > t	95% Confidence limits	
Δ (Post - Pre-shift) BADGE·2H$_2$O in urine	Intercept	−0.15	0.70	−0.94	0.64
	Mid-coat	1.09	**0.01**	0.23	1.96
	Top-coat	0.31	0.34	−0.34	0.96
	SPF	0	.	.	.

not straightforward due to differences in cohorts and exposure details. For example, the Wang et al. 2012 study did not specify study participant selection criteria, therefore some of those participants could have been occupationally exposed to epoxy products including through do-it-yourself epoxy applications. Furthermore, biomonitoring results depend on urine sampling collection time relative to exposure events, and the uptake and clearance kinetics of these biomarkers depend on exposure routes - ingestion in the general population vs. inhalation and/or potential skin exposures in the occupational cohorts. We have no explanation for the much higher method sensitivities reported in Wang et al. 2012 study. The authors of that study reported ~3× lower analyte detection limits than ours (10 vs. 25 pg/mL for BADGE·2H_2O), 4× less urine (0.5 mL vs. 2 mL), 1× concentration factor (0.5 mL urine brought to 0.5 mL final sample volume) vs. 10× (2 mL urine to 0.2 mL final concentration), in an API 2000 instrument, which is three generations older than our API 4000. Future urinary biomonitoring of BADGE biomarkers in other occupational cohorts and the general population will likely provide more clarity on this matter.

The exact biotransformation pathways and clearance kinetics of BADGE in humans are lacking. Limited data in animals using radiolabeled ^{14}C-BADGE (Climie et al. 1981 a, b) suggest very slow uptake by the skin route. Studies from the early 80 s found that BADGE is absorbed very slowly from the mouse skin, with ~90% of the administered dose (as ^{14}C-BADGE) remaining in the skin at 24 hrs post-application and 40% after 8 days (Climie el al. 1981a). Daily urinary elimination of radioactivity was at ~0.3%, with a maximum of 1.3% elimination that was facilitated by the solvent vehicle. The amount of BADGE chemically bound to the skin was small, 0.3% of the administered dose on day one to 1.3% on day 8. Since skin exposure is an important, if not dominant, pathway in this cohort of workers, it is possible that higher pre-shift BADGE·2H_2O concentrations in top-coat applicators relative to mid-coats (GM 0.67 vs. 0.5 ng/mL), and the cross-shift increase (GM 0.67–0.91 ng/mL) may reflect the delayed contribution of epoxy skin exposures from prior day/s in the total body burden. The presence of BADGE biomarkers in the samples of top-coat workers who were exposed 1–8 days before urine collection, is consistent with continued slow and steady BADGE uptake from the skin. In fact, high skin exposures to BADGE have been documented in our earlier work. Inhalation exposure, on the other hand, results in fast uptake, and it is less likely that observations in top-coat applicators related to prior inhalation exposures. A third explanation, also plausible, relates to non-occupational sources of BADGE exposures, including through foods and food products, which is best illustrated with background BADGE biomarkers in SPF workers.

Ingestion of epoxies from occupational exposures (primarily hand-to-mouth) is much less studied, but as likely as inhalation and dermal exposures. The only data on biokinetics of BADGE following ingestion exposures come from the aforementioned studies in mice. The oral administration of ^{14}C-BADGE in mice in that same study (Climie el al. 1981a) resulted in rapid uptake by the ingestion route and equally fast elimination – 88% in three days - mostly via feces (80%) and some via urine (11%), with ~0.1% of the administered oral dose of radioactivity remaining in the body of animals after 8 days post-ingestion. The metabolism of BADGE to various polar compounds, such as diols formed from BADGE hydrolysis by epoxide hydrolases in vivo and subsequent conjugation (e.g. by glutathione transferases and glucuronidases) was slow and followed similar profiles by both exposure routes (Climie et al 1981b). In F344 rats, plasma half-life was 8.8 h following a single intravenous administration and 4.4 h following an oral dose (DFG 2003).

There are no published studies on the biodistribution and clearance half-life of BADGE biomarkers via inhalation in humans and no exposure biomonitoring data to allow for accurate determination of these exposure half-lives or relative contribution of different exposure pathways. Our studies are the first in the literature of occupationally epoxide exposed cohorts to provide both inhalation and potential skin exposure data and urinary biomonitoring data. The observed relative abundance of different urinary BADGE biomarkers in workers is consistent with expectations from these earlier kinetic studies in animals which suggest an approximate proportion of 10:1:0.1 for bis-diol:mono-diol:free BADGE, respectively (Boogaard et al. 2000), with bis-diol being the predominant biomarker.

Very little is known about the exact origin of BADGE·HCl·H_2O biomarker, the second most abundant one, other than it is likely derived from further hydrolysis of BADGE·H_2O to BADGE·HCl·H_2O. The earlier seminal papers on the metabolism and clearance of BADGE in animals do not report on the identity of this biomarker. Partial hydrolysis of BADGE in the acidic environment of the stomach, followed by fast absorption into systemic circulation, may be one possible explanation. In mid-coat applicators, BADGE·HCl·H_2O concentrations did not increase cross-shift, and BADGE·H_2O levels were mostly non-detectable. This observation would support the BADGE → BADGE·H_2O → BADGE·HCl·H_2O sequence, which is apparently less favored than the conversion to BADGE·2H_2O. In top-coat (isocyanate) applicators, both BADGE·HCl·H_2O and BADGE·2H_2O GMs changed almost identically cross-shift (GM ratio = 1.37 ± 0.1, SG normalized). Follow-up studies to assess BADGE toxicokinetics and clearance half-lives in humans are warranted.

Urinary biomonitoring data from the three groups of workers demonstrate widespread exposure to BADGE, suggestive of common exposures from non-occupational sources, including ingestion and do-it-yourself consumer applications of epoxy resins. In light of this observation, establishing a reliable biological monitoring guidance value (BMGV) for BADGE·2H_2O for occupational exposures will require additional background biomonitoring and health effect data. We are proposing an initial guidance value for BADGE·2H_2O of 0.5 ng/mL (SG-normalized) or 180 nmol/mol creatinine as the threshold to discriminate occupational from non-occupational exposures based on the maximum values observed in the reference SPF group. Urinary biomarkers values higher than this threshold indicate likely BADGE occupational exposures and warrant the need for better occupational hygiene practices and more effective PPE. Values lower than this threshold would reflect overall good hygiene practices. In our cohort of coating workers, 75% of mid-coat workers and 45% of top-coat workers had post-shift BADGE·2H_2O levels higher than this guidance value of 0.5 ng/mL.

This study design did not include post-application follow-up sampling and biomonitoring of participants to assess biomarker changes as they transitioned from mid-coat to top-coat applications. Although desirable, such follow-up designs are extremely difficult to implement at construction sites due to the uncertainties in work schedules, personnel job/site reassignments, and limited access. However, modern LC-ESI-MS/MS technologies now offer adequate sensitivity and selectivity to measure extremely low levels of these biomarkers in different occupational settings, including other construction trades, which may provide better opportunities for time series studies.

5. Conclusions

Construction workers who apply epoxy mid-coats in industrial coatings are exposed to potentially high levels of airborne and skin BADGE exposures as documented in our earlier study. Not surprisingly, urinary BADGE·2H_2O in mid-coat applicators who worked with BADGE epoxies during the shift increased by 2.9-fold post-shift urine compared to pre-shift (GM ratios). Field observations in combination with urinary biomonitoring indicate that better exposure controls are needed at these construction sites. This conclusion is further substantiated by (yet to be published) urine analysis results of a panel of oxidative stress and kidney function biomarkers in these workers (subject of future reports), which are also elevated in painters relative to other groups. BADGE·2H_2O is a robust, sensitive, and adequate biomarker for routine biomonitoring of exposures to epoxies in occupational settings, and 9× more abundant than the next most common urinary biomarker BADGE·HCl·H_2O. An

initial urinary concentration of 0.5 ng/mL for BADGE·$2H_2O$ is proposed as a threshold for good hygiene practices for epoxy exposures in occupational settings based on current biomonitoring results in SPF construction workers.

CRediT authorship contribution statement

Anila Bello: Conceptualization, Investigation, Data curation, Visualization, Formal analysis, Writing - original draft. **Yalong Xue:** Methodology, Data curation, Visualization, Writing - original draft. **Dhimiter Bello:** Conceptualization, Writing - review & editing, Supervision, Project administration, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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