




# Household determinants of biocontaminant exposures in Canadian homes

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## Abstract

Exposure to biocontaminants, such as dust mites, animal dander, bacteria, and mold, is associated with a range of health effects. This study identified household characteristics associated with indoor biocontaminant loadings in four Canadian cities. Floor dust was collected in 290 Canadian homes in Edmonton, Halifax, Montreal, and Windsor. The dust samples were analyzed for house dust mite allergens (Der f 1 and Der p 1), cat allergen (Fel d 1), cockroach allergen (Bla g 1), beta-(1,3)-D-glucan, and endotoxin. Household information was obtained through questionnaires and home inspections. We performed univariate and multivariate analyses to identify household determinants of biocontaminant loadings and mold odor presence. We observed large regional variations for all biocontaminants, except for cockroach allergen. The ranges of the contaminants measured in loadings and concentrations were similar to that of previous Canadian studies. Household characteristics including presence of carpeting, low floor cleaning frequency, older home age, presence of pets, and indoor relative humidity above 45% were positively associated with the presence of multiple indoor biocontaminants. High floor cleaning frequency and use of dehumidifiers were negatively associated with the presence of multiple indoor biocontaminants. Mold odor was positively associated with older home age, past water damage, and visible mold growth.

## KEYWORDS

beta-(1,3)-D-glucan, cat allergen, dust mite allergen, endotoxin, house dust, mold

## 1 | INTRODUCTION

Biocontaminants are common in the built environment and include protein allergens from house dust mites, fungi, furry pets, rodents, and cockroaches, as well as endotoxin from gram-negative bacteria, and the inflammatory compound beta-(1,3)-D-glucan from the cell wall of fungi in damp or water-damaged buildings. Exposure to one or more of these biocontaminants has been identified as an environmental risk factor for the development and exacerbation of asthma and allergic diseases.<sup>1-3</sup>

Inhaled biocontaminants from indoor exposures occur as a function of the loading and composition of settled fine dust,<sup>4</sup> which is resuspended by human activities.<sup>5-7</sup> Because of this, biocontaminant loadings per square meter has been suggested to be a more reliable measure to reflect exposures than concentrations per gram of dust.<sup>8-10</sup> Dust mites and domestic cats are major sources of indoor allergens, contributing to the incidence of allergic respiratory disease.<sup>11,12</sup> In Canada, approximately 40% of the atopic population is allergic to one or the other of the two common house dust mite species, *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides*

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*farina* (Der f 1).<sup>13</sup> In countries with warm climates, sensitization to cockroach allergens is associated with asthma in children, notably in U.S. inner cities.<sup>14,15</sup> Inhalation exposures to beta-(1,3)-D-glucan (hereafter mold glucan) from damp building fungi and lipopolysaccharide (LPS) endotoxin are potentially inflammatory. In data from the 2018 Canadian housing survey, 5% of Canadian homes had patches of mold greater than one square meter.<sup>16</sup> Exposure to mold glucan is believed to contribute to the non-allergic respiratory impacts such as the promotion of innate and adaptive immune responses of individuals living or working in moldy buildings.<sup>17</sup> Exposure to endotoxin is a risk factor for lung disease. In the North American context, it increases the risk of sensitization to concurrent allergen exposure.<sup>18</sup> Although some studies in Europe have suggested that exposure to environmental endotoxin (e.g., commonly associated with rural living and farming) may protect against the development of atopy and asthma,<sup>19,20</sup> recent population studies in Europe have failed to confirm this hypothesis.<sup>21</sup> Other studies have reported a positive association between elevated endotoxin and mold glucan exposures and increased asthma severity and bronchial hyper-responsiveness.<sup>18,22</sup> Mold odor has been associated with fungal concentrations in homes and has been used as an indicator for hidden mold contamination.<sup>23–25</sup> The presence of mold odor has been consistently associated with the development and/or exacerbation of asthma and rhinitis among both children and adults.<sup>23,26,27</sup>

In order to support the development of residential indoor air quality guidelines, between 2005 and 2010, Health Canada and its collaborators conducted residential indoor air quality (IAQ) studies in four Canadian cities (Edmonton, Halifax, Montreal, and Windsor) to determine the sources and levels of a number of priority indoor air contaminants in homes. A unique feature of these studies we describe herein is that the identified biocontaminants were all collected using the same sampling protocol and analyzed at the same laboratory using well-characterized methods. This framework allowed the investigation of regional variations in biocontaminants in Canadian homes. The main objectives of this study were as follows: (i) to assess the loadings of two dust mite allergens, cat allergen, cockroach allergen, mold glucan, and endotoxin, (ii) to identify household characteristics that are associated with biocontaminant loadings and the occurrence of mold odor, and (iii) to inform evidence-based guidelines and advice to reduce exposures to biocontaminants.

## 2 | METHODS

### 2.1 | Study areas

The four cities are from different provinces across Canada, with diverse climates. Montreal is the principal metropolis of the province of Quebec and the second-most populous city in Canada. It has warm, humid summers, and cold winters with frequent snowfalls. Edmonton is the capital of the province of Alberta and is Canada's fifth-largest municipality. It has warm dry summers and cold dry winters. Halifax is the capital of the province of Nova Scotia and is the

regional economic center in Atlantic Canada. Windsor is the third-most populated city in Southwestern Ontario. Halifax is located on the shores of the Atlantic Ocean, and Windsor is in the Great Lakes region, both feature warm, humid summers, and relatively mild winters compared to much of Canada.

### 2.2 | Measurements

The IAQ studies were carried out from 2005 to 2010 in Windsor (2005, 2006),<sup>28</sup> Halifax (2009),<sup>29</sup> Montreal (2009, 2010),<sup>30</sup> and Edmonton (2010).<sup>31</sup> In Edmonton and Halifax, non-smoking homes were selected using stratified sampling according to home construction year (<1945, 1946–1960, 1961–1980, 1981–2000, and 2001–2008). In Montreal and Windsor, non-smoking homes with asthmatic children (7–12 years old in Montreal, and 6–14 years old in Windsor) were selected. The participating homes in the Montreal study included rental properties, but not in the other three studies. All research was approved by the Health Canada Research Ethics Board and informed consent was obtained from all participants.

All measurements were carried out by trained field technicians. A questionnaire was administered in-home to each participant to collect housing characteristics along with information on mold and dampness. Mold and dampness questions collected as part of this questionnaire have been described in detail elsewhere.<sup>32–34</sup> Indoor temperature and relative humidity (RH) were measured continuously for five to seven consecutive days using different instruments: YES-206LH monitors (Yes Environment Technologies Inc.) in Edmonton and Halifax; Smart Reader Plus 2 (ACR Systems Inc.) in Windsor; and Hobo sensors (Hobo U10, Onset Computer Corp., Hoskin Scientific Ltd.) in Montreal. Home air exchange rates were measured over a 24-h period using the perfluorocarbon tracer (PFT) technique.<sup>35</sup> The PFT emitters were placed in the four corners of the main floor, and a capillary absorption tube (CAT) detector was located in the center of the main floor.

The dust samples were collected from the living room floor. The sampling was mainly conducted in the first sampling season (winter), and each home was sampled once. A high-volume small surface sampler (HVS3, CS3 Inc.) was used in Windsor homes. Due to its heavy weight, it was replaced by a lightweight and easier to operate Omega high-efficiency particulate (HEPA) abatement vacuum cleaner, with a X-cell 100 dust sampling sock (Midwest Filtration Company) fitted to the hose to collect dust samples in Edmonton, Halifax, and Montreal homes. Although we used different vacuum devices, the impact on dust collection in the size fraction of less than 300  $\mu\text{m}$  was small as we followed the same vacuuming protocol.<sup>7</sup> To ensure adequate dust was available for collection, the participant was asked not to clean the floor in the living room for seven days before sampling. Two different vacuum brushes were used to vacuum the floor depending on whether it was carpeted or had a hard surface flooring. The brushes were washed with water between each sampling to avoid cross-contamination. Vacuuming was conducted for four consecutive minutes. For carpeted areas, the area sampled was

about 2 m<sup>2</sup> and for hard floor surfaces, the area sampled was about 4 m<sup>2</sup>. As a minimum of four grams was required per sample, if collected dust was insufficient after the 4 minute vacuuming, the procedure was repeated on a larger area until the minimum dust weight was collected, and the total area vacuumed was recorded. The pre-weighed filter sock with the dust sample was deposited in a sealed Ziploc bag and placed in a closed brown paper bag. The dust samples were stored in a freezer at -20°C until shipped to the laboratory in a cooler at the end of the sampling season.

The samples were sieved to preserve particles less than 300 µm in size and were weighed. The sieved dust was analyzed for dust mite allergens Der f 1 and Der p 1, cat allergen Fel d 1, cockroach allergen Bla g 1, mold glucan, and endotoxin. The allergen concentrations of Der p 1, Der f 1, Fel d 1, and Bla g 1 were determined using the enzyme-linked immunoadsorbent assay (ELISA) (Indoor Biotechnologies). Endotoxin concentrations were measured using the Pyrochrome Limulus Amebocyte Lysate (LAL) test (Associates of Cape Cod). Mold glucan was determined through the factor G in the LAL reagent.<sup>36</sup> The detection limits (DL) were 10 ng/g for Der p 1 and Der f 1, 4 ng/g for Fel d 1, 0.04 U/g for Bla g 1, 0.06 µg/g for mold glucan, and 6 EU/g for endotoxin. All analyses were performed by the same analysts at Paracel Laboratories, a laboratory accredited by the Canadian Association for Environmental Analytical Laboratories (CAEAL) based on an international standard (ISO/IEC 17025).

## 2.3 | Statistical analysis

Results are expressed as both loading (per square meter of floor sampled) and concentration (per gram of dust). Loadings were calculated by multiplying the concentration with the weight of collected dust and dividing it by the size of the sampling area. The biocontaminant data were best described by a lognormal distribution. Descriptive statistics summarized the biocontaminant loadings and concentrations, including the geometric mean (GM), geometric coefficient of variation (GCV), 95% confidence intervals (CI), and the interquartile range (IQR).

The biocontaminant data contained censored observations (i.e., concentrations below the detection limit). If censoring was less than 80%, we used a robust regression on order statistics (ROS) function in the NADA package in R to estimate non-detected values.<sup>37</sup> The ROS function computes a linear regression using the Weibull-type plotting positions and the normal quantiles of the log-transformed uncensored data. The censored data were then estimated using this model, as a function of their normal quantiles. The estimated censored data (if <DL) and uncensored data (if ≥DL) were used for analysis. We did not conduct analysis if 80% or more of the data were censored.

To identify determinants of biocontaminant loadings in participants' homes, we considered the following factors for analysis: general housing characteristics (dwelling type, size, construction year, main and back-up heating systems, ventilation systems, room or

central air conditioning, presence of storm windows, window frame material, and floor material); occupants' demographics (family size, total annual household income, and homeowners' highest education level); information on mold and dampness (presence of visual mold, mold odor, current or past (12 months) water damage); and other direct or indirect sources/factors (floor cleaning frequency, type of vacuum cleaners, presence of humidifiers or dehumidifiers, interior wood storage, presence of pets or pests).

All biocontaminants were analyzed as continuous variables, except for the presence or absence of mold odor. Mean indoor RH was dichotomized at 45%, as it has been reported that dust mites require a RH higher than 45 to 50% to survive.<sup>12,38,39</sup>

We carried out city-specific analyses for each biocontaminant. All biocontaminant loadings were log-transformed before analysis. Although our focus was on the biocontaminant loadings, which has been suggested to be a more accurate expression to reflect exposure<sup>8-10</sup>, we also conducted analyses on concentrations in order to compare our results to reported values in the literature and to notional thresholds of sensitization.

For each city, we used linear regression to identify variables associated with the log-transformed biocontaminant loadings. Variables that were significantly ( $p < 0.05$ ) associated with at least one measure of biocontaminants in at least one city were included in multivariate analysis. As the independent variables were dichotomous, multicollinearity was checked using Pearson's chi-square test and Fisher's exact test (where the chi-square test was unsuitable). In the presence of multicollinearity, only one variable with a stronger correlation with biocontaminant loadings was kept in the multiple regression model. Some variables were removed if they were no longer associated with biocontaminant loadings in any of the cities, after adjustment for other variables in the model. Predictor parameter estimates and 95% CI were exponentiated to provide estimates of percent change in exposure associated with the positive category of the dichotomous variable while holding other variables constant.

For the presence of mold odor, we used logistic regression to determine the effect of individual housing characteristics, and multiple logistic regression to determine independent associations after adjustment for other variables in the model. Similar variable selection methods to those described above were used. The odds ratios (OR) and 95% CI were converted to probabilities ( $100 \times \text{OR}/(1 + \text{OR})$ ) of finding mold odor associated with certain household characteristics. All the analyses were conducted using SAS Enterprise Guide 7.1 (SAS Institute Inc.) and R 4.0.3 (R Core Team, 2020).

## 3 | RESULTS

### 3.1 | Household characteristics

Two hundred and ninety dust samples were collected from the participating homes in Edmonton (25%), Halifax (20%), Montreal (23%), and Windsor (32%). Table 1 shows selected characteristics of the homes. (A full list of characteristics is shown in the supplemental

information (SI)(1) Table S1). The majority of the homes were detached houses (78%), built before 1990 (63%), and with no more than four residents (72%). A large percentage of the homes (79%) had carpets and about half of the homes had pets. One-fifth of the homes had experienced water damage (e.g., from broken pipes, leaks, or flood) in the 12 months prior to the study, and a small number of the homes (3%) had current wet or damp spots on surfaces (e.g., walls, ceilings, carpets, or basement floors). Visible mold was observed in 31% of the homes.

### 3.2 | Biocontaminant loadings, concentrations, and determinants

The geometric mean of the floor dust (<300  $\mu\text{m}$ ) loading was 0.127  $\text{g}/\text{m}^2$  (95% CI: 0.105–0.154  $\text{g}/\text{m}^2$ ) across all cities. Table 2 shows the range and variation of biocontaminant loadings and concentrations. In Halifax, Montreal, and Windsor, 10–62% of the homes had Der f 1 and Der p 1 concentrations below the DL. The concentrations of Fel d 1, mold glucan, and endotoxin were above the DL for almost all homes. We did not report the concentrations for cities in which more than 80% of data were below the DL. These included Bla g 1 (all cities), Der f 1 (Edmonton), and Der p 1 (Edmonton). Between-city variations in biocontaminant loadings were 1.1 to 4.5 times higher than variations in concentrations. The loadings of Der p 1 in Windsor homes and Fel d 1 in Edmonton homes had much larger within-city variations than biocontaminant loadings in other cities. Table 3 shows the results of the multivariable model for biocontaminant loadings by city. Table 4 shows the results of the multivariable model for the presence of mold odor by city. The results of biocontaminant loadings and presence of mold odor univariate analyses are presented in SI(1) Table S2 and S3, respectively. The results of univariate and multivariate analyses of biocontaminant concentrations are presented in SI(1) Table S4 and S5, respectively.

Der f 1 was more prevalent than Der p 1 in all homes sampled. Der f 1 loadings were highest in Windsor homes (GM 583  $\text{ng}/\text{m}^2$ ), and Der p 1 loadings were highest in Halifax homes (GM 16  $\text{ng}/\text{m}^2$ ). Although thresholds of exposure, such as 2  $\mu\text{g}/\text{g}$  total dust mite allergens (Der f 1 + Der p 1) have been proposed,<sup>40</sup> the current view is that exposure should be minimized as much as possible.<sup>12</sup> Nonetheless using the old standards, 24% of the homes in this study exceeded the putative sensitization threshold (>2  $\mu\text{g}/\text{g}$ ) and 14% of the homes exceeded the asthma symptom threshold (>10  $\mu\text{g}/\text{g}$ ) for dust mite. Indoor average RH above 45% was associated with higher allergen loadings (760–2649% increase) for both Der f 1 (Halifax and Windsor homes) and Der p 1 (Halifax homes).

The highest and lowest Fel d 1 loadings were shown in Windsor (GM 4091  $\text{ng}/\text{m}^2$ ) and Montreal (GM 24  $\text{ng}/\text{m}^2$ ) homes, respectively. The allergen concentrations in homes with cats were significantly higher than in homes without cats. About 49% of the homes (2% with cats and 23% without cats) had concentrations that exceed the proposed threshold (>1  $\mu\text{g}/\text{g}$ ) for sensitization to Fel d 1, and 24% of the homes (16% with cats and 8% without cats) had concentrations

that exceeded the proposed threshold (>8  $\mu\text{g}/\text{g}$ ) for asthma symptoms in sensitized asthmatics. The prevalence of Fel d 1 is important considering that only about 20% of the households had cats.

Unsurprisingly, the presence of cats was the strongest determinant for higher Fel d 1 loadings (2714%–11 106% increase) across all four cities. The presence of carpet was the next contributing factor for higher Fel d 1 loadings (336%–897% increase). Although having a university or higher degree was not correlated with cat ownership, it was associated with higher Fel d 1 loadings (376% increase) in Halifax homes.

Mold glucan loadings were highest in Windsor homes (GM 2273  $\mu\text{g}/\text{m}^2$ ), and lowest in Montreal homes (GM 28  $\mu\text{g}/\text{m}^2$ ). Having carpeted flooring was a strong determinate for mold glucan loadings (109%–846% increase) for all cities. Other factors associated with elevated mold glucan loadings include indoor average RH above 45%, presence of storm windows, and if the home was built before 1990.

Endotoxin loadings were highest in Edmonton homes (GM 12 191 EU/ $\text{m}^2$ ), and lowest in Montreal homes (GM 5603 EU/ $\text{m}^2$ ). Similar to mold glucan, the presence of carpet was the strongest factor for higher endotoxin loadings (88%–523% increase) for all cities. Other factors positively associated with endotoxin loadings included low floor cleaning frequency (Halifax homes), and the presence of dogs or pet rodents (Windsor homes). The presence of dehumidifiers was negatively associated with endotoxin loadings in Montreal homes.

Mold odor was present in about one-third of the participating homes (16% with visible mold). Halifax had the largest percentage of homes (57%) reporting mold odor, and Montreal had the least percentage of homes (16%). Basement (69%), other locations (e.g., laundry, attic, crawl space) (19%), and bathrooms (9%) were the top three responses for the location of mold odor. Homes built before 1990 had an 84%–89% possibility of having mold odor in most cities. Having water damage events in the past 12 months and the presence of visible mold were strongly associated with the likelihood (80% and 90%) of having mold odor in Windsor homes.

## 4 | DISCUSSION

A major challenge in comparing results from different studies of various allergens, mold glucan, and endotoxin is the use of different sampling methods and considerable interlaboratory variation (e.g., extraction protocols, assay methods, analytical reagents, and standards used). Before the introduction of recognized standards circa 2012, analytical methods for the allergens from the two common house dust mite species (Der f 1 and Der p 1), domestic cat (Fel d 1), and German cockroach (Bla g 1) were not reliably standardized.<sup>41</sup> The interlaboratory variation of endotoxin is a major challenge in quantitative comparisons of results in both indoor and occupational environments.<sup>42,43</sup> Measurements of fungal glucan are also method dependent.<sup>44</sup> As noted, the form of mold glucan found in fungi that grow on damp building materials is known to be health relevant.<sup>45</sup> Some assay methods (e.g., GlucateLL method)

TABLE 1 Distribution of selected characteristics of the homes

Household characteristics		City				Total N
		Edmonton N (%)	Halifax N (%)	Montreal N (%)	Windsor N (%)	
Total number of homes		72 (25%)	58 (20%)	68 (23%)	92 (32%)	290
Dwelling type	Detached house	67 (30%)	53 (23%)	21 (9%)	85 (38%)	226
	Townhouse, duplex or triplex	5 (17%)	0 (0%)	18 (62%)	6 (21%)	29
	Apartment	0 (0%)	0 (0%)	23 (100%)	0 (0%)	23
	Other	0 (0%)	3 (30%)	6 (60%)	1 (10%)	10
Construction year	Before 1949	14 (27%)	17 (33%)	4 (8%)	16 (31%)	51
	1949–1990	30 (23%)	23 (17%)	33 (25%)	47 (35%)	133
	After 1990	28 (30%)	18 (19%)	20 (22%)	27 (29%)	93
	Unknown	0 (0%)	0 (0%)	11 (85%)	2 (15%)	13
Home size (ft <sup>2</sup> )	<1000	1 (5%)	1 (5%)	10 (53%)	7 (37%)	19
	1000–2000	13 (16%)	22 (27%)	14 (17%)	33 (40%)	82
	2000–3000	34 (36%)	23 (24%)	3 (3%)	35 (37%)	95
	≥3000	20 (39%)	11 (22%)	6 (12%)	14 (27%)	51
	Unknown	4 (9%)	1 (2%)	35 (81%)	3 (7%)	43
Total annual household income (CAD) <sup>a</sup>	<35 000	1 (17%)	5 (83%)			6
	35 000–80 000	18 (50%)	18 (50%)			36
	≥80 000	47 (60%)	31 (40%)			78
	Prefer not to say	5 (63%)	3 (38%)			8
	Unknown	1 (50%)	1 (50%)			2
Homeowner's highest education level <sup>a</sup>	Elementary or high school	4 (50%)	4 (50%)			8
	Technical college	19 (59%)	13 (41%)			32
	University or higher	46 (53%)	40 (47%)			86
	Other	3 (75%)	1 (25%)			4
Number of residents	1–2	32 (48%)	25 (38%)	7 (11%)	2 (3%)	66
	3–4	34 (24%)	28 (20%)	38 (27%)	43 (30%)	143
	5 or more	8 (10%)	5 (6%)	23 (28%)	47 (57%)	83
Presence of carpet	No	3 (5%)	16 (26%)	37 (61%)	5 (8%)	61
	Yes	69 (30%)	42 (18%)	31 (14%)	87 (38%)	229
Presence of pets	No	29 (20%)	24 (17%)	32 (22%)	60 (41%)	145
	Yes	43 (30%)	34 (23%)	36 (25%)	32 (22%)	145
Presence of water damage events in the past 12 months	No	55 (24%)	49 (21%)	56 (24%)	69 (30%)	229
	Yes	17 (28%)	9 (15%)	12 (20%)	23 (38%)	61
Currently wet or damp	No	69 (25%)	58 (21%)	67 (24%)	87 (31%)	281
	Yes	3 (33%)	0 (0%)	1 (11%)	5 (56%)	9
Presence of visible mold	No	55 (27%)	34 (17%)	45 (22%)	67 (33%)	201
	Yes	17 (19%)	24 (27%)	23 (26%)	25 (28%)	89
Presence of mold odor	No	45 (23%)	25 (13%)	57 (29%)	72 (36%)	199
	Yes	27 (30%)	33 (36%)	11 (12%)	20 (22%)	91

<sup>a</sup> The information on total annual household income and homeowner's highest education level was not collected in Montreal and Windsor homes.

over-estimate exposure because of an exaggerated response to glucans that are not of fungal origins, such as plant-derived glucans that are present in house dust due to the presence of pollen.<sup>36</sup> As there is no commonly accepted protocol for the dust sampling method,

sieving, extraction, and assaying, comparisons of the results using different methodologies and laboratories are fraught with error. Therefore, we restrict the discussion comparing our results to only Canadian studies that used similar analytical methods.

**TABLE 2** Geometric mean (GM), geometric coefficient of variation (GCV), 95% confidence intervals (CI) of GM, and interquartile range (IQR) of indoor biocontaminants, expressed as loading and concentration

Biocontaminant	City	N	% BDL <sup>a</sup>	Loading			Concentration		
				GM (GCV), 95%CI of GM	IQR	IQR	GM (GCV), 95%CI of GM	IQR	IQR
Der f 1 (loading: ng/m <sup>2</sup> , concentration: ng/g)	Halifax	58	0	127 (13), 71–230	38–876	2068 (6), 1245–3437	581–12 682		
	Montreal	67	25	3.5 (15), 2.0–6.1	0.587–19	73 (7), 45–119	16–314		
	Windsor	92	5	583 (28), 341–997	118–3998	738 (22), 442–1234	158–2755		
Der p 1 (loading: ng/m <sup>2</sup> , concentration: ng/g)	Halifax	58	10	16 (59), 7.3–33	2.2–227	253 (16), 136–472	60–1088		
	Montreal	66	52	0.361 (3.5), 0.243–0.535	0.131–1.4	7.8 (3.2), 5.3–11.4	2.6–19		
	Windsor	91	62	3.5 (3988), 1.5–8.1	3.2–49	4.5 (2046), 2.0–10.1	0.288–75		
Fel d 1 (loading: ng/m <sup>2</sup> , concentration: ng/g)	Edmonton	72	0	74 (142), 35–155	8–275	1244 (46), 649–2384	160–5546		
	Halifax	58	0	122 (26), 62–238	15–675	1983 (9), 1139–3450	482–10 161		
	Montreal	67	0	24 (22), 12–41	2.9–76	477 (17), 267–852	77–1385		
	Windsor	92	2	4091 (54), 2179–7345	532–33 290	5179 (32), 2998–8944	624–64 736		
Mold glucan (loading: µg/m <sup>2</sup> , concentration: µg/g)	Edmonton	72	0	85 (3), 60–120	37–247	1427 (1), 1143–1781	764–2485		
	Halifax	58	0	142 (3), 97–207	47–381	2315 (1), 1844–2908	1310–3430		
	Montreal	68	0	28 (3), 12–39	13–82	620 (1), 491–784	347–1205		
	Windsor	91	0	2273 (2), 1736–2977	1019–5479	2825 (1), 2409–3313	1565–4273		
Endotoxin (loading: EU/m <sup>2</sup> , concentration: EU/g)	Edmonton	72	0	12 191 (4), 8333–17 833	4607–34 548	204 984 (1), 162 285–258 917	115 500– 337 000		
	Halifax	58	0	8372 (2), 6058–11 570	3685–20 523	136 395 (1), 116 230–160 058	92 500– 208 000		
	Montreal	68	0	5603 (3), 3965–7916	2074–13 505	125 723 (1), 103 436–152 814	62 100– 197 000		
Windsor	92	0	6992 (2), 5394–9063	3301–15 540	8851 (1), 7626–10 273	6485–12453			

<sup>a</sup> BDL: below the detection limit.



**TABLE 3** Associations between household characteristics and biocontaminant loadings as determined by multiple linear regression analysis

Biocontaminant	Household characteristic	Percent change (%) (95% CI) by city			
		Edmonton <sup>a</sup>	Halifax	Montreal	Windsor
Der f 1	High bedroom floor cleaning frequency ( $\geq 4$ times/month)		-56 (-94, 243)	<b>-90 (-98, -47)</b>	
	Indoor average RH above 45%		<b>1930 (146, 16 638)</b>	-56 (-94, 240)	<b>760 (43, 5063)</b>
	Presence of dehumidifiers		-36 (-79, 92)	-33 (-92, 504)	<b>-76 (-93, -22)</b>
	Home built before 1990		<b>391 (51, 1495)</b>	78 (-64, 772)	76 (-43, 448)
	Natural gas heating		<b>-92 (-99, -52)</b>	-96 (-100, 271)	19 (-78, 563)
	Presence of pet rodents		139 (-96, 13 187)	16 (-84, 742)	<b>2695 (198, 26 140)</b>
Der p 1	Indoor average RH above 45%		<b>2649 (86, 40 623)</b>	22 (-67, 354)	694 (-52, 12 908)
	Presence of mold odor in the past 12 months		<b>790 (125, 3418)</b>	49 (-63, 496)	340 (-42, 3212)
	More than 4 residents		-18 (-91, 672)	135 (-15, 550)	<b>562 (27, 3355)</b>
Fel d 1	Presence of pet cats	<b>11 106 (3369, 36 104)</b>	<b>3595 (722, 16 520)</b>	<b>2714 (610, 11 049)</b>	<b>11 048 (3545, 33 998)</b>
	Presence of carpet in the living room	<b>336 (53, 1145)</b>	<b>897 (153, 3824)</b>	17 (-60, 246)	125 (-9, 456)
	Home owner's highest education level-University or higher	0 (-67, 198)	<b>376 (55, 1358)</b>		
Mold glucan	Presence of carpet in the living room	<b>345 (112, 831)</b>	<b>846 (375, 1784)</b>	43 (-28, 184)	<b>109 (22, 259)</b>
	Presence of carpet at home	46 (-71, 638)	3 (-41, 81)	<b>142 (19, 392)</b>	31 (-59, 316)
	Indoor average RH above 45%		<b>365 (72, 1154)</b>	-8 (-66, 153)	-1 (-60, 142)
	Presence of storm windows	161 (-9, 647)	63 (-9, 190)	<b>123 (7, 365)</b>	43 (-35, 214)
	Home built before 1990	13 (-51, 160)	<b>111 (15, 287)</b>	50 (-25, 203)	60 (-10, 183)
Endotoxin	Presence of carpet in the living room	<b>341 (111, 820)</b>	<b>523 (211, 1147)</b>	<b>105 (4, 305)</b>	<b>88 (14, 210)</b>
	Low living room floor cleaning frequency ( $\leq 4$ times/month)	-50 (-78, 11)	<b>107 (18, 263)</b>	54 (-35, 264)	
	Presence of dehumidifiers		61 (-7, 178)	<b>-78 (-93, -29)</b>	-13 (-51, 56)
	Presence of pet dogs	21 (-40, 147)	26 (-27, 116)	3 (-50, 112)	<b>207 (14, 724)</b>
	Presence of pet rodents	27 (-80, 698)	84 (-76, 1321)	-22 (-71, 109)	<b>251 (19, 936)</b>

Note: Results are expressed by percent change (95% CI) in exposure associated with the positive category of the dichotomous variable. Statistically significant results ( $p < 0.05$ ) are highlighted in bold. Only factors significantly associated with biocontaminant loadings in at least one city are included. Blank spaces indicate no data were available for analysis.

<sup>a</sup> The associations between household characteristics and dust mite allergen (Der f 1 and Der p 1) loadings in Edmonton homes were not analyzed due to the large degree of censoring.

In the present study, large regional variations were observed for all biocontaminants, although loadings and concentrations were generally within the range of values reported from other regions in Canada,<sup>10,46-52</sup> as presented in SI(2).

Der f 1 allergen was much more prevalent than Der p 1 in these Canadian homes (Table 2), as was the case in other Canadian studies.<sup>10,47-49,52</sup> The low concentrations of both dust mite allergens in Edmonton homes may be due to the fairly dry climate.<sup>12</sup>

Cockroach allergen was not detected, consistent with only one home having reported the presence of cockroaches in the 12 months prior to the study. Similar results were reported by a study in Toronto homes.<sup>52</sup> In the USA, cockroach infestations are associated with high population areas and low socioeconomic status.<sup>53</sup> Most of the homes in the present study were owned single-family dwellings.

Mold odor is a reliable sign of water damage and fungal growth, as water-damaged materials can become contaminated with

**TABLE 4** Associations between household characteristics and the presence of mold odor as determined by multiple logistic regression analysis

Biocontaminant	Household characteristic	Probability (%) (95% CI) by city			
		Edmonton	Halifax	Montreal	Windsor
Mold odor	Home built before 1990	<b>84 (60, 95)</b>	<b>84 (59, 95)</b>	83 (35, 98)	<b>89 (57, 98)</b>
	Presence of visible mold in the past 12 months	74 (45, 91)	65 (33, 87)	76 (41, 94)	<b>90 (73, 97)</b>
	Presence of water damage events in the past 12 months	70 (40, 89)	31 (8, 70)	25 (3, 77)	<b>80 (52, 93)</b>

Note: Results are expressed by probability (95% CI) of the mold odor presence associated with certain household characteristics. Statistically significant results ( $p < 0.05$ ) are highlighted in bold. Only factors significantly associated with mold odor in at least one city are included.

microorganisms, such as mold, if not remediated immediately.<sup>54</sup> The prevalence of molds was similar to that reported (32.4%) by Dales et al.<sup>55</sup> for the 14 948 home in 30 communities across Canada. In this study, visible mold was detected in half of the homes which reported mold odor. It indicated that there were many cases where mold growth occurs in places not easily visible, such as behind cabinets, inside walls, under carpets or carpet padding, as well as under-floor framing.

Many household factors were found to be associated with biocontaminant loadings in floor dust, and some were associated with multiple biocontaminants. The presence of carpet was a strong determinant for cat allergen, mold glucan, and endotoxin loadings. For dust mite, carpet flooring was significant in univariate analysis but not in multivariate analysis. Carpet flooring provides reservoirs for dust, allergens, outdoor mold, and yeast accumulation.<sup>12,24,56,57</sup> Removal of carpets has been recommended as an allergy remediation and was shown to be effective to control asthma symptoms.<sup>58</sup> The positive effect of floor cleaning on reduced endotoxin loadings was in line with the findings of Wickens et al.<sup>59</sup> Increased floor cleaning frequency decreased mite allergen levels, but this was only significant for Der f 1 in Montreal homes.

Pet ownership (cat, dogs, and rodents) was strongly associated with cat and dust mite allergens, as well as endotoxin loadings. As expected, the presence of cats in the home was the strongest determinant for Fel d 1 loadings for all cities, which was in line with the findings of the Toronto study.<sup>52</sup> Presence of dogs or pet rodents (hamsters, guinea pigs, or gerbils) was associated with elevated endotoxin loadings in Windsor homes. This may be because dogs or cats tend to have gram-negative bacteria in the gut and on the skin and they may transport bacteria and endotoxin from outdoors to indoors. Furry pets were identified as important sources for airborne endotoxins in Regina<sup>60</sup> and Edmonton homes.<sup>61</sup>

Elevated indoor RH and the use of dehumidifiers were associated with dust mite allergens and mold glucan loadings. Most homes in this study had an average RH below 50%, only three homes had RH between 50% and 55%. Indoor average RH greater than 45% was positively associated with mite allergen loadings in Halifax and Windsor. The Der f 1 loadings were negatively associated with the presence of dehumidifiers in the humid summer climate of Windsor.

The benefit of using high-efficiency dehumidifiers and air conditioning to reduce mite populations has also been reported by other studies.<sup>12,62</sup>

Older houses (built before 1990) were associated with elevated loadings of dust mite allergens and the presence of mold odor. Older properties are more likely to have dampness issues due to possible cracks in walls or foundations, roof damages, inadequate ventilation, and degradation. Ginestet et al.<sup>63</sup> conducted a systematic review on mold exposure in French homes and found that the proportion of damp dwellings increased with the age of the building. Data from the US National Health and Nutrition Examination Surveys showed that mold odor was three to five times more likely to occur in houses that were built before 1990.<sup>64</sup> Chan-Yeung et al.<sup>47</sup> also reported significantly higher concentrations of dust mite allergens in older homes (>20 years) in Vancouver, but not in Winnipeg.

This study has some limitations. The homes were not randomly selected, with inclusion criteria such as non-smoking homes, homes with asthmatic children (Montreal and Windsor), and homes that were owned (Edmonton, Halifax, and Windsor). The results are therefore not generalizable to all Canadian homes. Because of the cross-sectional nature of the study, we could not assess temporal changes in indoor exposures to biocontaminants and possible seasonal effects on the association between biocontaminant loadings and household characteristics. We only sampled living room floor dust and might have missed other important allergen reservoirs such as bedrooms, beds, and sofas. Indoor environmental data (e.g., temperature, RH, and air exchange rate) measured for only five to seven days may not be good indicators for long-term indoor climate conditions. By excluding homes with smokers, an important source of endotoxin was not captured.

One of the main strengths of our study is the design. We used the same survey questions across all homes, and all samples were analyzed by the same analysts using the same method in a CAEAL accredited Laboratory. We made use of robust regression on order statistics to handle measurements under the limit of detection. Our analysis included settled dust endotoxin and mold glucan, which have not been well-characterized in Canadian homes.



## 5 | CONCLUSION

This study measured a range of indoor biocontaminants in the floor dust of 290 homes in four Canadian cities and identified factors that are important determinants of indoor biocontaminants in Canadian homes. Although large regional variations were observed for all biocontaminants, the ranges of the biocontaminants measured in loadings and concentrations were largely similar to that of previous Canadian studies. The presence of carpet was an important determinant for cat allergen, mold glucan, and endotoxin loadings. Pet ownership (cat, dogs, and rodents) was associated with cat allergen, dust mite allergens, and endotoxin loadings. Indoor RH above 45% contributed to higher dust mite allergens and mold glucan loadings. The use of dehumidifiers reduced dust mite allergens and endotoxin loadings. Older homes (built before 1990) were associated with elevated loadings of dust mite allergens and the presence of mold odor. Among these factors, we have identified practical actions to reduce exposure, including controlling indoor humidity (e.g., operating air conditioners or dehumidifiers during humid weather), frequent vacuuming (i.e., weekly) if it is not possible to remove carpets, as well as preventing and properly mitigating water damage to prevent subsequent biocontaminant exposures.

### ACKNOWLEDGEMENTS

The authors would like to thank Health Canada staff (Mélisha St-Jean, Morgan MacNeill, Jill Kearney, Ling Liu, Ryan Kulka, Hongyu You, and Tae Maen Shin) and their collaborators at Université de Montréal (Audrey Smargiassi), McGill University (Marie-France Valois), Dalhousie University (Judy R. Guernsey and Mark D. Gibson), University of Windsor (Xiaohong Xu), and the University of Alberta (Warren Kindzierski and Md. Aynul Bari) who contributed to the design, management, and implementation of the Edmonton, Halifax, Montreal, and Windsor Indoor Air Quality studies. We are grateful to the participants that generously provided their time and access to their home. This study was funded by Health Canada.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### AUTHOR CONTRIBUTIONS

All authors reviewed and contributed to the manuscript. Amanda J. Wheeler, Marie-Eve Héroux, and Mark S. Goldberg designed and supervised the four indoor air quality studies. J. David Miller contributed to the dust sampling method, and mold and dampness questionnaire development. Gary Mallach contributed to the study coordination. Liu Sun, Mark S. Goldberg, Gary Mallach, and Keith Van Ryswyk contributed to the statistical analysis. Liu Sun and J. David Miller prepared the original draft. All authors approved the final version of the manuscript.

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#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Sun L, Miller JD, Van Ryswyk K, et al. Household determinants of biocontaminant exposures in Canadian homes. *Indoor Air*. 2022;32:e12933. <https://doi.org/10.1111/ina.12933>