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RESEARCH ARTICLE

A paired comparison between human skin and hairless guinea pig skin *in vitro* permeability and lag time measurements for 6 industrial chemicals

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Abstract

The purpose of the present study was to measure and compare permeability coefficients (k_p) and lag times (τ) in human skin and hairless guinea pig (HGP) skin. Paired experiments employed heat-separated epidermal membranes from human and HGP sources mounted on static *in vitro* diffusion cells. Infinite-dose, saturated aqueous solutions of 6 industrial chemicals were used as donors: aniline, benzene, 1,2-dichloroethane, diethyl phthalate, naphthalene, and tetrachloroethylene. No significant differences were found between human and HGP skin for either k_p or τ for any of these chemicals ($p \geq .24$). HGP vs. human k_p measurements, and HGP vs. human τ measurements, were highly correlated. For k_p, the slope of the linear correlation was close to unity (1.080 ± 0.182) and the intercept close to 0 (0.015 ± 0.029 cm/h), with a correlation coefficient (r^2) = 0.898. For τ, the slope was also close to unity (0.818 ± 0.030) and the intercept close to 0 (–0.014 ± 0.023 h), with $r^2 = 0.994$. These results suggest that HGP skin may serve as an excellent surrogate for human skin in *in vitro* dermal penetration studies.

Keywords: *Skin absorption; permeability; lag time; aniline; benzene; 1,2-dichloroethane; diethyl phthalate; naphthalene; tetrachloroethylene*

Introduction

In vitro measurements of the transdermal penetration of chemicals are important elements of occupational and environmental dermal risk assessments. While it may seem obvious that human skin is the preferred skin for risk assessments related to human health, the use of human skin has its drawbacks. Traditionally, cadaver skin has been used; however, there can be wide variability among donors due to differences in gender, race, and age (1–7). Furthermore, freshness is a concern, as harvesting and shipment may not be timely. Some of these issues have been mitigated by the increasing use of skin harvested from breast reduction and panniculectomy ("tummy tuck") procedures. Still, availability and timeliness remain significant problems.

The hairless guinea pig (HGP) offers several advantages over other rodent models for dermal penetration studies. The skin is smooth and devoid of hair, the animal is large enough to provide sufficient skin for multiple replicates, and the skin structure is similar to human skin (8,9). However, it is widely regarded that rodent skin is more permeable than human skin, even though several studies have reported similarities between human skin and HGP skin in measured permeability. Recently we undertook a quantitative review of the use of pigs or guinea pigs as surrogates for human *in vitro* dermal penetration studies (10). For both haired and hairless guinea pig skin compared with human skin, a highly significant correlation was found when we pooled studies in which both species were tested in the same laboratory using similar methods. Of the 12 studies examined, 6 used HGPs. Panchagnula et al.

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found a < 10% difference in permeabilities for water and 7-hydroxycoumarin in human and HGP skin. Valiveti et al. also found similar permeabilities and lag times of tetrahydrocannabinol (11) and a synthetic cannabinoid (12). Stinchcomb and colleagues also found 2- to 6-fold differences in permeabilities of naltrexone and its active metabolite, with HGP being more permeable (13), but similar permeability and lag time for charged naltrexone salt (14).

In the present study, we augment this body of knowledge through a paired comparison of human and HGP skin permeability and lag time measurements. Six chemicals of industrial use were studied, covering a moderate range of lipophilicity (log K_{ow} 0.90–3.40). Skin preparation (heat-separated epidermal membranes) and all other experimental methods were identical in these paired studies, in an attempt to minimize variability arising from sources other than the skin.

Materials and methods

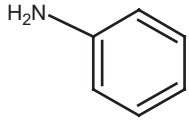
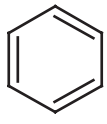
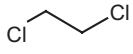
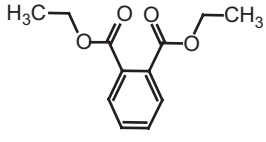
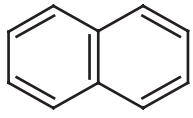
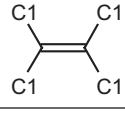
Chemicals and supplies

Properties of the 6 donor chemicals are listed in Table 1. The chemicals were obtained through Sigma-Aldrich (St. Louis, MO, USA) or subsidiaries as noted. Claimed purities were as follows: aniline (ANI) (Fluka, >99%), benzene (BEN) (≥99.9%), 1,2 dichloroethane (DCE) (≥99.8%), diethyl phthalate (DEP) (99.5%), naphthalene (NAP) (Supelco, 99.9%), and tetrachloroethylene (TCE) (≥99.5%). In addition, *n*-hexane (>99%), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) free acid (>99.5%), and sodium bicarbonate ($NaHCO_3$) (>99.5%) were obtained from Sigma-Aldrich. Hanks' balanced salt solution was purchased from Gibco Life Technologies (Rockville, MD, USA). Nalgene vacuum filter units (pore size, 0.2 μm) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Autosampler vials and crimp caps (Teflon-silicone-Teflon) were obtained from MicroLiter Analytical Supplies (Suwanee, GA, USA). Solid-phase microextraction (SPME) fibers were purchased from Supelco (Bellefonte, PA, USA).

Buffer and donor solutions

Buffer consisted of HEPES-buffered Hanks' balanced salt solution: 5.96 g of HEPES was stirred into 1,000 mL of Hanks' solution. Then 0.32 g of $NaHCO_3$ and 0.05 g of gentamicin sulfate were added. The pH was brought to 7.4 at 37°C by dropwise addition of 6 N sodium hydroxide (NaOH). Buffer was filtered (Nalgene) and degassed prior to use by warming to 40°C and stirring under laboratory vacuum.

Table 1. Some chemical properties of the studied compounds.

Chemical	Abbreviation	CAS No.	Structure	MW	Log K_{ow}	Solubility[a] (μg/mL)
Aniline	ANI	62-53-3		93.1	0.90	37,909 ± 1,318
Benzene	BEN	71-43-2		78.1	2.13	1,896 ± 108
1,2-Dichloroethane	DCE	107-06-2		99.0	1.48	6,731 ± 452
Diethyl phthalate	DEP	84-66-2		222.2	2.47	975 ± 194
Naphthalene	NAP	91-20-3		128.2	3.30	33.3 ± 4.6
Tetrachloroethylene	TCE	127-18-4		165.8	3.40	223 ± 43

[a]Solubility was measured by the authors in buffer solution, pH 7.4, 32°C (mean ± standard deviation [SD], $n = 4$).
CAS = Chemical Abstract Society; Log K_{ow} = base 10 logarithm of the octanol-water partition coefficient, taken from the Hazardous Substances Data Base; MW = molecular weight.

Donor solutions for penetration studies consisted of saturated solutions of the individual chemicals in buffer. Excess of chemical was added to buffer and vortexed overnight at room temperature, and then warmed to 32°C. When added to donor cells, small excess of chemical remained to maintain saturation. Saturation quantities of the organic base aniline (negative logarithm of the acid ionization constant [pK_a] 4.8) increased solution pH to 7.5. Therefore, more than 99% of aniline existed in its nonionized form, and we assumed that the applied concentration of nonionized aniline equals the total aniline donor concentration.

Skin sources

Male HGPs (~500 g) of the strain Crl:IAF(HA)-hrBR were obtained from Charles River Laboratories (Wilmington, MA, USA) and their use was approved by our Animal Care and Use Committee. The HGPs were euthanized with carbon dioxide (CO_2), and abdominal skin was harvested and used on the same day.

Human skin samples from caucasian females (age range 19–57 years) were obtained from breast reductions or panniculectomies from the West Virginia University Skin Bank. The Skin Bank maintains Human Subjects Review Board approval for all collections, but the specific use of this tissue in our experiments was deemed "not human subject research" and therefore not subject to approval. Skin was frozen (–85°C) on the same day as surgery and used within 191 days.

Skin preparation

Heat-separated epidermal membranes were used. The thawed skin was submersed in 60°C buffer for 45–60 seconds, and epidermis was teased from the dermis using cotton swabs. Skin disks were obtained using a 5/8-inch diameter stainless-steel punch. Visual inspection of each skin disk under dissecting microscope (20 × power) was performed to eliminate any samples with obvious defects (holes).

Diffusion cell studies

Vertical, static diffusion cells (PermeGear, Bethlehem, PA, USA) were used. Receptor compartments (5 mL) were filled with warmed, degassed buffer. From each of 4 donors, 3 human skin disks and 3 HGP skin disks were mounted on the cells. The diameter of exposed skin was 9 mm. The water-jacketed cells were maintained at 37°C via a recirculating water bath; this maintained temperature at the skin surface at 32°C. At time 0, 0.5 mL of saturated donor solution of 1 of the 6 chemicals was added to the 6 diffusion cells. Donor compartments were covered with parafilm. Receptor fluid samples were taken at time 0 and at set times through the duration of the experiment. Sample times differed according to chemical: ANI: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 hours; BEN: 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hours; DCE: 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 hours; DEP: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 hours; NAP: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 hours; and TCE: 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hours. Sample volumes varied according to the chemical being studied (specified subsequently), but in all cases the exact volume was replaced with fresh buffer. Donor solutions were replaced periodically to maintain "infinite" dose conditions. Receptor fluid concentrations for all chemicals were less than 10% of their measured saturation quantities.

Chemical quantification

Gas chromatographic (GC) and sample preparation methods were optimized for 5 of the 6 chemicals. The gas chromatograph was a Varian CP-3800 (Varian Inc., Walnut Creek, CA, USA) equipped with a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland). For ANI, an in-vial, liquid-liquid extraction was used: 1.0 mL of *n*-hexane was added to 0.5-mL of sample in a 2-mL autosampler vial. The contents were vigorously vortexed for 30 minutes, then centrifuged at 2,500 rpm for 30 minutes. Two microliters of the upper organic phase was injected into the GC injection port, and flame ionization detection (FID) was used. For DEP, SPME methods were used, as described previously (15,16). Briefly, an 85-μm polyacrylate fiber was equilibrated in 1.5 mL of sample in a 2-mL vial at 40°C for 45 minutes with agitation. The fiber was desorbed in the injector for 10 minutes and analyte was detected with FID. For BEN, DCE, and TCE, 0.2-mL samples were placed in 10-mL headspace vials. The samples were heated to 50°C for 5 minutes with agitation, and then 0.1 mL of headspace was injected into the GC, with FID for BEN and electron capture detection (ECD) for DCE and TCE.

For NAP, quantification was via direct reading of fluorescence (excitation 275 nm, emission 332 nm) using an LS 50B Luminescence Spectrometer (PerkinElmer, Waltham, MA, USA), with appropriate dilution of samples as necessary.

Calibrations were performed for each experiment from freshly prepared solutions covering the full range of measured values. Calibration correlation coefficients were > 0.98.

Donor solutions were sampled in triplicate from each experiment. Solutions were centrifuged at 2,500 rpm for 30 minutes at 32°C, and clear solutions were subjected to analysis following appropriate dilution. Donor concentrations are listed as solubilities in Table 1; values are means ± standard deviations (SDs) of 4 determinations.

Data analysis

The total amount of chemical penetrated as a function of time of exposure was calculated from the measured concentrations for each skin disk. Permeability and lag time were calculated by nonlinear regression of the following equation, as described previously (15):

$$m(t) = k_p Ct - k_p C_1 \tau - \frac{12 k_p C \tau}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(\frac{-n^2 \pi^2 t}{6\tau}\right) \quad (1)$$

Here, $m(t)$ is the cumulative amount of chemical penetrated per unit area of skin at time t, and C is the donor concentration. Two unknown parameters determine the solution of this equation: permeability coefficient, k_p, and lag time, τ. Nonlinear regressions of the mass accumulation data with equation 1 were performed using SigmaPlot 9.01 (Systat Software, Inc., Chicago, IL, USA). The equation was truncated to 7 terms in the series. Use of equation 1 is mathematically equivalent to calculating k_p from the slope of the steady-state accumulation curve and τ as the intercept of this asymptote with the time axis. However, use of equation 1 is quantitatively precise and eliminates subjectivity of the analyst in these determinations.

The means of the calculated permeabilities and lag times for each skin disk were taken as the values for each donor. For each chemical, 4 human and 4 HGP donors were used. Pairwise comparisons were made between HGP and human permeability and lag time measurements using the *t*-test (SigmaStat 3.1, Systat Software, Inc.).

Linear regressions were performed on the HGP vs. human data means for both k_p and τ (SigmaPlot 9.01). If the HGP were a perfect surrogate for human skin, then the slope m of the regression line would be 1, the intercept b would be 0, and the correlation coefficient r^2 would be 1. The linear regressions also give standard errors for the intercept and slope; these are measures of the precision of the estimates.

Coefficients of variation for k_p and τ measurements were calculated as the SD divided by the mean. The means of these quantities (i.e., the means for all 6 chemicals) for human k_p, HGP k_p, human τ, and HGP τ are reported in order to assess the variability in human skin measurements compared with HGP skin measurements. These comparisons were evaluated for statistical significance using the *t*-test (SigmaStat 3.1).

Results

Results for all 6 chemicals are presented in Table 2. Means and SDs of permeability coefficients and lag times for both HGP and human skin are given. No significant differences between HGP and human skin were detected in either quantity for any of the 6 chemicals tested. For all comparisons, $p \geq 0.24$. Human skin permeability measurements exhibited more variability than HGP. The average CV was 40% for human and 22% for HGP ($p = 0.05$). For lag time measurements, human skin and HGP skin exhibited similar variances (~60%).

Figure 1 presents the comparison between HGP and human skin permeability measurements. An excellent linear correlation ($r^2 = 0.898$) was found, with a slope close to unity (1.080 ± 0.182) and an intercept close to 0 (0.015 ± 0.029 cm/h).

Figure 2 presents the comparison between HGP and human lag time measurements. Again, an excellent linear correlation ($r^2 = 0.994$) was found, with a slope close to unity (0.818 ± 0.030) and an intercept close to 0 (-0.014 ± 0.023 h).

Discussion

These data support the use of HGP skin as a surrogate for human skin for human dermal risk assessments. For both permeability coefficient and lag time, there were excellent correlations between HGP and human skins when considering all 6 of the chemicals studied here. No significant

Table 2. Permeability and lag time measurements[a] for human skin and hairless guinea pig skin

Chemical	Permeability (k_p) (cm/h)			Lag time (τ) (h)		
	Human	HGP	p	Human	HGP	p
ANI	0.064 ± 0.043	0.079 ± 0.009	.53	0.19 ± 0.14	0.18 ± 0.01	.85
BEN	0.167 ± 0.077	0.230 ± 0.057	.24	0.15 ± 0.13	0.08 ± 0.05	.35
DCE	0.259 ± 0.070	0.295 ± 0.093	.56	0.12 ± 0.05	0.09 ± 0.06	.55
DEP	0.043 ± 0.019	0.057 ± 0.012	.24	0.71 ± 0.16	0.62 ± 0.32	.62
NAP	0.177 ± 0.033	0.158 ± 0.014	.32	1.68 ± 0.55	1.35 ± 0.85	.53
TCE	0.141 ± 0.049	0.192 ± 0.061	.24	0.25 ± 0.21	0.14 ± 0.16	.46
CV (%)	40 ± 17	22 ± 10	.05	57 ± 28	61 ± 35	.84

[a]Data shown are mean ± standard deviation (SD); $n = 4$ donors for each measurement.

ANI = aniline; BEN = benzene; CV (%) = coefficient of variation of measured quantity for the 6 chemicals; DCE = 1,2-dichloroethane; DEP = diethyl phthalate; HGP = hairless guinea pig; NAP = naphthalene; TCE = tetrachloroethylene.

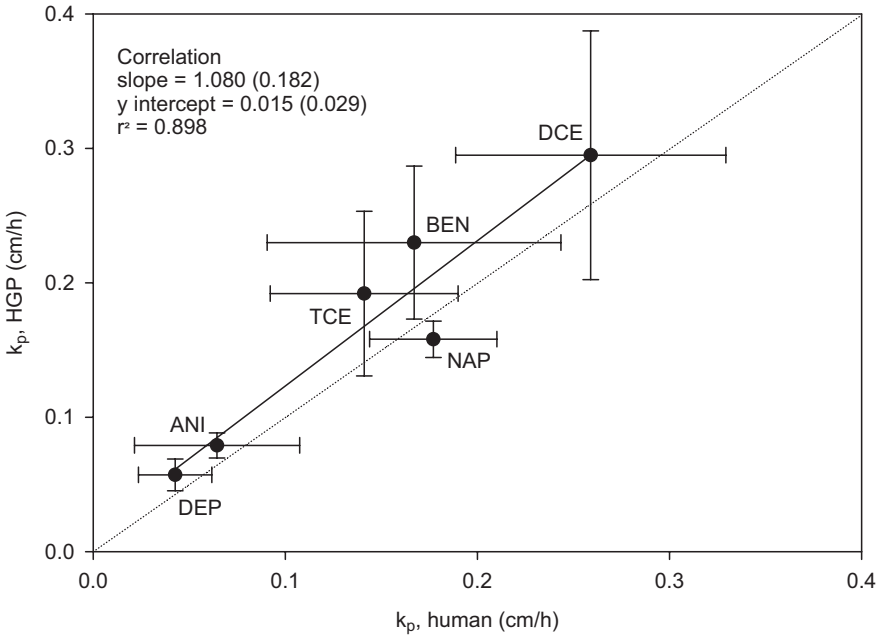


Figure 1. Comparison of permeability (k_p) measurements of hairless guinea pig (HGP) and human skin for the 6 chemicals. Shown are means ± standard deviations; $n = 4$ donors per chemical. Dashed line is the line of identity; solid line is linear regression. Correlation slope and intercept are listed (means, standard errors), as is correlation coefficient r^2. ANI = aniline; BEN = benzene; DCE = 1,2-dichloroethane; DEP = diethyl phthalate; NAP = naphthalene; TCE = tetrachloroethylene.

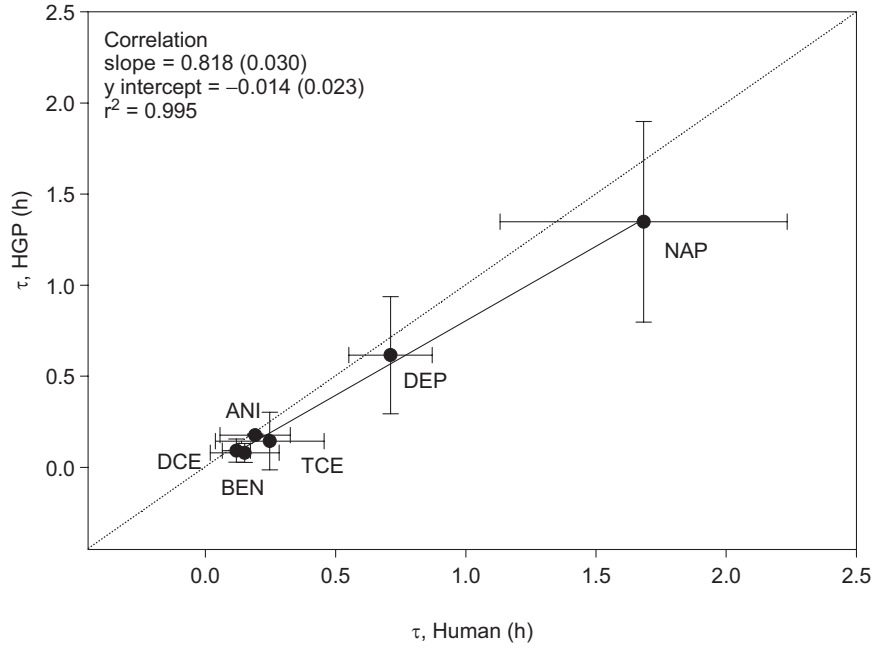


Figure 2. Comparison of lag time (τ) measurements of hairless guinea pig (HGP) and human skin for the 6 chemicals. Shown are means ± standard deviations; $n = 4$ donors per chemical. Dashed line is the line of identity; solid line is linear regression. Correlation slope and intercept are listed (means, standard errors), as is correlation coefficient r^2. ANI = aniline; BEN = benzene; DCE = 1,2-dichloroethane; DEP = diethyl phthalate; NAP = naphthalene; TCE = tetrachloroethylene.

differences were observed in either of the measured quantities for any of the 6 chemicals. That is, these experiments provide no evidence that there is a difference in penetration rate or time lag for any of these chemicals.

Several studies have compared permeability of guinea pig and human skin. In addition to those cited in the introduction, other studies report a factor of difference in percentage absorbed or non–steady-state absorption

rate between the 2 species. A factor of difference (FOD) > 1 indicates that HGP skin is more permeable, while an FOD < 1 means that human skin is more permeable. In a recent review of the literature (10), we found 14 measurements reporting FOD. Of these, 9 found less than a 3-fold difference between HGP skin and human skin. Others found differences that were substantial, with 4 of 5 finding HGP skin to be more permeable than human skin.

Moody et al. found an FOD of 28 for benzo(*a*)pyrene (BaP) (17), although Storm et al. found an FOD of 3.7 for the same compound (18). In Moody et al.'s study, if one includes the residual BaP in skin, the FOD is < 2. BaP is a polycyclic aromatic, whereas NAP studied here is a bicyclic aromatic. Therefore, one might expect similar FODs for these structurally similar compounds. However, our measured FOD of 0.89 for NAP (Table 2) differs substantially from these FODs for BaP. BaP is highly lipophilic (log K_{ow} 5.97, compared with 3.30 for NAP); this factor may have contributed to the differences in BaP permeability between human skin and HGP skin.

Hood et al. found FODs of 26 and 8 for musk xylol from different vehicles (19). Here again, if one includes the residual skin amounts measured at the end of the experiment, the FODs are much smaller (< 3). When the authors continued these studies in time, most of this residual amount was found in receptor fluid, suggesting that total absorption between HGP and human skins were similar, but with different time courses.

We (10) recently reported that Moody and Nadeau (20) found an FOD of 0.2 for the insect repellant *N*,*N*-diethyl-*m*-toluamide (DEET) when comparing cumulative absorption of a finite dose. However, a rereading of the article suggests an FOD of 0.75, considering reported differences in the applied dose between the 2 species. In a subsequent report on DEET penetration from 3 commercial formulations (21), the same authors reported FODs exceeding 1. Thus, it appears that our original designation of DEET FOD as an outlier is in error.

This article deals with *in vitro* studies only; however, it may be pointed out here that Anderson et al. (22) found the haired guinea pig to be a reasonable *in vivo* model for human skin absorption for 3 tested compounds. Overall, the literature suggests that both haired and HGP may be good substitutes for human skin for many but not all chemicals. Based on studies reported here and analysis of published data, HGP skin permeability is similar to or exceeds human skin permeability, supporting the use of the HGP as a conservative surrogate for human *in vitro* penetration studies for dermal risk assessments.

We found that HGP skin exhibits less variability than human skin for permeability measurements. This may relate to the genetic uniformity present in an inbred animal colony. This may be an important consideration in studies designed, for example, to identify differences in response to specific exposure conditions. Less intersubject variability increases the statistical power of an experiment, which means that significant differences can be observed with fewer subjects. For example, in order to detect a 1.5-fold difference in the mean via an unpaired *t*-test with a minimum power of 0.8, only 7 HGPs would be required, whereas skin samples from 19 humans would be necessary, assuming the variances found here (22% for HGP, 40% for human; calculation using SigmaStat 3.11).

In summary, these studies suggest that skin from the HGP can be used in place of human skin for *in vitro* penetration studies for the chemicals tested. A review of the literature comparing human skin and HGP skin confirms that the latter may serve as a conservative surrogate for human *in vitro* penetration studies for dermal risk assessments.

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***Disclaimer*:** The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the National Institute for Occupational Safety and Health.

***Declaration of interest*:** The authors report no conflicts of interest.

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