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## Original Article

# Evaluation of Matrix Effects in Quantifying Microbial Secondary Metabolites in Indoor Dust Using Ultraperformance Liquid Chromatograph–Tandem Mass Spectrometer

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## ABSTRACT

**Background:** Liquid chromatography–tandem mass spectrometry (LC-MSMS) for simultaneous analysis of multiple microbial secondary metabolites (MSMs) is potentially subject to interference by matrix components.

**Methods:** We examined potential matrix effects (MEs) in analyses of 31 MSMs using ultraperformance LC-MSMS. Twenty-one dust aliquots from three buildings (seven aliquots/building) were spiked with seven concentrations of each of the MSMs (6.2 pg/μl–900 pg/μl) and then extracted. Another set of 21 aliquots were first extracted and then, the extract was spiked with the same concentrations. We added deepoxy-deoxynivalenol (DOM) to all aliquots as a universal internal standard. Ten microliters of the extract was injected into the ultraperformance LC-MSMS. ME was calculated by subtracting the percentage of the response of analyte in spiked extract to that in neat standard from 100. Spiked extract results were used to create a matrix-matched calibration (MMC) curve for estimating MSM concentration in dust spiked before extraction.

**Results:** Analysis of variance was used to examine effects of compound (MSM), building and concentration on response. MEs (range: 63.4%–99.97%) significantly differed by MSM ( $p < 0.01$ ) and building ( $p < 0.05$ ). Mean percent recoveries adjusted with DOM and the MMC method were 246.3% (SD = 226.0) and 86.3% (SD = 70.7), respectively.

**Conclusion:** We found that dust MEs resulted in substantial underestimation in quantifying MSMs and that DOM was not an optimal universal internal standard for the adjustment but that the MMC method resulted in more accurate and precise recovery compared with DOM. More research on adjustment methods for dust MEs in the simultaneous analyses of multiple MSMs using LC-MSMS is warranted.

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## 1. Introduction

Most of the construction materials used for indoor living spaces and offices are made from or contain hydrocarbons that are prone to supporting microbial growth under excessive moisture conditions after water incursion. Water damage can occur owing to improper maintenance [1] or high indoor humidity due to the lack of proper ventilation or poor architectural design [2], as well as natural disasters such as flooding from hurricanes.

Dampness and mold in water-damaged buildings are associated with various respiratory illnesses in occupants [3–5]. Public

concerns of respiratory illnesses associated with fungal exposure have been on the rise through the last 40 years or more [3–5]. Recently, studies on occupants of water-damaged buildings have attempted to link occupants' health to fungal secondary metabolites [6–8]. In a study by Kirjavainen et al [6], microbial secondary metabolites (MSMs) were detected in floor dust samples collected from 93 homes where 1-year-old children lived; the study did not find clear evidence of association of the MSMs in the dust with childhood asthma at the age of six years. Cai et al [7] reported a negative association between the presence of verrucarol in dust and daytime breathlessness among school pupils in damp

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classrooms in Malaysia. Auger et al [8] presented a short communication article in which they proposed a potential link between the fungal spore-borne low-molecular-weight compounds (MSMs) and respiratory and nonrespiratory symptoms.

In such epidemiological studies, the sample analytical method is critical in examining associations of exposures to secondary metabolites with health outcomes. The selected method should accurately and precisely quantify the metabolites to minimize exposure misclassification. Studies on MSMs in food and agricultural chemistry have quantified them using different analytical techniques, such as thin-layer chromatography [9], enzyme-linked immunosorbent assay [10], and liquid chromatography (LC), with different detection techniques such as ultraviolet [11], fluorescence [12], and tandem mass spectrometry (MSMS) [13,14]. Of these, the liquid chromatography-tandem mass spectrometry (LC-MSMS) method has been widely used because of the capability of simultaneously quantifying multiple analytes. Ultraperformance (UP) LC-MSMS is an advanced analytical tool with increased sensitivity, speed and resolution through use of smaller (1.7  $\mu\text{m}$ ) particle packing material in a chromatographic column that can handle higher column pressure compared with high-performance LC-MSMS [15]. UPLC-MSMS has been widely used in biological analyses for the purpose of detection and quantification of small biological molecules [16–18]. However, LC-MSMS has its own limitations, such as the alteration of ionization efficiency by coeluting substances, called matrix effects (MEs) [19,20]. Owing to interfering materials in the sample matrix, ion enhancement or suppression can occur, which produces altered responses in the MSMS.

The present study evaluates the extent of the signal suppression/enhancement (ME) in the UPLC-MSMS method for simultaneously quantifying MSMs in floor dust samples collected from three buildings and explores two adjustment methods [the use of an internal standard (ISTD) and the matrix-matched calibration method] to minimize quantification errors.

## 2. Materials and methods

### 2.1. Standard materials and chemicals

Standard materials of 31 MSMs and one ISTD (deoxydeoxynivalenol, also known as DOM or DOM-1 as a deoxynivalenol metabolite) were used to investigate MEs. DOM was purchased from Sigma-Aldrich, St. Louis, MO, USA, dissolved in acetonitrile as a 25  $\mu\text{g}/\text{ml}$  solution. Table 1 lists the standard materials of the MSMs, their suppliers, and the abbreviated names that were assigned to each one and will be used throughout the text. Methanol (>99.9%, LCMS grade), acetic acid ( $\geq 99.7\%$ , LCMS grade), and ammonium acetate as mobile phase buffer ( $\geq 99.0\%$ , LCMS grade) were purchased from Fisher Scientific (Waltham, MA, USA). Acetonitrile ( $\geq 99.5\%$ , LCMS grade) was purchased from EMD Millipore (Burlington, MA, USA). Ultrapure water was collected through an Advantage 10 ultra-filtration assembly (EMD Millipore) with resistivity of 18.2  $\text{M}\Omega \cdot \text{cm}$  at 25°C.

### 2.2. Dust collection

Floor dust was collected in two office buildings (buildings 2 and 3) and a school administration building (Building 1) located in three different cities in the northeastern region of the United States. The Building 1 (a historic building built in 1914) was located in a seaport city, the Building 2 (built in 1978), in close proximity to downtown of a city, and the Building 3 (built in 1978/annex built in 1991), in a small town in rural area. All three buildings had a history of water damage. Dust collection took place in June and August 2007, as well

**Table 1**

List of MSMs, abbreviations, the suppliers, and CAS numbers

No.	Analyte	Abbreviation	Supplier*	CAS no.
1	3-Nitropropionic acid	NITP	Sigma-Aldrich	504-88-1
2	Aflatoxin B1	AFB1	Sigma-Aldrich	1162-65-8
3	Aflatoxin B2	AFB2	Fermentek	7220-81-7
4	Aflatoxin G1	AFG1	Sigma-Aldrich	1165-39-5
5	Aflatoxin G2	AFG2	Sigma-Aldrich	7241-98-7
6	Alternariol	ALT	Sigma-Aldrich	641-38-3
7	Alternariol Monomethylether	AME	Adipogen	26894-49-5
8	Asperglauclide	ASPG	ChemFaces	56121-42-7
9	Chaetoglobosin A	CTGA	Adipogen	50335-03-0
10	Citreoresein	CITRO	ChemFaces	481-73-2
11	Citrinin	CIT	Sigma-Aldrich	518-75-2
12	Cyclo(L-Pro-L-Tyr)	CYCLO	Bioaustralis	4549-02-4
13	Deoxynivalenol	DON	Sigma-Aldrich	51481-10-8
14	Diacetoxyscirpenol	DAS	CAYMAN	2270-40-8
15	Emodin	EMOD	Sigma-Aldrich	518-82-1
16	Fumonisin B1	FUB1	Sigma-Aldrich	116355-83-0
17	Integracin A	INTA	Santa Cruz	224186-03-2
18	Integracin B	INTB	Santa Cruz	224186-05-4
19	Neoechinulin A	NEOA	ChemFaces	51551-29-2
20	Neosolaniol	NEO	Sigma-Aldrich	36519-25-2
21	Nivalenol	NIV	Fermentek	23282-20-4
22	Ochratoxin A	OTA	Sigma-Aldrich	303-47-9
23	Roquefortine C	ROQC	Santa Cruz	58735-64-1
24	Skyrin	SKY	Sigma-Aldrich	602-06-2
25	Stachybotrylactam	STCH	Santa Cruz	163391-76-2
26	Sterigmatocystin	STEG	Sigma-Aldrich	10048-13-2
27	T-2 toxin	T2	Fermentek	21259-20-1
28	Valinomycin	VAL	Sigma-Aldrich	2001-95-8
29	Verrucaric acid	VERA	Sigma-Aldrich	3148-09-2
30	Verrucarol	VERO	Sigma-Aldrich	2198-92-7
31	Zearalenone	ZEA	Sigma-Aldrich	17924-92-4

MSMs, microbial secondary metabolites.

\* Sigma-Aldrich, St. Louis, MO, USA; Fermentek, Jerusalem, Israel; Adipogen, San Diego, CA, USA; ChemFaces, Hubei, China; Bioaustralis, Smithfield, NSW, Australia; Cayman, Ann Arbor, MI, USA; Santa Cruz, Dallas, TX, USA.

as August 2008. The sampling method involved vacuuming a 2 square meter ( $\text{m}^2$ ) carpeted-floor area for 5 minutes using a Li'l Hummer backpack vacuum cleaner (100 CFM, 1.5 horse power, Pro-Team Inc., Boise, ID, USA) with polyethylene filter socks. The detailed sampling methodology was described in earlier publications [21–23]. We selected four or more fine dust samples from each study building that had 120 or more dust samples collected and combined them by building to secure enough dust for the experiments.

### 2.3. Experimental design

To evaluate dust MEs, extraction efficiencies (EEs), and experimental procedure efficiencies (PEs), we analyzed spiked neat solutions (standard solutions), dust samples spiked before extraction, and dust extracts spiked after extraction. First, we homogenized the pooled dusts by rotating on a 360-degree rotary arm shaker at 65 r.p.m. for 2 hours. Then, we made 16 dust aliquots (average weight = 30.6 mg/aliquot; range = 30.0–31.8 mg) from pooled dust for each of the three buildings. Fourteen dust aliquots for each building were randomly assigned into two different groups (seven aliquots per group): one group for spiking before extraction and the other group for spiking after extraction. Seven aliquots in each group were spiked with 31 MSMs at seven spiking concentrations (0.00625, 0.0125, 0.025, 0.075, 0.15, 0.45, and 0.9  $\text{ng}/\mu\text{l}$ ) and analyzed with a set of neat solutions at the same concentrations in mobile phase solvent (30% methanol, 69% ultrapure water, and 1%

acetic acid, by volume). We established the range of the spiking concentrations to make sure that the spiked MSMs are reliably detected even under the influence of MEs and extraction loss. Spiked ISTD concentration was 0.15 ng/ $\mu$ l for all samples and neat solutions. Two additional dust aliquots without spiking (plain dust) from each building were also extracted and analyzed as controls for the experiment to confirm amounts of the target MSMs in dust before spiking. Therefore, the total number of samples analyzed for all three buildings was 48.

The response of the mass spectrometer to each concentration of the MSMs in these neat solutions represents the responses without interference of the dust matrix and without extraction loss. Spiked extracts were dust extracts that were spiked with MSMs and ISTD after sample extraction. Because spiking occurred after extraction, the response of the mass spectrometer for these samples represents results that were influenced only by MEs without extraction loss. Finally, spiked dusts were dust aliquots that were spiked before extraction. The response of the mass spectrometer for these samples represents results that were influenced by both MEs and extraction loss.

#### 2.4. Sample preparation and extraction

We dissolved each powdery standard material in 1 ml of acetonitrile to prepare initial stock solutions at 1 or 5 mg/ml, depending on the supplied amount. From the stock solution, a working standard was prepared with all 31 MSMs at 25 ng/ $\mu$ l. Then, we used volumetric serial dilution for the working standard to prepare spiking solutions at concentrations of 0.035, 0.0695, 0.139, 0.417, 0.833, 2.5 and 5 ng/ $\mu$ l. We also prepared an ISTD spiking solution at 1 ng/ $\mu$ l. To prepare these serial dilutions, we used 5- to 50- $\mu$ l pipettes (average uncertainty 1.9%, Thermo Fisher Scientific, Joensuu, Finland), 10–100  $\mu$ l (1.9%), 20–200  $\mu$ l (1.2%), or 100–1000  $\mu$ l (1.2%), accordingly.

We prepared seven spiked dust samples before extraction by spiking each dust aliquots with 36  $\mu$ l of the intermediate working standard solutions to achieve the desired spike level. After spiking, we vortexed the aliquots vigorously to ensure complete mixing between the spiked MSMs and dust. We left the aliquots to dry overnight in a chemical hood. Once dried, we added 30  $\mu$ l of the initial working solution of DOM, vortexed the aliquots, and left them to dry in the chemical hood. We extracted the MSMs spiked into dust by adding 1 ml of extraction solvent (a mixture of 79% acetonitrile, 20% water, and 1% acetic acid by volume) to each aliquot, vortexing, and then shaking on a rotary shaker for 90 minutes. After shaking, we centrifuged at 1962  $\times$  g for 3 minutes. Then, we transferred 800  $\mu$ l of the supernatant to a centrifuge glass tube and dried the contents using a gentle nitrogen stream. We also prepared seven dust extracts spiked after the extraction for each building dust. First, we extracted dust aliquots as described previously and then spiked 36  $\mu$ l of working standard solutions and 30  $\mu$ l of ISTD spiking solution into dust extracts. And these dust extracts were allowed to dry under a gentle stream of nitrogen.

We reconstituted all the dried extracts with 200  $\mu$ l of mobile phase solvent and vortexed until the dried deposit at the bottom of the centrifuge glass tube was dissolved in the solvent. We centrifuged the tubes once more at 1962  $\times$  g for 3 minutes and transferred 150  $\mu$ l of the supernatant to a UPLC injection vial for analysis. Ten microliters of the extracted solutions were injected twice (duplicate injection) into the UPLC.

#### 2.5. Chromatographic conditions and MSMS transitions

We used a UPLC (Acquity H Class, Waters, Milford, MA, USA) equipped with Acquity UPLC BEH C18 column (2.1  $\times$  1500 mm, 1.7

$\mu$ m packing) for chromatographic separation of the MSMs. The programed gradient flow starting at 90% aqueous and 10% organic was pumped at a flow rate of 0.2 ml/min. The aqueous solvent was composed of 99% ultrapure water and 1% acetic acid (by volume), with the addition of 10 mM ammonium acetate as a buffer. The organic solvent is a combination of 99% methanol and 1% acetic acid (by volume) with 10 mM ammonium acetate as a buffer. The initial flow combination was 10% organic and 90% aqueous kept for 0.5 minute and then ramped to 50% organic within the next 0.5 minute. During the initial 4 minutes, the organic phase was increased from 10 to 97% and held for 5 minutes until total run time of 9.5 minutes. Then, the flow was reversed to 10% organic (the initial flow) within 0.5 minutes and maintained the initial flow until the end of the run (12.5 minutes).

We used a tandem mass spectrometer (MSMS) (Acquity Xevo TqD Quadrupole Tandem Mass-Spectrometer, Waters) for quantification of the MSMs. First, we developed multiple reaction monitoring methods for the MSMs by directly infusing each metabolite into the tandem mass spectrometer to determine mass transitions as well as the cone voltages and collision energies required to obtain the maximum ion intensities for each metabolite. We selected two transitions, one for qualification and one for quantification for each metabolite, except for 3-nitropropionic acid (NITP) for which we obtained only one transition. The selected transitions were mostly those with the two highest intensities. The tune method within the MSMS included capillary voltage of 0.5 kV, a desolvation temperature of 350  $^{\circ}$ C, and a desolvation flow of 650 L/hr. Extended MSMS parameters are the following: RF lens 2.5 V, extractor 3 V, source temperature 150  $^{\circ}$ C, and cone gas flow 0 L/hr. Table 2 shows a complete list of the MSMS parameters and retention time involved in the analyses with the UPLC-MSMS.

#### 2.6. Calculations

The response (summation of peak areas of the two transitions selected from the multiple reaction monitoring method) was used to calculate ME, EE, PE, and percent recoveries of the MSMs in each sample. ME is a measure of the extent of signal enhancement or suppression due to interference of matrix components with the targeted metabolite in the ionization process. It was defined as ion suppression in our study, i.e., percentage of the response of metabolite in the sample extract spiked after the extraction to the response of the same metabolite in neat solution and then subtracted from 100 as expressed in equation (1). EE is the efficiency of extracting the MSMs from the spiked dust samples. It was calculated by the following equation (2), i.e., the ratio of the response of the MSMs spiked in dust before extraction to the response of the same metabolite in sample extract spiked after the extraction expressed as a percentage. PE is a measure of efficiency of the whole experimental procedure combining EE and ME and was calculated from the following equation (3) [24].

$$ME(\%) = 100 - (100 \times A/N) = 100 \times (1 - A/N) \quad (1)$$

$$EE(\%) = 100 \times B/A \quad (2)$$

$$PE(\%) = 100 \times B/N = 100 \times (A/N) \times (B/A) \quad (3)$$

In these equations, N is the response obtained from spiked neat solution, A the response obtained from dust extracts spiked after extraction, and B the response obtained from dust samples spiked before extraction.

Three sets of neat solutions for the three buildings were prepared, and each set was used to generate a calibration curve for each building. The linear standard regression curves were

**Table 2**  
Precursor and product ions, MSMS conditions, and LC retention time (RT) of all MSMs and ISTD

Abbreviation	Precursor ion (m/z)	Cone voltage (V)	Product ions (m/z) (1st, 2nd)	Collision energy (V) (1st, 2nd)	RT (min)
AFB1	313.1 [M+H] <sup>+</sup>	62	241.1, 284.9	40, 24	5.4
AFB2	315.2 [M+H] <sup>+</sup>	58	287.1, 259.1	28, 30	5.3
AFG1	329.1 [M+H] <sup>+</sup>	58	243.0, 311.0	28, 22	5.2
AFG2	331.1 [M+H] <sup>+</sup>	60	313.0, 245.0	26, 32	5.0
ALT	257.0 [M-H] <sup>-</sup>	56	212.3, 189.0	24, 24	5.9
AME	273.2 [M+NH <sub>4</sub> ] <sup>+</sup>	54	127.9, 115.0	50, 54	6.8
ASPG	445.5 [M+H] <sup>+</sup>	50	367.2, 349.2	18, 18	5.9
CIT	251.2 [M+H] <sup>+</sup>	28	233.0, 191.0	18, 26	5.6
CITRO	285.1 [M-H] <sup>-</sup>	62	224.0, 241.0	34, 26	6.2
CTGA	529.5 [M+H] <sup>+</sup>	32	130.0, 511.3	38, 10	6.6
CYCLO	261.2 [M+H] <sup>+</sup>	36	136.0, 154.1	18, 28	4.3
DAS	384.3 [M+NH <sub>4</sub> ] <sup>+</sup>	24	307.2, 105.0	12, 32	5.6
DOM	297.2 [M+H] <sup>+</sup>	28	249.1, 203.1	10, 16	4.4
DON	281.2 [M+H] <sup>+</sup>	26	109, 233.1	22, 10	4.7
EMOD	269.0 [M-H] <sup>-</sup>	66	225.0, 240.9	28, 28	7.5
FUB1	722.6 [M+H] <sup>+</sup>	62	352.3, 704.4	38, 32	5.8
INTA	629.7 [M+H] <sup>+</sup>	26	349.1, 289.2	16, 30	8.4
INTB	587.6 [M+H] <sup>+</sup>	24	307.2, 167.0	14, 26	7.9
NEO	400.3 [M+NH <sub>4</sub> ] <sup>+</sup>	20	305.1, 215.1	12, 20	4.7
NEOA	324.3 [M+H] <sup>+</sup>	28	156.9, 130.0	42, 52	5.8
NITP*	117.8 [M-H] <sup>-</sup>	20	45.9	6	2.1
NIV	313.2 [M+H] <sup>+</sup>	26	205.1, 125.0	12, 12	4.0
OTA	404.3 [M+H] <sup>+</sup>	32	239.0, 358.1	22, 14	6.3
ROQC	390.2 [M+H] <sup>+</sup>	16	193.0, 322.1	30, 22	6.3
SKY	539.3 [M+H] <sup>+</sup>	68	521.1, 504.0	30, 46	7.7
STCH	386.4 [M+H] <sup>+</sup>	70	178.1, 150.1	38, 46	7.0
STEG	325.2 [M+H] <sup>+</sup>	50	310.1, 281.0	22, 54	6.8
T2	484.4 [M+NH <sub>4</sub> ] <sup>+</sup>	26	305.1, 185.1	14, 20	6.3
VAL	1128.7 [M+NH <sub>4</sub> ] <sup>+</sup>	66	343.3, 1083.7	71, 43	9.7
VERA	520.4 [M+NH <sub>4</sub> ] <sup>+</sup>	24	249.1, 457.2	18, 14	6.2
VERO	267.1 [M+H] <sup>+</sup>	16	249.1, 231.1	6, 12	4.9
ZEA	317.1 [M-H] <sup>-</sup>	52	130.9, 174.9	30, 26	6.6

AFB1, aflatoxin B1; AFB2, aflatoxin B2; AFG1, aflatoxin G1; AFG2, aflatoxin G2; ALT, alternariol; AME, alternariol monomethylether; ASPG, asperglaucide; CIT, citrinin; CITRO, citreorosein; CTGA, chaetoglobosin A; CYCLO, cyclo(L-Pro-L-Tyr); DAS, diacetoxyscirpenol; DOM, deepoxy-deoxynivalenol; DON, deoxynivalenol; EMOD, emodin; FUB1, fumonisin B1; INTA, integracin A; INTB, integracin B; ISTD, internal standard; LC, liquid chromatography; MSMS, tandem mass spectrometry; MSMs, microbial secondary metabolites; NEO, neosolaniol; NEOA, neochininulin A; NITP, 3-nitropropionic acid; NIV, nivalenol; OTA, ochratoxin A; ROQC, roquefortine C; SKY, skyrin; STCH, stachybotrylactam; STEG, sterigmatocystin; T2, T-2 toxin; VAL, valinomycin; VERA, verrucaric acid; VERO, verrucarol; ZEA, zearalenone.

\* We found only one transition for the MSM.

generated using  $1/x$  (inverse of concentration) weighting factor to minimize the impact of high concentrations on the fitted line [24,25].

To estimate repeatability of the readings by the instrument, we calculated percent coefficient of variation (%CV) of the responses for the duplicate injections as follows [26]:

$$\%CV = \frac{\text{Standard Deviation of the duplicate responses}}{\text{Average of the duplicate responses}} \times 100 \quad (4)$$

Finally, the percent recovery of each metabolite is calculated from the following equation (5):

$$\%RE = \frac{\text{Estimated} - \text{Estimated}_{\text{plain dust}}}{\text{Spiked concentration}} \times 100 \quad (5)$$

In equation (5) 'Estimated' refers to the concentration of spiked dust samples measured by the instrument, 'Estimated<sub>plain dust</sub>' to the measured concentration in plain dust with no spiked MSMs, and 'Spiked concentration' to the known spiking concentration. Plain dust sample results showed no detectible quantities of the targeted MSMs in all three buildings, with the exception of chaetoglobosin A (CTGA). We adjusted the measured responses of the spiked samples for CTGA response in the plain dust by subtracting the response of plain dust from those of spiked dust as shown in equation (5). We also calculated percent recovery of each metabolite spiked before the extraction using DOM as an ISTD and using a matrix-matched calibration curve instead of the neat solution calibration curve. The matrix-matched calibration curve was constructed with the responses of dust extract spiked with the seven different concentrations of each metabolite after extraction. The limit of detection was estimated based on the signal-to-noise ratio of three. Limit of detections for the majority of the metabolites in the study were less than 0.01 ng/ $\mu$ l, except for alternariol, citrinin, CTGA, deoxynivalenol (DON), fumonisin B1 (FUB1), and sterigmatocystin (STEG) (between 0.01 and 0.1 ng/ $\mu$ l); verrucarol, citreorosein (CITRO), nivalenol, and zearalenone (ZEA) (between 0.1 and 0.3 ng/ $\mu$ l); and alternariol monomethylether (AME) and skyrin (SKY) (between 0.3 and 0.5 ng/ $\mu$ l).

## 2.7. Statistical analysis

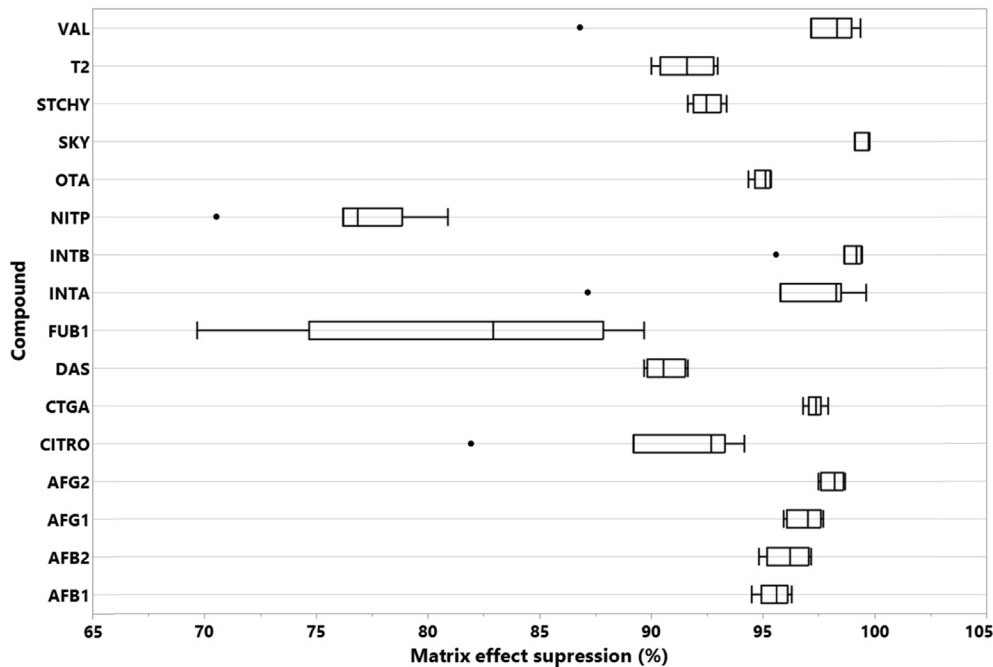
We considered any response with the signal-to-noise ratio less than 3 to be invalid; these were mostly from the lowest three concentrations (0.00625, 0.0125, and 0.025 ng/ $\mu$ l). For many MSMs at these lowest concentrations, the responses were extremely high or low, which did not follow the trend of the responses of the other higher four concentrations, although the signal-to-noise ratio was greater than 3. Therefore, we deleted the lowest three concentrations for statistical analysis to obtain consistency across the MSMs. This constraint left not enough data points for certain MSMs, and thus, some of the box plots were incomplete for those MSMs. However, we also conducted sensitivity analyses using data including all responses with the signal-to-noise ratio greater than 3 in the lowest three concentrations to confirm that our study findings and conclusions were the same. We used multifactor analysis of variance (ANOVA) to examine if there was any effect of metabolite, building, and spiked concentration on ME, EE, PE, and percent recovery or if there was any effect of metabolite, building, spiked concentration, and spiking type (before, after, or no spiking) on %CV of responses as main effect models [27]. We also evaluated interaction effects between metabolite and building. Multiple comparisons among levels within each factor were performed with the Tukey Honest Significance Differences procedure; least square means (LSMeans) were estimated for each level within the factor from ANOVA models adjusted for other factors. We considered  $p < 0.05$  as statistically significant. All analyses were performed using SAS 9.4 and JMP 13.0.0 (SAS Institute Inc., Cary, NC).

## 3. Results

### 3.1. Matrix effect

In our study, MEs appeared to be due to signal suppression because all percent MEs were positive. The results show that substantial suppression was present for all MSMs in the studied buildings, and our main effect ANOVA model indicated that the suppression differed by dust collected from different buildings ( $p < 0.0001$ ) and MSM ( $p < 0.0001$ ). Fig. 1 shows example results of calculated MEs for 16 representative MSMs (the complete set of results is shown in the supplemental material Figs. SM1, SM2, and





**Fig. 1.** Percent suppression due to matrix effects of selected sixteen MSMs for Building 1. MSMs, microbial secondary metabolites.

SM3 for buildings 1, 2, and 3, respectively). Most MSMs showed more than 90% suppression (range of LSMeans: 80.5–101.2%; median of LSMeans: 95.7%). However, 3-nitropropionic acid (NITP), FUB1, diacetoxyscirpenol (DAS), stachybotrylactam (STCHY), T-2 toxin (T2), CITRO, and CTGA were among the MSMs that showed the lowest MEs (range: 80.5–89.9%). The results also showed that for buildings 1 and 2, the median of percent suppression was generally higher than 90%, with the exception for NITP and FUB1. For Building 3, the median values of MEs for CTGA, DAS, CITRO, ochratoxin A, NITP, STCHY, T2, and ZEA, in addition to NITP and FUB1, were lower than 90%. Dust collected from Building 2 generally showed significantly ( $p < 0.05$  for multiple comparison in the main effect ANOVA model) larger MEs (LSMeans = 96.1%) over all MSMs compared with those from the buildings 1 and 3 (LSMeans = 95.2 and 90.9%, respectively). In addition, the effect of building on matrix suppression appeared to be somewhat different by the metabolite ( $p$ -value for interaction effect  $< 0.05$ ). The substantial MEs were also concentration dependent. The lower concentrations appeared to be more susceptible to MEs [LSMean of MEs for 0.9 ng/ $\mu$ l (93.3%) was significantly ( $p < 0.05$ ) lower than those for 0.15 and 0.075 ng/ $\mu$ l (94.6% and 94.3%, respectively) in multiple comparison], but the difference was only 1%.

### 3.2. Recoveries of MSMs

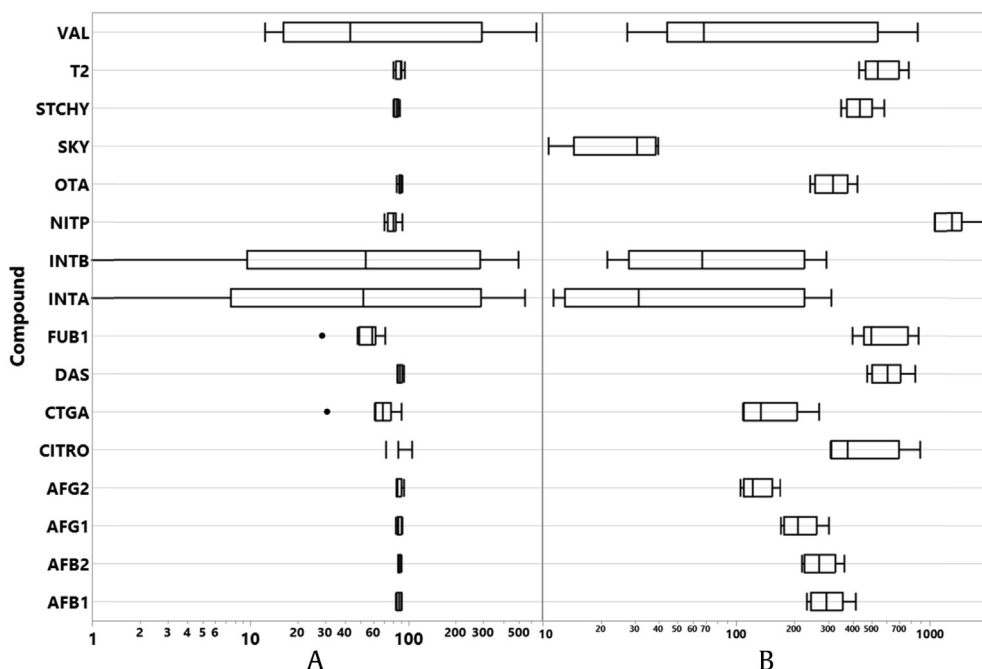
We examined two methods for adjusting MEs—application of a universal ISTD (DOM) for all MSMs and a matrix-matched standard curve (MMC). We calculated percent recovery for each metabolite with each of the two methods in dust samples from all three studied buildings. Fig. 2 shows examples of calculated recoveries of selected sixteen MSMs for Building 1 (the complete set of results is shown in the supplemental material Figs. SM4, SM5, and SM6 for buildings 1, 2, and 3, respectively). When we used DOM as an ISTD, the percent recovery tended to be substantially overestimated (range = 20.3–863.9%, average = 246.3%, and median = 185.5%). On the other hand, when we compensated for the MEs with an MMC curve, the percent recovery was much closer to 100%, with smaller

variation (range = 51.9–135.5%, average = 86.3%, and median = 80.4%) compared with those by DOM adjustment. LSMeans of percent recovery adjusted by the MMC curve for each building were similar (83.0–88.1%) to each other ( $p = 0.74$ ). Our main effect ANOVA models also showed that percent recoveries adjusted with an MMC were not statistically different by the metabolite ( $p = 0.55$ ). We observed no interaction effects between the metabolite and building on the MMC-adjusted percent recovery either. However, concentration was a significant factor on percent recovery in the matrix-matched calibration adjustment, whereby the highest concentration (0.9 ng/ $\mu$ l) showed 102.8% recovery that was significantly higher than that of 0.075 ng/ $\mu$ l (% recovery = 73.5%;  $p$  value  $< 0.05$  in multiple comparison).

### 3.3. Extraction and PEs

Calculated EEs of selected sixteen MSMs for Building 1 are presented as examples in Fig. 3 (the complete set of results is shown in the supplemental material Figs. SM7, SM8, and SM9 for buildings 1, 2, and 3, respectively). LSMeans of EE for individual MSMs including DOM ranged from 87.7 to 132.8%, except for integracin A (INTA) and integracin B (INTB), SKY, and valinomycin (VAL), which showed exaggerated EEs (268.9, 256.2, and 583.8, 176.1%, respectively) and also differed by metabolite in both ANOVA models with or without those four outlier MSMs. In an ANOVA model without those 4 MSMs, Building 3 had significantly ( $p < 0.05$ ) higher EE (LSMeans = 110.9%) than Building 1 (LSMeans = 104.9%) but no difference from that of Building 2 (110.6%). However, EE did not vary by concentration ( $p = 0.14$ ) in an all-MSM model, and no interaction effect between the metabolite and building on EEs was observed ( $p > 0.05$ ).

PE is a combination of ME and EE as shown in equation (3). Because we observed substantial MEs in the spiked sample analysis, PE was expected to be poor as well. Without any adjustment, PE ranged from 1.0 to 20.0% over the 31 MSMs and DOM. Most MSMs showed very low PEs, smaller than 10%, due to large MEs. Averages of PEs for each MSM presented with



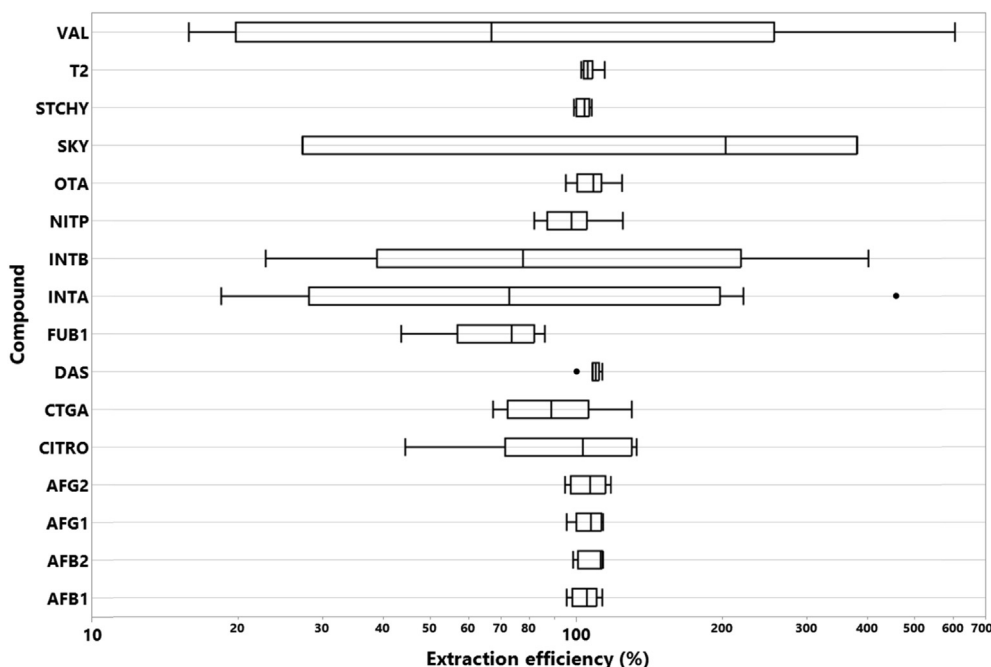
**Fig. 2.** Percent recoveries of selected sixteen MSMs for Building 1 after adjustment using matrix-matched calibration (denoted as A) and internal standard DOM (denoted as B). DOM, deoxy-deoxynivalenol; MSMs, microbial secondary metabolites.

confidence intervals of 2 x standard deviation are shown in the supplemental material [Fig. SM10](#).

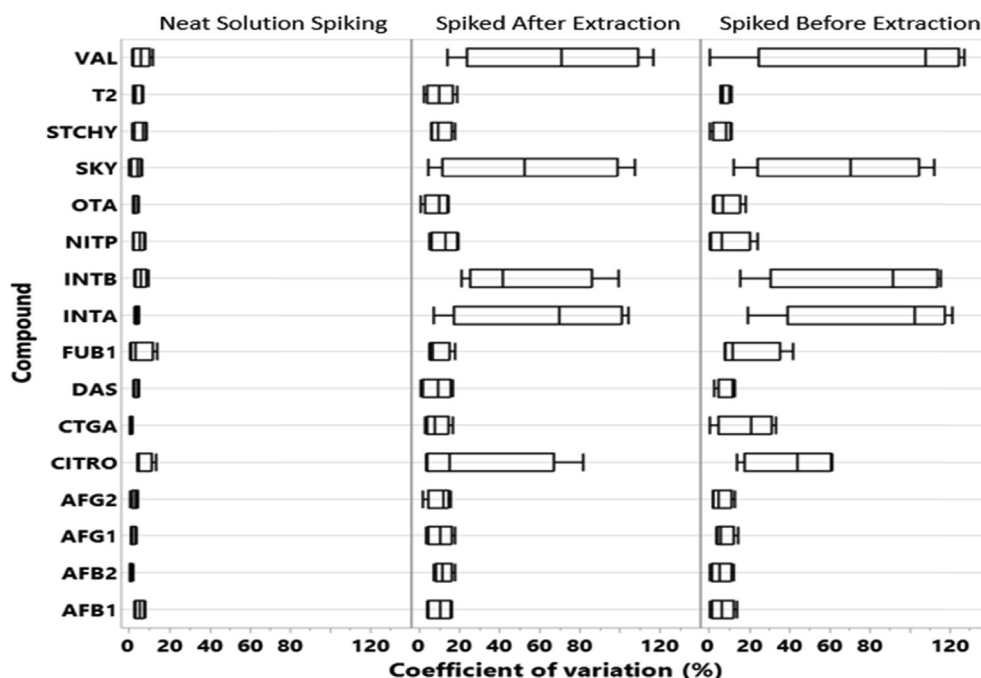
### 3.4. Percent coefficient of variation

The %CV of the responses differed by the metabolite ( $p < 0.01$ ), and the LSMeans of the %CV for metabolites from the main effect ANOVA model ranged from 5.6 (aflatoxin G2 (AFG2)) to 20.4% (CITRO) for most of the MSMs, except for INTB, INTA, SKY, and VAL for which the %CVs were 36.5, 39.2, 42.6, and 44.8%, respectively.

[Fig. 4](#) shows examples of the calculated %CV of the selected sixteen MSMs for Building 1 (the complete set of results is shown in the supplemental material [Figs. SM11, SM12, and SM13](#) for buildings 1, 2, and 3, respectively). The LSMean of the %CV of the responses for the MSMs in neat solution (%CV = 3.4) was significantly ( $p$ -values < 0.05) lower than those in spiked samples before (% CV = 18.8) and after (%CV = 16.1) extraction. In addition, the LSMean of the %CV of the duplicate responses of the spiked samples from Building 3 (10.9%) was significantly ( $p$ -value < 0.05) lower than those from buildings 1 and 2 that were not different from each



**Fig. 3.** Extraction efficiencies of selected sixteen MSMs for Building 1. MSMs, microbial secondary metabolites.



**Fig. 4.** Percent coefficient of variations of responses of duplicate injections for selected sixteen MSMs by the spiking type (spiking in neat solvent and spiked in samples before and after extraction) for Building 1. MSMs, microbial secondary metabolites.

other (%CV 14.5 versus 13.0). The %CV of spiked dust samples from different concentrations was not statistically different from one another. Our sensitivity analyses with ANOVA models including all seven concentrations also showed that results and conclusions presented here did not change, although adjusted LSMeans and variations of the measurements were slightly different.

## 4. Discussions

### 4.1. ME and adjustment

Interference in ionization can occur when samples for mass spectrometry analyses are composed of a complex matrix, such as food [28] or dust [29], and these complex matrices may contain compounds with interfering MS ionization that coelute with the target metabolite. An earlier study by Vishwanath et al [30] reported that building materials such as mortar and carton-gypsum board did not produce MEs but that settled house dust was a challenging matrix that produced large MEs. In our study, we also found substantial MEs (suppression) in the simultaneous quantification of 31 MSMs spiked in office building floor dust using UPLC-MSMS. The present study finding is consistent with our previous report of MEs in the analysis of verrucarol in floor dust using the gas chromatography-tandem MS method [31]. Our present study also showed that the ME was different by the building where dust was collected, the spiked concentration, and the specific MSM. Our findings indicate that the significant suppression would result in substantial underestimation in quantity of the MSMs in floor dust samples using UPLC-MSMS, unless an appropriate compensation method is applied. Therefore, evaluating MEs during method development and exploring appropriate adjustment methods would be critical to obtain accurate quantification of the MSMs in floor dust samples. All the MSMs in floor dust collected from buildings 1 and 2 were affected by substantial MEs (more than 90%), except for NITP. On the other hand, MSMs spiked in dust extract from Building 3 were generally less influenced by MEs than those in buildings 1 and 2. This finding indicates that each

distinctive dust matrix is likely to differently affect signal suppression and that every metabolite may also differently react to a certain dust matrix in MSMS ionization. Different locations and building materials might have contributed to dust components, which could have made such difference. Our study also indicates that dust samples, especially those containing a low level of MSMs, are likely to be determined as being below the limit of detection without proper adjustment.

To reduce MEs in quantitative bioanalysis in the pharmaceutical industry, improvement of sample preparation such as protein precipitation, solid-phase extraction, and liquid-liquid extraction has been suggested by Chambers et al [32]. Combining mobile phase alteration with solid-phase extraction was also shown to provide significant benefits in reducing the ME. Shou and Naidong [33] reported that a change in chromatographic conditions, such as extending the analysis time to allow better separation of the coeluting compounds, may decrease the ME. Another suggested strategy to compensate for the ME is applying different calibration methods such as external matrix-matched calibration, standard addition, or ISTD calibration [34,35]. In our study, the matrix-matched calibration method compensated reasonably well for extraction loss and MEs, and the recovery rates for all MSMs quantified were between 51.9 (CITRO) and 135.5% (VAL). The major benefit of using the matrix-matched calibration would be the construction of a calibration curve specifically adjusted for interference of the particular matrix components in the same sample [36]. Zrostlíková et al [37] evaluated two compensation methods and showed that matrix-matched calibration yielded comparable results to those found with the ISTD adjustment. However, adjustment of MEs using matrix-matched calibration may not always be a practical choice, especially if there is no blank matrix (free of analytes of interest) available. In our study, we used the same pooled dust to prepare multiple aliquots for the experiment, which made it possible to evaluate the matrix-matched calibration method. Preparation of a matrix-matched calibration curve for each sample significantly increases the number of samples analyzed and relevant cost. These limitations make the application of the matrix-

matched calibration method challenging in our field. On the other hand, a standard addition method (a calibration method using one or more additional sample aliquots spiked with known amounts of standard materials for each sample) was also suggested if samples are expected to contain a detectable amount of analytes from screening methods [34,35]. French et al [35] reported that accurate estimation of sample concentration can be achieved even by a single-point standard addition in the analysis of pesticide residue in food samples. While our method is useful for in-depth survey purposes, single-point standard addition method, suggested by French et al [35] that was not tested in our study, seems to be more practical for adjusting MEs in floor dust samples for routine screening, although it increases the sample size by twofolds.

The ISTD is a compound that does not exist in the matrix that is being studied and behaves similar to, or the same, as the target analyte during the analytical procedure and has been used to adjust for the loss of analyte during sample preparation as well as the interfered response in MS due to MEs [38]. The most suitable ISTD is an isotopically labeled analyte because it will behave exactly same as the analyte during the extraction and analysis, except for slightly different molecular mass [39]. Unfortunately, it is extremely difficult and expensive in many instances to develop such isotopes, and therefore, they are not easily available for the most MSMs. As an alternative, DOM has been used as a nonisotope-labeled universal ISTD (one of the DON derivatives) [40] in simultaneous analysis of multiple mycotoxins in previously published studies [32,33,37,41–43]. In those studies, authors did not evaluate DOM as an appropriate ISTD to adjust MEs. Our study showed that DOM as a universal ISTD for 31 MSMs did not appropriately adjust MEs for many of these MSMs, except for INTB, AME, roquefortine C, STEG, aflatoxin G<sub>2</sub>, VAL, asperglaucide, and emodin. These MSMs showed reasonable recovery rates between 50% and 150%. Indeed, 19 of the 31 MSMs were overadjusted (recovery rate >150%) with DOM (Fig. 2, SM2, and SM3). Our findings show that adjustment of MEs using DOM may work for a few MSMs but not as a universal ISTD for all MSMs in an analysis that simultaneously quantifies multiple MSMs in floor dust samples.

#### 4.2. Extraction efficiencies

We found that our extraction method performed reasonably well (EEs ranged from 87.7 to 132.8%) for most of the MSMs in all three building samples, except for INTA, INTB, VAL, and SKY. Those four MSMs showed exaggerated EEs (>133%) and appeared to be heavily influenced by high outliers due to poor repeatability between duplicate injections because most of their median EEs were still smaller than 200%. The purpose of the extraction in our simultaneous analysis of the multiple MSMs was to keep the extraction procedure as simple as possible, thus minimizing potential loss of certain MSMs during the extraction [44]. Sample preparation with the simple extraction method might have resulted in the presence of more interfering materials in the final extract; however, introduction of solid phase extraction or liquid–liquid extraction into sample preparation to produce cleaner extract as suggested by Chambers et al [32] may result in loss of some MSMs. In general, our extraction method appeared to be appropriate for the simultaneous analysis of the multiple MSMs using UPLC-MSMS.

#### 4.3. Repeatability of instrument

The CV represents the repeatability of the instrument's readings from duplicate injections of the same sample extract. Percent CVs of most of the MSMs were below 20%, indicating desirable repeatability of our instrument [26]. However, we did not obtain good repeatability for four MSMs INTA, INTB, SKY, and VAL in the dust

matrices. We also found that %CV of extract containing dust matrix showed lower repeatability by 12.7–15.4% compared with those in neat solvent and that %CV can also differ by dust collected from different buildings, indicating that the dust matrix can also influence repeatability of instrument. Lagerwerf et al [45] also reported similar findings that human plasma matrix substantially reduced repeatability of repeated injections of their ISTD (acycloguanosine) and discussed that the level of contamination in the ion source or the HPLC column condition may be associated with low repeatability. However, it is unclear why our instrument differentially affected repeatability of those four MSMs.

## 5. Conclusions

We found substantial MEs in the simultaneous analysis of 31 MSMs in floor dust samples collected from office or school administration buildings using UPLC-MSMS. There was strong suppression in MS ionization of the MSMs. This suppression led to substantial underestimation of the concentrations of the MSMs spiked in the dust samples. Proper quantification of these MSMs in floor dust samples cannot be achieved by external calibration alone because it does not compensate for the ME. The selection of an ISTD to compensate for the MEs that are not isotopically labeled is challenging, especially in simultaneous analysis of multiple MSMs, because there is no single ISTD that compensates for all MSMs as shown in the case of DOM in our study. Although the matrix-matched calibration method provided reasonable adjustment for the MEs, creating the matrix-matched calibration curve with blank matrix for every single dust sample is impractical. More research is needed to find more cost-effective and practical ways to adjust for the substantial dust MEs in the analyses of the MSMs using LC-MSMS. Considering our study findings of large dust MEs and limitations in application of the matrix-matched calibration method and in use of ISTDs, more research on a single-point standard addition method is warranted.

## Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

## Competing interests

The authors declare no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.shaw.2018.12.004>.



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