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β(1→3)-GLUCAN EXPOSURE LEVELS AMONG WORKERS IN FOUR BRITISH COLUMBIA SAWMILLS

Lisa A. Ronald[2], Hugh W. Davies[1], Karen H. Bartlett[1], Susan M. Kennedy[1,2], Kay Teschke[1,2], Jacques Spithoven[3], Martine Dennekamp[1], Paul A. Demers[1,2]

[1]School of Occupational and Environmental Hygiene, University of British Columbia, Vancouver, BC, Canada
[2]Department of Health Care and Epidemiology, University of British Columbia, Vancouver, BC, Canada
[3]Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

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Abstract: β(1→3)-glucans were extracted from wood dust samples taken during the summer of 1997 at four British Columbia sawmills. Personal dust samples were collected using a GSP-sampler for inhalable dust and the sampling strategy targeted all production and maintenance jobs at least once at each mill. Potential exposure determinants data were documented concurrently, including weather conditions, log storage methods, wood conditions, species, production level, jobs and tasks. β(1→3)-glucans were measured by enzyme inhibition immunoassay (EIA). A total of 223 personal β(1→3)-glucan samples were analyzed. 45.7% were below the limit of detection (LOD). Geometric mean concentration ranged from 3.5 to 18.9 μg/m^3 across the four mills. The highest levels were measured at the Interior mills, particularly in the log processing and sawmill areas. Multivariate regression models indicated that land-based log storage, clean-up jobs, high wood dust concentration, lumber yard department and the interaction between land-based log storage method and log processing department were associated with increased β(1→3)-glucan concentration.

Address for correspondence: Dr. Paul A. Demers, PhD, School of Occupational and Environmental Hygiene, University of British Columbia, 2206 East Mall, Vancouver, BC, V6T 1Z3, Canada. E-mail: pdemers@interchange.ubc.ca

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INTRODUCTION

Employment in woodworking occupations has been associated with a range of non-malignant respiratory diseases, including bronchitis, occupational asthma, extrinsic allergic alveolitis (also referred to as hypersensitivity pneumonitis) and chronic airflow obstruction [8, 17, 18, 22]. While most respiratory studies of woodworkers have focussed on the effects of wood dust exposure, inhalation of moulds has been implicated as a possible causal factor of some of these observed non-malignant respiratory diseases [8]. Mould exposure has been linked previously to cases of chronic and acute non-malignant respiratory diseases among workers in sawmills [2, 20, 27] , wood chip and debarking operations [30, 35], plywood factories [4], logging [14] and landscaping [3].

Conditions favourable for mould growth are commonly found in wood processing operations, with wood providing an excellent substrate for mould growth [37] (particularly in bark and sapwood layers of logs as compared to the more durable heartwood) [12, 13]. Studies measuring the concentration of airborne viable fungi in wood processing operations have found high concentrations within debarking departments [6, 35], woodchipping operations [1] and in areas where moist piles of wood chips or bark mulch are stored [23, 24, 34]. Several studies (predominantly of

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Scandinavian origin) have also measured high mould levels within trimming departments [5, 16], though it has been suggested that this reflects lower kiln temperatures used in some Scandinavian kilns (compared to North American kilns) [26].

In addition to studies of viable moulds, a number of studies have also examined mould exposure in terms of total spore counts and other fungal cell markers. β(1→3)-glucan (a polyglucose compound present in the cell wall of fungi, certain bacteria and vegetable materials) has been examined as a possible marker for total mould exposure. Elevated levels of β(1→3)-glucans have been detected within woodworking operations in Australia [1], sawmills in New Zealand [11] and debarking operations in Sweden [29], and high correlations between viable mould concentrations and β(1→3)-glucan concentrations have been measured in some woodworking operations [1]. Health effects have also been directly associated with exposures to β(1→3)-glucans, including an increased risk of respiratory disease symptoms and airways inflammation [29, 33], and β(1→3)-glucans have been suggested as a cause of 'sick building syndrome' [28].

This study was conducted to identify potential determinants of mould exposure among sawmill workers for use in a retrospective cohort study, using glucan as a mould marker. This assessment of glucan exposures is a continuation of a previous study assessing levels of endotoxin exposures among sawmill workers [9]. Both the endotoxin and glucan exposure assessments were conducted as part of a larger, ongoing study aimed at determining the health effects of a variety of occupational exposures among a large cohort of sawmill workers from 14 lumber mills in the province of British Columbia, Canada [32].

MATERIALS AND METHODS

Study population and sampling strategy. Wood dust samples were collected at four BC lumber mills during the summer of 1997. The four mills were selected to represent the range of geographic areas and variety of conditions in the industry in this province. Two of the mills were located on the BC coast [one on Central Vancouver Island (mill A) and one on the South Coast (mill B)] and two were located in the interior of the province [one in the Southern Interior (mill C) and one in the Northern Interior (mill D)].

Species processed at the two Interior mills (C and D) were primarily Engelmann Spruce (*Picea engelmannii*) or White Spruce (*Picea glauca*), Lodgepole Pine (*Pinus contorta*), and Balsam Fir (*Abies lasiocarpa*), while the Coastal mills (mills A and B) processed predominantly Western Hemlock (*Tsuga heterophylla*) and Douglas Fir (*Pseudotsuga menziesii*). Logs at mill D were stored on land, while logs at other mills were water-stored (though following debarking, logs at mill C were stored on land for a short time prior to processing). Among the mills using water-storage methods, the two Coastal mills stored logs in salt water while the Interior mill C stored logs in fresh water. Mills included in the study also differed by yard condition, and type and location of debarking machines.

Personal sampling was conducted over a two-week period in each mill. The sampling strategy targeted all production and maintenance jobs at least once in each mill. Individuals were randomly selected from all available job title/shift combinations and full-shift sampling periods were utilized for the majority of samples (ie. 8 or 12 hours).

In order to assess the determinants of exposure to moulds and other wood dust components, data was recorded at the level of the mill, department, job, and individual. For each sampling day, weather information was collected from the government environmental monitoring station closest to each mill. Production information (i.e. species processed, production level) was collected for each department of the mill, based on reports from production management personnel. Over the sampling period, information was gathered for each job sampled including department in which the job was located, whether it was designated as a clean-up job, an estimate of the percentage of enclosure (in a booth) associated with the job, and whether the job was located indoors or outside. Temperature and relative humidity at the site and time of sampling were also collected. At the end of each sampling period, individual participants were questioned about the jobs performed that day, the number of times that compressed air was used for "blowing down" sawdust, whether they spent time in a cab or booth during their shift, and whether the wood processed was kiln-dried or green.

Glucan sampling and analysis. All airborne dust samples were collected using a GSP-sampler for inhalable dust (Deha-Haan & Wittmer GmbH, Friolzheim, Germany). Filters used were Teflon (polytetrafluorethylene) with a 0.45 µm pore size and 25 mm diameter (Costar, USA). Filters were pre-conditioned in a climate-controlled room (temperature 22 ± 2ºC, relative humidity 65% ± 5%) and weighed in triplicate on a Sartorius microbalance (Sartorius M3P, Germany). Personal air sampling pumps (SKC model 224-44XR, Eighty-Four, PA, USA) were calibrated to a flow rate of 3.5 litres per minute. After sampling, each filter was immediately refrigerated at 4ºC. Before weighing to determine dust concentration, the filters were desiccated at 4ºC for approximately 48 hours and then re-equilibrated in the climate-controlled room for 48 hours at 22ºC. After equilibration, the filters were again weighed in triplicate and stored in 50 ml pyrogen-free centrifuge tubes until extracted for endotoxins and β(1→3)-glucans.

Glucan analyses were performed in the laboratory of Gert Doukes at Wageningen Agricultural University in the Netherlands. All filters were initially extracted for endotoxin analysis (results described elsewhere by Dennekamp *et al.* [9]). Subsequent to endotoxin extraction, dust samples were heat-extracted in 5 ml pyrogen-free

water with 0.05% Tween-20 (polyoxyethylenesorbitan monolaurate; Merck, Germany) (see method described by Douwes *et al.* [10]). Samples were shaken for 15 minutes, autoclaved at 120ºC for 60 minutes, then shaken again for 15 minutes. Sample suspensions were centrifuged at 1,000 × g for 15 minutes and stored frozen at -20ºC until analysis.

β(1→3)-glucan was assayed with a specific enzyme inhibition immunoassay (EIA) as developed by Douwes *et al.* [10]. Microtitre plates (Costar 96 well cell culture cluster 3596, USA) were coated overnight (200 µl/well) at 4ºC with 16 µg/ml laminarin (Fluka AG, Buchs SG, Switzerland) in PBS (see Douwes *et al.* [10] for a description of the characteristics of laminarin). Plates were washed with PBT (PBS washing buffer solution containing 0.05% Tween-20), then coated with PBTG solution (300 µl of 0.5% gelatine in PBT) and incubated for 30 minutes at 37ºC. Test sample or laminarin standard (100 µl diluted in PBTG) were added to microwells and mixed with 100 µl 1:75,000 purified polyclonal rabbit anti-laminarin antibodies in PBTG. Microtitre plates were then incubated at 37ºC on a rotating platform for one and a half hours. After extensive washing, 200 µl 1:5,000 horse anti-rabbit peroxidase (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands, No. M1234) was added to each well and shaken for 1 hour at 37ºC. After further extensive washing, 200 µl OPD substrate (2 mg/ml *o*-phenylenediamine (Sigma Chemicals, St. Louis, MO) in 0.05 M citrate/ phosphate buffer and with 0.015% H_2O_2) was added and incubated for 30 minutes at 20ºC in dark conditions. The enzyme reaction was terminated by adding 50 µl 2M HCl in wells and optical density (OD) was read at 492 nm. On each microtitre plate twelve dilutions of the reference laminarin preparation were included as well as four control wells (containing only antibodies mixed with PBTG). Samples were tested in four different dilutions. β(1→3)-glucan concentrations were determined by interpolating the OD_{492} of each test sample dilution on a semi-log calibration curve obtained with the twelve dilutions of the reference preparation and calculated with SOFTmax (Molecular Devices Corporation, Menlo Park, Ca, USA), a 4-parameter curve fitting program.

The limit of detection (LOD) for the assay was determined using the laminarin calibration curves of 40 assays performed on individual microtitre plates. The detection limit of each microtitre plate was determined by calculating the corresponding glucan concentration at 15% inhibition. The mean LOD of the 40 plates was calculated as 0.059 µg/ml (SD = 0.054 µg/ml). The mean concentration of field blanks (calculated as 0.16 µg/ml, SD = 0.40 µg/ml) was subtracted from all values, in order to correct for background glucan levels.

STATISTICAL ANALYSIS

The intended purpose for the final model was prediction of historical exposures for a retrospective cohort study. Therefore, the strategic plan for the development of the predictive model was to 1) identify factors that are strong predictors of mould in current time; 2) identify which of these have historical data; 3) create the best fit model(s) for strong predictors with historical data; and 4) retrofit them to past circumstances.

All statistical analyses were conducted using the statistical program SPSS (Release 10.0.5, Chicago, USA). Descriptive statistics (counts for categorical data, and means, ranges, standard deviations, and frequency distributions for continuous data) were calculated for all variables thought to influence exposure levels. Continuous variables which appeared to be log-normally distributed based on frequency histograms (wood dust concentration, glucan concentration) were log-transformed (base e) prior to analysis. All categorical variables were re-coded, so that one category from each variable was selected as baseline (based on the *a priori* assumption that the category selected for baseline represented a low exposure category). Variables with a large proportion of missing data (i.e. relative humidity and temperature at the time and site of sampling) were excluded from further analysis. The self-reported variable for whether wood was kiln-dried or green was also partially incomplete, but it was included in further modelling procedures after attempts were made to interpolate the missing data based on the knowledge of specific mill drying procedures. The department variable was re-coded by an Industrial Hygienist into a 'process group' variable to better reflect jobs with similar tasks.

Using simple linear regression modelling procedures, variables that were not highly predictive of the outcome variable ($p > 0.25$) were excluded from further analysis. Among pairs of work area variables with a correlation coefficient ≥ 0.7 (using Pearson's r), only one variable was selected for inclusion in the model. Since the major intended purpose for the final model was its usage in prediction of historical exposures for a retrospective cohort study, variables were selected for inclusion based on the range of data available historically for the 14 BC cohort mills. Variables were also selected based on ease of interpretability of the final model.

Interactions between log storage method and process group were selected based on the *a priori* hypothesis that fungal growth rates on land-stored logs would be higher than on water-stored logs, and that exposures would be highest for jobs located close to the debarking machines at land-storing mills. No other interactions were included in the modelling as there were no other *a priori* hypotheses. Multivariate regression modelling was then conducted using backward and forward stepwise procedures and variables with $p < 0.05$ were retained in the model; the best fitting model was selected (based on R^2 values).

RESULTS

There were 275 samples analysed for glucans; 48 were area samples (mean sample time = 382 minutes) and 227 were personal samples (mean sample time = 467 minutes).

Four personal samples were excluded: two because the workers were rotating through multiple jobs during the shift; and two because the sample time was less than three hours. Of the 223 personal samples, 45.7% were below the LOD and of the 46 area samples, 52.1% were below the LOD. Concentrations below the limit of detection were included as LOD/√2 [21].

Characteristics of each of the four study mills are summarized in Table 1. Mean temperature during sampling was highest at the Coastal mill A and lowest at Interior mill D, while mean relative humidity during sampling was highest at Coastal mill A and lowest at Interior mill C. Precipitation was most frequently recorded during sampling at the Interior mill D and most infrequently recorded at Coastal Mill A. For personal samples, the majority of sampled workers at all mills were located indoors (ranging from 61.4% of workers at Coastal mill B to 75.5% of workers at Interior mill D) and were less than 50% enclosed in a booth/cab for the duration of their shifts (ranging from 65.7% of indoor workers at Coastal mill B to 97.5% of indoor workers at Interior mill D). Approximately one-third of all workers used compressed air during their shift to blow sawdust off equipment (ranging from 26.3% of sampled workers at Coastal mill B to 42.6% at Interior mill C). Mean wood dust levels and lumber production levels were highest at the Interior mill D, followed by the Interior mill C and the two Coastal mills A and B.

A summary of glucan concentrations at the four study mills is presented in Table 2. Mean personal exposures to glucans were highest in the two Interior mills (GM = 2.52 at mill C and GM = 4.00 at mill D). A greater percentage of samples were below the LOD at the two Coastal mills (67.3% at mill A and 59.6% at mill B). Area samples, collected in mill C and mill A only (not shown in Tab. 2), showed a similar pattern of β(1→3)-glucan exposures to personal samples in these mills, with higher mean levels recorded in the Interior mill C (GM = 4.5 µg/m^3) *versus* the Coastal mill A (GM = 0.8 µg/m^3).

Variations were also observed by process group within Interior versus Coastal mills (Tab. 3). The highest β(1→3)-glucan levels were measured among workers in the log yard (GM = 12.6 µg/m^3), clean-up/labour (GM = 8.94) and log processing (GM = 5.91) groups at the Interior mills. Glucan levels below a geometric mean of 1 µg/m^3 were observed in the majority of Coastal mill process groups, including log processing, mill maintenance/ sawfilers, non-mill maintenance, planing, sawmill-cutting, sorting/packaging, and administration/boom. At the Interior mills, only the administration/boom workers had a geometric mean exposure below 1 µg/m^3.

Examination of simple linear regression modelling results (Tab. 4) suggests that log storage method, process group, wood dust concentration, debarker type, species, yard condition, and production level were all associated with β(1→3)-glucan concentration. Working in a booth/cab

Table 1. Characteristics of study mills and samples*

Characteristic	Coastal Mills		Interior Mills	
	Vancouver Island (A)	South Coast (B)	South Interior (C)	North Interior (D)
Main species of wood processed	hemlock, fir	hemlock, fir	spruce, pine, balsam	spruce, pine, balsam
Primary log storage methods	fresh water	salt water	fresh water; land (debarked logs)	land
Yard condition	paved	paved	paved	dirt
Type/location of debarking machine	mechanical; in mill	hydraulic; in mill	mechanical; in mill	mechanical; outside
Sampling month (# of samples taken)	July (52)	June (36); July (21)	August (61)	May (13); June (40)
Conditions (during sampling)				
Mean temperature, °C (range)	20.5 (17-22.2)	14.6 (12-19)	18.1(15-22.6)	12.1(9.5-19.9)
Mean relative humidity, % (range)	64.7 (44-90)	58.0 (40-69)	50.1(40-62)	52.0 (41-77)
Precipitation recorded-sampling day	14.3% of days	42.9% of days	42.9% of days	66.7% of days
Mean inhalable wood dust, mg/m^3	0.8	1.9	2.0	3.7
Mean daily production, 10^3 FBM (foot board metre)	339.2	89.2	552.2	771.3
% of sampled workers				
Indoors	75.0%	61.4%	67.2%	75.5%
<50% enclosure in booth/cab	84.6%	65.7%	87.8%	97.5%
Outdoors	25.0%	38.6%	32.8%	24.5%
<50% enclosure in booth/cab	53.8%	40.9%	68.4%	30.8%
% of samples with wood conditions				
Kiln-dried	0%	0%	11.5%	24.5%
Mixed (kiln-dried and green)	17.3%	0%	18.0%	9.4%
Green	82.7%	100%	70.5%	66.0%
% workers using compressed air	30.8%	26.3%	42.6%	35.8%

*Variable frequencies may not total 100% due to rounding.

Table 2. Summary of β(1→3)-glucan concentrations (μg/m^3) at the four study mills.

	Coastal Mills		Interior Mills	
	A	B	C	D
N	52	57	61	53
AM	3.84	3.54	10.41	18.94
GM	0.84	1.02	2.52	4
GSD	3.32	3.86	5.16	5.96
Range	<LOD-77.2	<LOD-53.6	<LOD-120.8	<LOD-226.3
% below LOD	67.3%	59.6%	31.1%	24.5%

AM - Arithmetic mean, GM - Geometric mean, GSD - Geometric standard deviation

Table 3. Mean β(1→3)-glucan concentrations (μg/m^3) by process group in Coastal versus Interior mills.

Process group	Coastal mills			Interior mills		
	n	AM	GM	n	AM	GM
boom/administration	9	0.86	0.59	5	0.51	0.51
chip and hog	5	3.86	2.17	3	8.14	3.93
clean-up/labour	7	5.38	2.1	13	26.98	8.94
log processing	8	0.72	0.59	7	37.7	5.91
log yard	-[a]	-	-	3	23.42	12.6
lumber yard	9	12.99	1.93	8	2.45	1.32
mill maintenance/saw filing	20	0.85	0.72	31	20.66	3.97
non-mill maintenance	2	0.51	0.51	4	4.28	2.23
planning	3	0.51	0.51	6	8.61	4.24
sawmill-non-cutting	8	14.7	3.03	4	15.15	2.72
sawmill-cutting	18	1.61	0.64	5	2.79	1.16
sorting and packaging	20	2.4	0.81	25	4.94	2.14

[a] Data not collected at this location; AM - Arithmetic mean; GM - Geometric mean

(defined as 50–100% enclosure during shift), precipitation recorded, number of compressed air blowdowns, relative humidity, and outside temperature were also significant or borderline significant predictors of β(1→3)-glucan concentrations in simple linear regression modelling, and were thus retained for further modelling. Species, yard condition, outside temperature and production level were later removed from further modelling procedures due to correlation with other predictor variables.

Two determinants of exposure models were created, the first excluding wood dust concentration and the second adjusting for wood dust concentration. The first model accounted for 29.3% of the variance, as measured by the R^2 value for the model, and included process group, log storage, and the interactions between land storage/log processing and land storage/lumber yard. When wood dust concentration was added to the model, it was a highly significant predictor variable of glucan concentration. The R^2 value for the second model increased to 39.6% and the parameter estimates changed slightly (though the variables included in the model did not change). Parameter estimates for the two models are shown in Table 5.

Table 4. Results of simple linear regression modelling of exposure variables as independent predictors of β(1→3)-glucan concentration (log transformed, base e).

	df	p-value	Amount of variance explained (R^2)
Process group (12 categories)	11	0.001	0.139
Log storage (water/land/mixed)	2	<0.001	0.148
Drying condition (kiln/green/mixed)	2	0.868	0.001
Precipitation on sample day (no/yes)	1	0.069	0.015
# of compressed air blowdowns/shift (0-5, 6+)	1	0.035	0.020
Relative humidity (continuous)	1	0.013	0.027
Wood dust concentration (continuous; log-transformed, base e)	1	<0.001	0.254
Location of worker (indoor/outdoor)	1	0.517	0.002
Working in booth/cab during shift (yes/no)	1	0.021	0.024
Debarker type (mechanical/hydraulic)	1	0.005	0.036
Species (5 categories)	4	<0.001	0.152
Yard condition (paved/dirt)	1	<0.001	0.083
Production level (continuous)	1	<0.001	0.103
Debarker location (inside/outside or enclosed)	1	0.563	0.002
Outside temperature (continuous)	1	0.080	0.014

DISCUSSION

In the assessment of determinants of β(1→3)-glucan exposure, one pattern that emerged in our study was the higher overall mean β(1→3)-glucan levels observed at the Interior mills (particularly the Northern Interior mill) compared to lower levels at the Coastal mills. There are a number of characteristics associated with BC Interior versus Coastal mills which could help to explain these differences, several of which were shown to be strong determinants in the regression models.

In British Columbia, the Coast mountain range divides province into coastal and interior zones, each having distinctive forest types and industries. In the Coastal industry, sawmills are generally located on rivers, lakes or on the sea where transportation of logs is facilitated by water. Logs are often stored in water prior to processing. This contrasts with a number of the Interior mills, where land storage of logs prior to processing is more prominent. In our study, log storage method was a strong determinant of β(1→3)-glucan exposure, with the highest exposures measured within mills processing land-stored logs and the lowest exposures measured within mills processing water-stored logs. The interaction between land storage and log processing department was also

Table 5. Linear regression models estimating β(1→3)-glucan concentration (log transformed, base e).

	Exposure Model #1 (wood dust excluded) R^2 = 0.293		Exposure model #2 (wood dust adjusted) R^2 = 0.396	
	B	p-value	B	p-value
Intercept	-0.916	0.019	-0.177	0.641
Wood dust concentration (continuous; log transformed, base e)	-	-	0.51	0.007
Log storage (categorical)				
Ref = water stored (n = 109)				
land stored (n = 53)	1.340	<0.001	0.656	0.017
water / land stored (n = 59)	0.836	<0.001	0.429	0.060
Process group (categorical)				
Ref - boom/administrative (n = 14)				
chip and hog (n = 8)	1.601	0.012	1.096	0.064
cleanup/labour (n = 20)	1.927	<0.001	1.299	0.007
log processing (n = 15)	0.466	0.406	0.482	0.352
log yard (n = 3)	2.276	0.014	1.847	0.031
lumber yard (n = 17)	1.329	0.016	1.107	0.030
mill maintenance/saw filing (n = 51)	0.970	0.026	0.503	0.220
non-mill maintenance (n = 6)	0.587	0.400	0.121	0.853
planning (n = 9)	0.874	0.156	0.143	0.806
sawmill-non cutting (n = 12)	1.668	0.003	0.940	0.078
sawmill-cutting (n = 23)	0.346	0.475	0.080	0.860
sorting and packaging (n = 45)	0.625	0.156	0.292	0.477
land storage × log processing (n = 3)	2.553	0.008	2.407	0.007
land storage × lumber yard (n = 4)	-1.901	0.026	-2.163	0.007

statistically significant, with higher levels observed in the log processing areas where the bark from land stored-logs is removed prior to further processing. Water storage has been shown to inhibit the development of fungi due to the low concentration and slow diffusion rate of oxygen in water [37]. The potential for mould growth on land-stored logs is much higher (depending on conditions such as log spacing, yard condition, length of storage and climate). Since mould has been shown to grow predominantly within the bark and outer sapwood layers of logs, it might be expected that the highest mould levels (and thus β(1→3)-glucan levels, assuming β(1→3)-glucan is a marker for mould exposure) would be in debarking areas of mills where logs are land-stored. This pattern was observed in our study (for β(1→3)-glucans) and has been observed in other studies assessing viable mould concentrations in BC mills [6, 7]. High β(1→3)-glucan levels have also been reported in the debarking areas of a paper manufacturing plant in Sweden [29], although the log storage methods at this plant were not specified.

Differences in species processed at the mills in our study could also help to explain some of the observed differences in β(1→3)-glucan levels. While not included in the final regression modelling, species was a strong predictor of exposure in univariate modelling. Specifically, pine (which was processed at the Interior mills in our study) is known to have particularly low durability to microbial attack, while species processed at the Coastal mills tend to be of higher durability [36]. As a marker for mould exposure, it might be expected that β(1→3)-glucan levels would be slightly higher at Interior mills based on species processed. However, it is unlikely that species alone could fully explain the extent of the differences observed between Coastal and Interior mills, particularly the large differences observed within the log processing areas of the mills. Durability ratings of softwoods generally refer to the durability of heartwood rather than sapwood layers and the sapwood of all species is considered to have low resistance to decay. Therefore, levels of mould growth within sapwood would not be expected to differ markedly between species and as a predictor of mould growth, the impact of species would more likely be evident further into the mill (where heartwood is being cut and the dust is allowed to settle). Differences in species durability could help to explain the slightly higher glucan levels observed in the sawmill-cutting areas of the Interior mills compared to the Coastal mills, however, the differences were not marked and thus the effect of species, if any, is likely to be small.

Production levels were also higher at the two Interior mills and production was a strong predictor in univariate regression modelling. It might be expected that higher production would produce greater levels of airborne moulds (due to increased debarking). Higher production would also likely produce more wood dust, thus leading to even higher concentrations of moulds if wood dust acted as a substrate for secondary mould growth within the mill. However, it is not possible to determine the impact of production (adjusting for wood dust concentration) since it was not offered in the final regression models (due to concerns about colinearity).

Wood drying methods also tend to differ between Coastal and Interior mills. Kiln-drying is generally considered less economical (and is thus less common) among the Coastal

mills than the Interior mills, due in part to the fact that hemlock is a naturally wet wood requiring longer drying times than the naturally drier spruce-pine-fir combination. In our study, both of the Interior mills used kiln-dried wood (with highest levels observed at the Interior mill D) whereas only one of the Coastal mills used kilns (to a lesser extent). Several studies have reported high mould concentrations (both viable and non-viable) in trimming departments processing kiln-dried wood [5, 16], while others have reported low levels in mills that use kiln-dried wood [1]. In a study of two New Zealand sawmills, the highest levels of β(1→3)-glucan were measured in the planer mills [11]. This contrasts with the results of our study, where relatively low glucan exposures were measured in the planing mills. Differences in findings between our study and those of the recent New Zealand study could be due to possible differences in kiln-drying temperatures or drying times used in their study mills or differences in ventilation systems within their planer mills (since personal exposures to microorganisms have been shown to be very low at workplaces with efficient dust control systems [1]). Whether wood was kiln-dried or green was also not a strong predictor of β(1→3)-glucan levels in univariate regression modelling our study, however, this information was collected by self-reporting from workers and the accuracy of the data is unknown. It is noteworthy that relatively low levels were also observed in the sorting/packaging and lumber yards in our study, with regression modelling indicating that particularly low levels may be observed in the lumber yard of Interior mill D (where kiln-drying is most common).

Within mills, levels of personal β(1→3)-glucan exposure were significantly elevated for clean-up workers/labourers. This is not surprising, since clean-up workers would likely be re-suspending particulates during the clean-up process. Elevated bioaerosol exposure levels have been measured among clean-up workers in other studies [9, 15], suggesting that clean-up workers without effective respiratory protection in the sawmill environment could be at an increased risk for bioaerosol-related respiratory diseases.

Booths have been shown to markedly decrease exposures to wood dust in sawmills [31], however, working within a booth/cab was not a significant predictor in the final regression models in this study. One possible explanation for this discrepancy is that, while booths may be effective in reducing exposures to wood dust, they may be less effective in reducing exposures to glucans. It is also possible that the variable did not adequately reflect the amount of time spent in the booth/cab during the shift or the amount of enclosure that the booth/cab provided. Though the booth/cab variable was not a significant predictor in the final regression models, the fact that booths/cabs were more commonly reported as being used by workers within the sawmill-cutting process groups (61% of workers in booth/cab) than sawmill non-cutting process groups (8% of workers in booth/cab) may help to explain why personal exposure levels of β(1→3)-glucan were lower for workers in the cutting areas of the sawmill than in the non-cutting sawmill areas.

This analysis of β(1→3)-glucan exposure included a fairly large number of samples and included an assessment of some of the determinants of β(1→3)-glucan exposure within the sawmill environment. The amount of variability explained by the regression model ranged from approximately 29% to 40%. It is interesting to consider potential additional sources of variability not explained in our models. Some of the key determinants for β(1→3)-glucan exposure may have either not been collected or were not collected in a meaningful way for the purposes of this exposure assessment. Whether wood was kiln-dried or green, for example, is a likely and important determinant for β(1→3)-glucan exposure; however, it was collected by self-reporting and was somewhat incomplete. Attempts were made to fill-in missing data for the variable based on our knowledge of mill-specific drying practices; this, however, may have led to some misclassification within this variable. Length of log storage time (both in the forest after felling and in the yard or boom) and general yard hygiene are additional exposure determinant variables that may have been important determinants to examine but were not documented as part of this assessment. Within-mill data at the time of sampling, such as relative humidity and temperature at the sampling location (rather than as collected for the general mill location), could also be important determinants for β(1→3)-glucan exposure, particularly as determinants for secondary sources of mould growth within the mill environment (assuming that β(1→3)-glucan represents a marker for mould exposure). However, these variables were not included in the regression modelling since the data collection for these variables was incomplete. As a result, this analysis tended to focus on between-mill differences as opposed to job-specific or sample-specific determinants, which could account for much of the unexplained variance in the models. Our study design was also not the best for examining all between-mill differences, since only one such difference could be included in the final models (due to colinearity concerns). With only four mills included in the study, it was difficult to distinguish whether log storage, species, or production levels had independent effects.

Since no viable mould measurements were conducted as part of this study we were unable to directly assess the potential for using β(1→3)-glucan as a marker for mould exposure. The distribution of β(1→3)-glucan within our study mills does correspond with distributions of viable moulds observed in other studies of British Columbia sawmills; however, it is not possible to conclusively state that β(1→3)-glucan represents an effective marker for mould exposure in this study. One drawback of the EIA method used in this study is the potential cross-reactivity with other plant β(1→3)-glucans [10]. Wood dust itself represents a large possible source of β(1→3)-glucan, however, we were able to rule out the possibility that all of the β(1→3)-glucans measured in our study were

derived from wood dust since some adjustment was made for wood dust concentration in the regression models. Wood dust concentration accounted for about 25% of the variance in β(1→3)-glucan levels in our study samples (through simple linear regression modelling), suggesting that other sources of β(1→3)-glucan were present in these mills. In the log and lumber yards it is possible that organic debris (other than from mould sources) contributed to the levels of β(1→3)-glucan observed in our study. However, within the sawmill itself, it is likely that mould represented a large proportion of the observed β(1→3)-glucan. It is not at present known how comparable results are from the EIA (used in this study) and the much more sensitive Limulus amebocyte lysate (LAL) assay for β(1→3)-glucan [19, 25]. The LOD of the EIA method was felt to be adequate for the levels of exposure found in lumber mills. Levels observed in our study were similar to other occupational environments (compost workers and pig farmers) where workers are known to experience organic dust-related respiratory symptoms [10], however no direct associations between β(1→3)-glucan exposure levels (using EIA) and health effects have been published to our knowledge.

CONCLUSION

This study provides quantitative estimates of exposure to β(1→3)-glucans among lumber mill workers in British Columbia. Higher levels of β(1→3)-glucans were measured in mills that process land-stored logs compared to mills processing water-stored logs, with the highest personal exposure levels occurring in the log processing areas (where the bark is removed) of mills that land-store logs. Clean-up workers were also at risk for higher β(1→3)-glucan exposures compared to other mill workers. Results from this study will be used as a retrospective exposure assessment to examine health effects of wood dust and bioaerosol exposures among a large cohort of sawmill workers, including workers at these four mills.

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