RESEARCH REPORT

Additional Analysis of Wallaceburg Data

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REPORT

Additional Analysis of Wallaceburg Data

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ABSTRACT

Data on moisture sources, winter condensation potential, CO_2 levels, levels of biological contamination and blood lymphocytes from fifty nine houses subjected to detailed testing and inspection in a previous phase of the Wallaceburg study were subject to more detailed statistical evaluation to determine the relationships between moisture sources, fungal growth and objective measures of health. The work showed a correlation between measurements of mold growth and immunological reactions of occupants and that mold growth appeared to be more related to local moisture sources than air change levels.

DISCLAIMER

EXECUTIVE SUMMARY

A previous study carried out in the Town of Wallaceburg, Ontario measured biologically-active contaminants in houses and explored the health of occupants. In a second phase of the study, a group of 39 houses with the worst levels of biological contamination and a group of 20 with the least contamination were subject to more detailed study including detailed field inspections and testing, monitoring of indoor environmental condition, and simulation to predict condensation formation potential in winter conditions. Subjective measures of health were gained through questionnaires and brief physical exams of all occupants and objective measures of health included analysis of blood samples drawn from a child in each house for T-lymphocyte and B lymphocyte structure using a Fluorescence Activated Cell Sorter (FACS).

Preliminary analysis undertaken for this second phase consisted of contrasting of the house and health data of the "good" and "bad" house sample sets, and determining correlation of measured and combined variables considered significant measures of house and health performance. This analysis indicated that there was a link between measurements of mold growth and objective measures of health, and that the presence of indoor moisture sources is a more significant factor in fungal growth than air change.

This report summarizes some additional analysis of the Wallaceburg data which included:

- determining a calculated moisture source strength for 55 houses based on monitored humidity levels and AIM-2 simulation using AES supplied weather data,
- estimating condensation potential (estimated hours of condensation and length of condensation periods) using a condensation simulation program developed for CMHC for this project,
- Statistical analysis blood lymphocyte levels of children in the "good and "bad" house sample sets controlling for child's age, dust mite antigen, presence of furry or feathered pets, weather the child had a cold, CO_2 and VOC levels and the presence of a humidifier,
- evaluation of levels and variations in bedroom CO_2 concentrations by house, occupancy and whether the bedroom door was open or closed.

The analysis indicated that the calculated moisture source strength was one of the strongest predictors of biological contamination levels and that condensation potential appeared to be a strong predictor of some lymphocyte measures.

High levels of CO_2 in the child's bedrooms at night in many of the houses showed that in these houses the bed room was poorly ventilated. CO_2 levels did not correlated with increased levels of biological contamination or lymphocyte measures.

SOMMAIRE

Au cours d'une étude antérieure menée dans la ville de Wallaceburg (Ontario), on a mesuré les polluants biologiques dans les maisons et examiné la santé des occupants. Cette étude a permis de constater un écart considérable dans l'importance de la contamination par des polluants biologiques et une corrélation de ces mesures avec les mesures subjectives de la santé obtenues au moyen de questionnaires sur la santé. Pour la phase II du projet de Wallaceburg, on a choisi un groupe de maisons affectées des pires niveaux de contamination par des polluants biologiques, ainsi qu'un autre groupe parmi les moins affectées, aux fins d'une étude plus détaillée.

Trente-neuf maisons fortement contaminées par des polluants biologiques et 20 maisons comprenant de faibles niveaux de ces polluants ont fait l'objet d'inspections détaillées des lieux, d'essais pour déterminer les paramètres de fonctionnement de la maison, d'un suivi des conditions environnementales intérieures et d'une simulation visant à estimer la formation potentielle de condensation dans des conditions hivernales. On a effectué des mesures subjectives de la santé au moyen d'un questionnaire et d'un examen physique sommaire de chaque occupant; on a aussi procédé à des mesures objectives de la santé par des prélèvements de sang et de sécrétions nasales chez l'enfant «indicateur» dans chaque maison aux fins d'analyse de la structure de leurs lymphocytes T et B au moyen d'un trieur de cellules à fluorescence (FACS).

Les protocoles d'inspection et les essais étaient fondés sur ceux que l'on avait élaborés lors de contrats antérieurs octroyés par la Société canadienne

d'hypothèques et de logement, mais comportaient certaines modifications visant à améliorer leur application à la présente situation. Les protocoles comprenaient l'utilisation d'instruments de cueillette de données homogènes pour toutes les phases du travail. Les données cueillies ont été introduites dans une base de données relationnelles (ACCESS de Microsoft) pour qu'elles se prêtent bien à un traitement par d'autres logiciels que pourront utiliser d'éventuels utilisateurs. Cette base de données comprend actuellement au-delà de 400 zones de données.

L'analyse préliminaire entreprise pour ce projet consiste à comparer les données sur les maisons et sur la santé cueillies pour la catégorie des «bonnes» maisons et pour celle des «mauvaises» maisons, puis à déterminer les corrélations entre les variables mesurées et combinées que l'on considère être des mesures significatives du rendement de la maison et de l'état de santé.

L'examen des données préliminaires indique qu'il existe un lien entre les mesures de la prolifération de moisissures et les mesures objectives de la santé, et que ce lien n'est expliqué ni par les facteurs potentiellement confusionnels pour lesquels on a cueilli des données, notamment le tabagisme, le niveau de composés organiques volatils (COV) ou de CO_2 dans l'air à l'intérieur de la maison, ni par une possible contamination par des polluants biologiques, notamment les antigènes d'acariens ditriticoles et d'animaux domestiques dans la poussière de maison.

L'analyse préliminaire indique aussi que le niveau de ventilation générale dans les maisons ne constitue pas un facteur significatif du niveau de contamination par des polluants biologiques. Il semblerait que la présence de sources locales de moisissures constitue un facteur beaucoup plus important.

Lors de la détermination de la portée du projet, on a reconnu la possibilité et la nécessité d'analyser de façon beaucoup plus détaillée les données cueillies. Nous recommandons des analyses supplémentaires pour confirmer les relations constatées lors de l'analyse préliminaire et pour examiner davantage les relations apparentes constatées entre le rendement de la maison, les paramètres de la prolifération de moisissures et les problèmes de santé qui ne semblent pas liés directement à la prolifération de moisissures. Cette analyse devrait comprendre un examen statistique plus rigoureux et des études plus détaillées sur certains des rapports qu'ont révélés les corrélations déterminées au cours de l'examen préliminaire.

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1. INTRODUCTION

In the Wallaceburg Project, 59 houses were selected based on previous measurements of mold levels. These were then subjected to field inspection, testing and monitoring and the occupants subjected to health evaluation questionnaires and testing of blood and nasal secretions. Data on house performance, mold growth and health were compiled in an electronic data base. In the previous report[1], preliminary analysis of the data showed correlations between measurements of mold growth and the immunological reactions of occupants and that the mold growth appeared to be more related to local moisture sources than ventilation levels.

This document reports on additional analysis of the Wallaceburg data, undertaken to provide a more detailed and extensive look at factors related to moisture sources, potential for condensation, CO_2 levels in the house and how these related to the quantitative measures of health.

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1 Moldy Houses: Why they Are & Why We Care, Morrison Hershfield Limited for Canada Mortgage and Housing Corporation, November ,1995

2. OBJECTIVES

The objectives for the additional data analysis could be listed as follows:

a) Undertake further investigation as to why air change rates determined by AIM-2 simulations differed from those measured by tracer gas decay testing carried out on the house.

b) Using newly available data, reasonable assumptions for missing data and any corrections deemed necessary upon detailed examination of the data, determine a calculated moisture source strength for as many houses as possible.

c) Compare calculated moisture source strength with estimates of moisture source strength based on occupancy and reported occupant activity.

d) Using the FPLAIM5.XLS program developed for CMHC, with some refinements to algorithms deemed necessary, develop measures of condensation potential and combination variables to compare house performance.

e) Evaluate the relationships between CO_2 levels, RH occupancy and door status in the Index child's bedroom.

f) Examine the impact of confounding factors in the relationship between mold growth factors and measurements of health.

3. PROCEDURES AND FINDINGS

The following sections describe the processes and outcomes of the additional data analysis. Appendix A contains hard copy output of the revised data fields (also provided in electronic spreadsheet form in EXCEL 3 and Lotus 123). The correlation tables are provided in section 3.5.1.

3.1 Review of Air Change Test Results

In the preliminary data analysis, the air change rate, as measured by a tracer gas decay test, was compared with the air change predicted by an AIM-2 [2] simulation for the same period. It was found that the average of the *Tracer Gas ACH / AIM2 ACH* ratio was very close to unity but the maximum and minimum of the ratio differed from the mean by a factor of four. This ratio was used as a correction factor during the preliminary simulations used to evaluate condensation potential of the houses. Therefore, determining the reasons for the variation and how to react to them in further data analysis, was important.

A house by house review of field data was undertaken to validate data and determine reason for the variation.

Revisions to Tracer Gas Air Change Data

The review of tracer gas decay test data included an evaluation of the data points (tracer gas concentration) of each test.

The tracer gas data for two households were discarded. In house 024 the chimney flue was left open during the tracer gas test and in household 154, there were very questionable readings which were at the low end of the instrument's range.

In other houses there were specific anomalous data points which should be ignored and the air change determined from the decay curve recalculated. This affected the results from households: 010, 030, 096, 100, 102, 138, 206, 215, 267, 352, 377 and 419.

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2 Walker I.S. and Wilson, D.J., University of Alberta, *The Alberta Air Infiltration Model AIM-2*, 1990

Revisions to Aim-2 Air Change Simulation Data

The inputs to AIM-2 can be classified into four categories:

- air leakage data as determined from a fan depressurization test,
- leakage location data, based on envelope component areas,
- data on exposure to wind, and
- weather data, including wind and temperature.

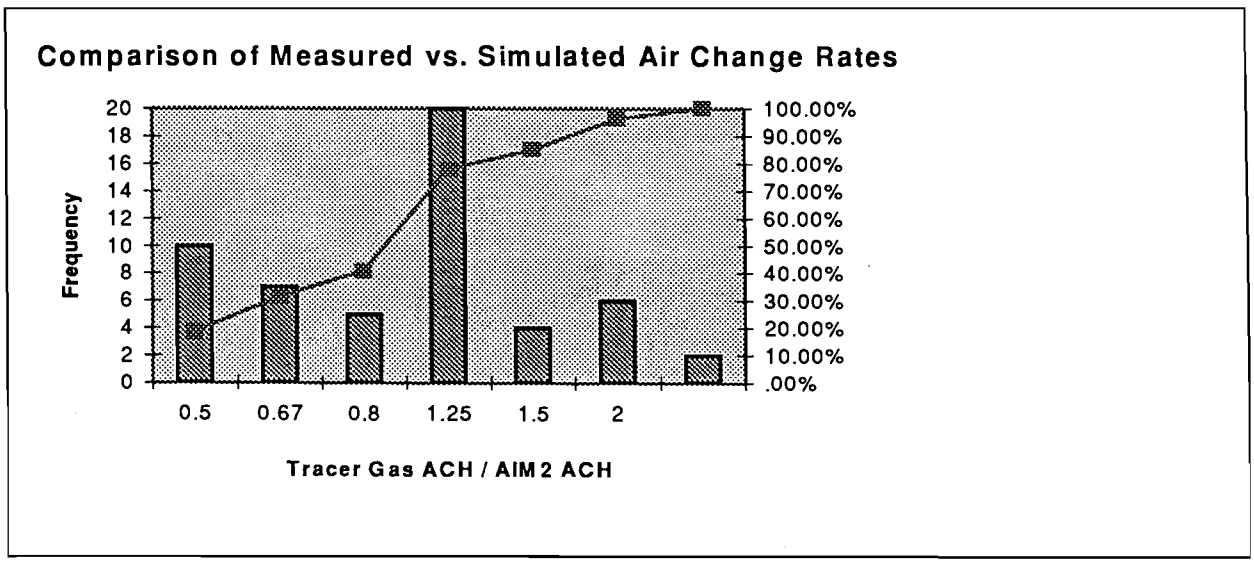
The source of weather data was Environment Canada data from the Windsor weather station. No measurements were undertaken at the site. This data category could not be improved.

The exposure and leakage location data was reviewed for obvious errors and consistency in assumptions. No reason to adjust data was found.

The fan depressurization test results were reviewed and it was determined that in any comparison of simulated and predicted air change the results for houses 419 and 432 should be ignored because these houses were halves of duplexes. The fan test results would incorporate interior leakage and therefore result in incorrectly-high simulated air change rates.

Impact of Revisions

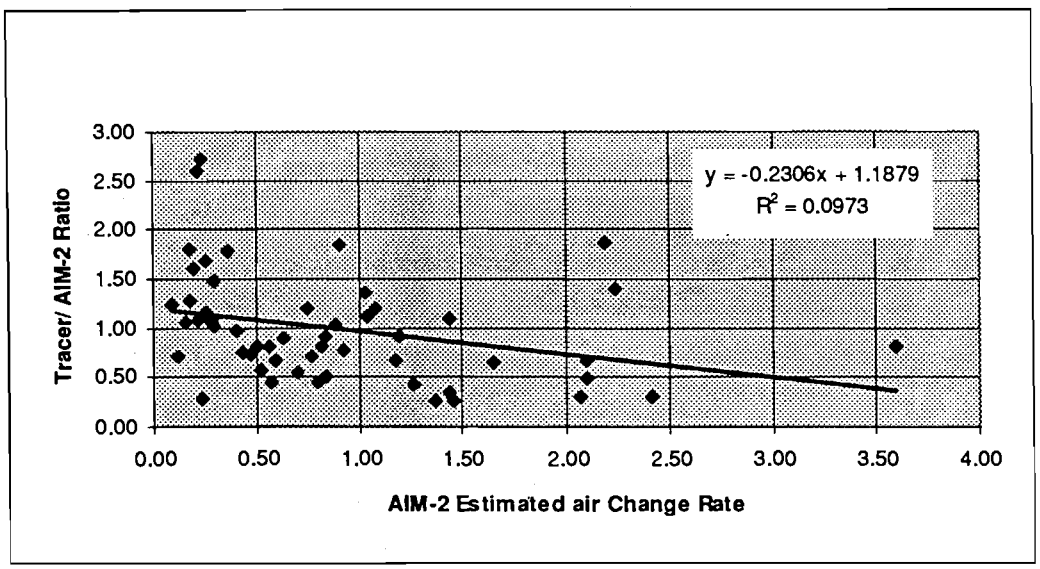
The following chart shows the frequency distribution of the *Tracer Gas ACH / AIM2 ACH* ratio with the revised data.



The average of the *Tracer Gas ACH / AIM2 ACH* ratio in the 54 considered houses was 0.99 (versus 1.08 in the preliminary results). The revisions reduced the variation, but it remains significant.

One factor that we feel contributes to the variation is the assumed wind speed. A review of field notes established that, in a number of cases (notably households 034, 078, 216, 232, 396 and 431), the measured wind speed at the Windsor weather station differed significantly from the observations in the field. No measurement of the wind speed were undertaken at the site. However, the field crew typically recorded general comments on the prevailing wind conditions (i.e. calm, light breeze, etc.). In general the notes could be interpreted to indicate that local wind speed in these houses at the time of test was significantly less that the airport data used for AIM-2 input. Further review of the data revealed a strong correlation (0.25) between the Tracer/Aim ratio and the assumed wind speed. This factor could account for the relatively high number of cases where the *Tracer Gas ACH / AIM2 ACH* ratio is lower than unity.

Another factor may be how well AIM-2 predicts air change as the driving force varies. The Graph below plots the *Tracer Gas ACH / AIM2 ACH* ratio against the AIM-2 estimate for the hour of the tracer gas test. The trendline indicated that AIM-2 may underestimate air change at low estimated air change rate values and overpredict it at high values.



Impact on Further Analysis

The key question arising from this analysis is whether a correction for tracer gas decay test results should be used in calculations that are used to estimate moisture source strength and condensation potential.

The indication that AIM-2 may underestimate air change at periods of low driving forces is significant since air change calculated by AIM-2 was used to calculate moisture source strength and condensation potential. In spite of this, our analysis lead to the opinion that, with one exception noted below, the uncorrected simulation result was a more reliable value for air change than a value corrected by the *Tracer Gas ACH / AIM2 ACH* ratio. The reason for this opinion can be summarized as follows.

1. The one hour air change test is subject to test errors and the impacts of local, short term conditions that should not be ascribed to long term analyses.
2. Differences in wind speed between a specific location and the airport are not consistent over time. One cannot assume that because there was a difference at one specific hour it could be assumed to exist over each hour of a heating season.
3. AIM-2 attempts to address local conditions by shielding and terrain factors.

The one exception to this rationale concerns two houses, 419 and 432, which were halves of duplexes. Fan test results, and therefore AIM-2 results, would incorporate interior leakage and therefore the AIM-2 result could be expect to be higher than reality. These results were adjusted by the *Tracer Gas ACH / AIM2 ACH* ratio.

3.2 Moisture Source Analysis

One of the key factors in predicting humidity levels is the magnitude of moisture sources in the house. In the preliminary analyses, the source of moisture into the indoor house environment was estimated from occupancy data collected in the occupant interviews. When Environment Canada weather data for February and March became available, the moisture source strength was estimated from environmental data recorded in the one week monitoring. The hourly source strength was estimated by the equation:

$$S = ACH * V * 1.2 * (W_i - W_o)$$

where

S	is the moisture source strength (kg/hr)
ACH	is the AIM2 simulated Air Change Rate for the house using the Environment Canada supplied wind and temperature data for the hour.
V	is the heated volume of the house (m^3)
1.2	is the assumed density of indoor air (kg/m^3)
W_i	is the moisture ratio of indoor air, calculated from monitored temperature and relative humidity in the particular hour (kg_{H20}/kg_{air})

W_o is moisture ratio of outdoor air, calculated from Environment Canada supplied temperature and dew point temperature for the hour (kg_{H20}/kg_{air})

The average hourly moisture source strength for the monitoring period in 47 houses was determined for the period over which indoor humidity monitoring results were available.

In this extension to the project, the data on calculated moisture source strength was increased and improved by:

- using addition weather data affecting 8 houses (43, 81, 86, 96, 177, 247, 377, 414) in which the monitoring period extended into April, 1996 (April weather data was not available at the time of the preliminary analysis).
- using the adjustment factors for AIM-2 as discussed in Section 3.1
- in two houses (134 and 414), in which indoor temperature data was not available, assuming a constant temperature of 21°C.
- correcting house characterization errors found in AIM-2 input for houses 109, 132, 177, 232.

The end result was that the calculated moisture source strength was determined for 55 of the 59 houses. In the other four houses (030, 154, 157, 276), critical data for the calculation was missing and impossible to estimate.

The calculated moisture source strength for each house was compared to the moisture source strength estimated from reported occupancy and occupant activities. The mean of the ratio of calculated versus previously-estimated moisture source strength for all 55 houses was 1.143. The mean of the difference between the calculated and previously estimated moisture source strength for all 55 houses was 0.056 kg/hr. (1.3 kg/day). The same mean difference for the 35 "bad" houses was 0.177 kg/hr (4.2 kg/day) and for the 20 "good" houses it was -0.156 kg/hr (-3.7 kg/day). The difference of 8 kg/day is statistically significant (p=0.030). This indicates that the "bad" house group likely had more sources of moisture which were not related directly to occupancy. This difference was, however, typically small in proportion to the moisture sources estimated from occupancy data. For the whole set of 55 houses the range estimated values was 2.6 kg/day to 58.4 kg/day and the calculated values ranged from 11.2 kg/day to 22.6 kg/day.

One concern with the validity of the calculated moisture source strength calculation related to the location of the humidity monitor. This was placed in the Index Child's bedroom. The moisture source strength calculation ascribes the humidity measured in this location to the whole house. One should question if the humidity in this bedroom was significantly different from the rest of the house, due to occupancy factors. As one check, we compared the measured humidity during the night (22:00 h to 6:00 h), when the bedroom can be expected to be occupied, and the humidity measured during the day (10:00 h to 18:00 h). The ratio between the two was consistently close to unity, ranging from 0.86 to 1.10 with a mean of 1.01. This does not prove that the humidity measured in the bedroom was the same as the rest of the house, but increases confidence in the assumption by having it pass one validity test.

In the previous report on the Wallaceburg project, the authors expressed some concerns about whether the calculated moisture source strength is a real measure of moisture source strength in the houses. These concerns remain. We point to the very strong positive correlation between the calculated moisture source strength and estimated air change rate over the monitoring period (0.511) and the Equivalent Leakage Area (0.347), from which air change is derived, as suspicious. Physically, this was evidenced by the observation that humidity levels measured in the houses were much more consistent across the sample group than the estimated air change rates. The calculated moisture source strength had to show high positive correlation with air change rate for this pattern to occur. We believe that the moisture source strength calculation over-emphasizes the relationship to air change. This could be related to our previously noted observation that AIM-2 may underestimate air change at low driving forces.

In spite of these concerns, one can accept calculated moisture source strength as an artificial variable relating humidity, air leakage area and outdoor weather conditioning. The data shows that the calculated moisture source strength turns out to be one of the strongest predictors of some key independently measured results. The calculated moisture source strength of the bad house sample set was higher than the good house sample set ($p=0.023$) and the correlation tables show significant correlations with ranked levels of dust mite antigens and Colony Forming Units.

3.3 Evaluation of Condensation Potential

The prime focuses of the Wallaceburg study were to determine if there were relationships between house construction and operating characteristics, condensation on exterior surfaces in the house, biological contamination levels and health factors. Many of the factors affecting house performance are interrelated and vary over time. This greatly complicates statistical analysis based on measurements that were not concurrent.

One way of addressing this is to create a summary variable relating the various factors and comparing this with the independently-measured health and contamination variables.

The potential for condensation formation in a house can be considered a complex function relating moisture sources, moisture storage effects, factors affecting air change, outdoor weather condition, indoor temperature and factors affecting the temperature of surfaces. These were related using a simulation program FPLAIMv5.XLS developed by Sheltair Scientific for CMHC foruse in this project. It uses hourly weather data to predict air change rate using AIM-2 algorithms and determines periods when condensation will occur using the algorithms of FLPRH2 [3], which predicts the occurance of condensation based on air change, moisture sources and the thermal resistance of the building envelope being modeled.

We used a modification of this program to produce summary variables, by modeling the window surfaces in the house with input parameters selected to force condensation formation at some time during the heating season in most houses (a realistic assumption). The output in total hours of condensation, length of longest condensation period, and number of periods with condensation or high surface humidity could then be considered summary variables of condensation potential.

To accomplish this the following modifications to the FPLAIMv5.XLS program and its input assumptions were made.

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[3] TenWolde, Anton, *A Mathematical Model for Indoor Humidity In Homes During Winter*,

- The analysis macro (which calculates the number of periods with condensation, the longest period of condensation and the number of periods likely to promote condensation) was modified and the calculation of the number of hours of condensation was included. A period likely to promote mold growth was redefined as any 24 hour period during which there was 100% RH or an RH greater than 70% following a period of 100% RH at the surface being modeled.

- The calculated moisture source strength determined for each house was assumed to be constant over the heating season. For houses with no calculated source strength, the estimated moisture source strength from occupancy factors based on the home owner questionnaire was used.

- The assumed indoor temperature was determined from mold growth site reports. For houses with no mold growth site reports, the dry bulb temperature measured in the living room was used.

- the moisture storage coefficient was set to zero.

- the interior air film was excluded from the calculation of the window RSI.

- the natural ventilation correction factor (Tracer/AIM-2 ratio) was set to 1 with the exception of the two duplexes where the modified Tracer/AIM-2 ratio was used.

The output values of condensation potential created by this process were similar for "good" and "bad" houses sample sets. The differences were not statistically significantly. In the subsequent statistical analysis there was a remarkably high correlation of this factor with changes in one of the lymphocyte testing measures, CD45O3CT.

3.4 Analysis of CO_2 Monitoring Data

Field measurement of CO_2 concentration was carried out for approximately one week period in the index child's bedroom in each house. In the previous report, the data was reported as mean values over the monitoring period. Additional analysis carried out for this extension included the following:

a. Determining the mean measured CO_2 concentration over the periods of 10:00 h to 18:00 h (day) and 22:00 h to 6:00 h (night).

b. Determining the occupancy of the rooms for these periods and whether the bedroom door was open or closed as reported on log sheets kept by the occupants.

c. Reviewing the graphical output of the data to visually determine the lowest readings of CO_2 concentration, which could be assumed to be close to outdoor ambient readings (with or without an offset caused by instrumentation error).

d. Determining the average elevation of CO_2 level ,for both day and night periods (above the low identified in c above).

The data shows that the CO_2 concentrations in the good house sample set were higher than for the bad house sample set, but not by an amount that was statistically significant. This is consistent with the finding that the good house sample had lower leakage areas. The CO_2 concentrations in the bedrooms at night were higher than in the day by about 45% (984 vs. 680), which could be expected from the occupancy patterns.

In our opinion, the best comparative data is the elevation of CO_2 concentrations over the lowest value as determined in item c. This modified variable minimize the impact of instrumentation offset errors and provide the best surrogate for ventilation per occupant or level of metabolism in the room. We found that the CO_2 elevation averaged about 448 ppm at night (standard deviation 291). This was about 3.6 times higher than the daytime mean of 154 ppm (standard deviation 88). In 13 of the 58 houses, the night elevation of CO_2 concentrations was greater than 600 ppm.

One would expect that the degree of CO_2 elevation at night would be strongly dependent on occupancy and whether the door to the room was open. In examining these relationships, independently on the whole data set, the differences were not statistically significant. In looking at the subset of house with one bedroom occupant (to eliminate the influence of occupancy), the ratio of nighttime CO_2 elevation to daytime CO_2 elevation was higher by about 30 %, but with enough variation that the difference cannot be considered statistically significant.

3.5 Evaluation of relationships between Moisture, Mold, Health and Confounding Factors

3.5.1 Correlation Coefficients of Revised Data

Table 1 provides statistical information contrasting revised results for the "good" and "bad" house sample sets and Table 2 provides correlation coefficients between the revised data and previous measures of contamination and health. In both tables the measured data from Phase I (Endotoxin to CFU Glys) is in ranked form. In Table 2 the output has been formatted to identify correlations which have held up to statistical tests of significance (z = fisher(r)

* $(n-3)^{0.5} > 1.96$ to reject null hypothesis at $\alpha = 0.05$, or >1.64 to reject null hypothesis at $\alpha = 0.1$).

Discussion on many of the correlations is included in previous sections. The correlations that were of most interest were those which related calculated moisture source with measures of biological contamination (mite antigens and mold colony forming units (CFUs)) and those relating measures of condensation potential with the lymphocyte variable CD4503CT. Simple analysis indicates that these correlations exist and are statistically significant. A review of the data by Dr. Corinne Dulberg, who carried out statistical analysis of health related factors, found some data distribution problems which could possibly discount the significance of these simple correlations but found that the moderate association between these variables was retained even when data was tranformed to counteract the effect of skew and with use of non parametic evaluation methods.

Table 1 Contrast of Results between "Bad and "Good" Houses

	"Bad" Houses			"Good" Houses				
	N	Mean	Std. Dev	N	Mean	Std. Dev.	TTest (p)	
Average Air Change Rate	38	1.030	0.712	20	0.658	0.373	0.034	*
Calculated Moisture Source	35	0.850	0.497	20	0.513	0.543	0.023	*
Estimated Moisture Source	39	0.682	0.142	20	0.669	0.158	0.746	
Ratio Calc./Est.	35	1.276	0.722	20	0.912	1.312	0.190	
Difference Calc.-Est.	35	0.177	0.472	20	-0.156	0.625	0.030	*
No. of periods with condensation	38	51.32	68.00	20	23.25	59.77	0.126	
Longest condensation period	38	21.87	38.49	20	12.10	16.44	0.284	
Total hours of condensation	38	277	627	20	144	463	0.407	
No. of periods likely to promote mold growth	38	6.921	8.059	20	4.000	9.481	0.222	
Cond. Related GPS	39	1329	2876	20	1060	2318	0.718	
Other Mold Growth	39	9228	29960	20	832	2242	0.218	
CO2 NightAvg minus LowPtAvg	38	419	257	20	534	343	0.156	
CO2 Ratio Night/Day minus LwPtAvg	38	3.650	3.224	20	3.493	1.833	0.842	
Endotoxin	39	33.15	16.54	20	23.85	17.10	0.048	*
Ergosterols	39	38.72	13.64	20	13.00	0.00	0.000	*
Mite F	39	32.23	18.18	20	25.65	14.33	0.164	
Mite P	39	32.19	17.26	20	25.73	16.47	0.172	
CFU Glys	39	35.74	17.64	20	18.80	8.86	0.000	*
am peak flow (% of estimated)	38	94.32	22.56	20	96.60	33.72	0.760	
am-pm average peak flow/predicted	38	-4.59	11.41	20	-5.91	16.20	0.719	
CD4CD8CT	36	1.556	0.366	20	1.813	0.566	0.044	*
CD45O3CT	36	1.472	0.553	20	1.140	0.635	0.046	*
Mean RH	37	0.345	0.095	20	0.379	0.103	0.214	
Mean CO2	39	814.6	238.5	20	892.9	391.4	0.344	
Mold Area	39	1.173	3.044	20	0.414	0.964	0.283	

* $p \leq 0.05$

See Table 2 for Units

Table 2 Correlation Coefficients

	Average Air Change Rate	Calculated Moisture Source	No. of periods with condensation	Longest condensation period	Total hours of condensation	No. of periods likely to promote mold growth	Cond. Related GPS	Other Mold Growth	CO2 NightAvg minus LowPtAvg
Units	AC/hr	kg/hr		hrs	hrs		wk*cm^2	m^2	ppm
Average Air Change Rate	1.000								
Calculated Moisture Source	0.511	1.000							
No. of periods with condensation	-0.202	0.532	1.000						
Longest condensation period	0.041	0.584	0.647	1.000					
Total hours of condensation	-0.011	0.638	0.808	0.946	1.000				
No. of periods likely to promote mold growth	-0.206	0.539	0.960	0.522	0.711	1.000			
Cond. Related GPS	-0.207	0.133	0.505	0.159	0.244	0.496	1.000		
Other Mold Growth	-0.145	-0.099	-0.028	-0.049	-0.052	-0.018	-0.007	1.000	
CO2 NightAvg minus LowPtAvg	***-0.277***	0.019	0.203	0.212	0.174	0.158	***0.320***	0.031	1.000
Endotoxin	***0.283***	-0.020	-0.143	0.001	-0.051	-0.152	-0.076	-0.072	-0.187
Ergosterols	**0.227**	0.197	0.217	0.146	0.120	0.172	0.051	0.133	-0.107
Mite F	0.177	0.215	0.101	-0.031	-0.010	0.111	0.076	0.187	0.173
Mite P	**0.222**	***0.463***	0.214	***0.260***	**0.231**	0.181	**0.252**	-0.085	0.131
CFU Glys	***0.367***	***0.436***	0.092	0.205	0.160	0.046	-0.119	0.049	-0.150
am peak flow (% of estimated)	0.038	-0.101	-0.168	-0.120	-0.141	-0.197	-0.085	-0.038	-0.191
am-pm average peak flow/predicted	0.071	0.052	-0.013	-0.003	-0.026	0.014	-0.127	0.129	0.048
CD4CD8CT	-0.158	0.082	0.133	0.122	0.094	0.140	0.162	-0.054	**0.253**
CD45O3CT	0.022	0.124	***0.370***	***0.424***	***0.365***	***0.361***	0.177	-0.007	-0.201

Values formatted as ***2.60*** reject null hypothesis at 0.05 level of significance
Values formatted as **2.21** reject null hypothesis at 0.1 level of significance
based on two tailed student T distribution

Prepared by Morrison Hershfield Ltd. 7/5/96

	Endotoxin	Ergosterols	Mite F	Mite P	CFU Glys	am peak flow (% of estimated)	am-pm average peak flow/predicted	CD4CD8CT	CD45O3CT
Units	rank	rank	rank	rank	rank				
Average Air Change Rate									
Calculated Moisture Source									
No. of periods with condensation									
Longest condensation period									
Total hours of condensation									
No. of periods likely to promote mold growth									
Cond. Related GPS									
Other Mold Growth									
CO2 NightAvg minus LowPtAvg									
Endotoxin	1.000								
Ergosterols	0.030	1.000							
Mite F	-0.097	0.262	1.000						
Mite P	-0.009	0.178	***0.622***	1.000					
CFU Glys	0.158	***0.268***	***0.299***	***0.403***	1.000				
am peak flow (% of estimated)	0.129	-0.189	0.191	0.022	0.134	1.000			
am-pm average peak flow/predicted	-0.193	0.055	0.007	-0.071	0.036	0.002	1.000		
CD4CD8CT	**-0.254**	-0.165	-0.007	0.214	-0.180	***-0.359***	0.103	1.000	
CD45O3CT	0.161	0.138	0.047	0.063	**0.230**	0.043	**0.227**	-0.204	1.000

3.5.2 Detailed Statistical Analysis of Measured Health Parameters

A key component of the work undertaken in this extension was a more detailed examination of the apparent statistical relationship in levels of biological contamination and lymphocyte counts made from blood samples of the index children. This work was undertaken under subcontract by Dr. Corinne Dulberg. She worked closely with Dr. Robert Dales, the Health Canada Advisor on this project. A detailed discussion of the methods and findings of this work is presented in a joint paper prepared by Drs. Dale and Dulberg[4]

The following is a summary of her methods and findings. A more detailed description of methods is included in Apppendix B

"T-tests indicated statistically significant ($p<0.05$) differences in CD4CD8, CD45RO3CT and CD45RO3 between good and bad houses. Between group mean differences were moderate in size (about half a standard deviation) for CD4CD8 and CD45RO3CT, and large (0.8 standard deviation) for CD45RO3. Point biserial correlations between house group and lymphocytes indicated that about 0.07 of the variance in CD4CD8 and CD45O3CT and 0.14 of the variance in CD45RO3 was explained by house group.

To determine if the univariate results were influenced by other characteristics of the child or the environment, multiple regression analyses controlled for factors which could have a biologically-plausible association with lymphocyte differentiation and which differed in prevalence between the home categories: child's age, dust mite antigens (der p 1 and der f 1), furry/feathered pets in the home (no/yes), presence of a "cold" of the child at the time of sampling (no/yes), and presence of a humidifier in the child's bedroom (no/yes). Mean CO_2 and concentration of Volatile Organic Compounds (VOC) measured during the monitoring period in each house were considered for entry as potential control factors in separate regression models.

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4 Dales, R., Miller, D., White, J., Dulberg, C. and Lazarovitz A.,*The Influence of Residential Fungal Contamination on Peripheral Blood Lymphocyte Populations in Children, Submitted*

In view of the limited available sample size, only subsets of control factors were included in the regression models. Age was always controlled and forward stepwise entry (with a liberal entry criterion) was used to select other control factors relevant for each lymphocyte subtype.

In the regression models, lymphocyte subtype was the dependent measure; mold and fungi (CFU Glys and bedroom ergosterol) the independent variables. The primary statistic of interest in these analyses was the increment in R^2, or the unique contribution to the variance in the lymphocyte made by inclusion of mold area and the measure(s) of fungal contamination.

Controlling for age and dust mites, observable mold area and measures of fungi accounted for small, non-significant ($p<0.10$) proportions of variance in CD4CD8 ($R^2 = 0.03$ and 0.08, respectively). The inclusion of CO_2 level as a control factor increased the proportion of variance in CD4CD8 explained by mold area ($R^2 = 0.05$), but decreased the proportion of variance accounted for by the two measures of fungi ($R^2 = 0.04$).

CD45RO3CT was not associated with observable mold area, before or after controlling for other factors. The two fungi measures accounted for small, non-significant ($p<0.10$) proportions of variance in this lymphocyte before and after controlling for age and whether the child had a cold ($R^2 = 0.08$ and 0.09, respectively). Neither CO_2 nor VOCs were relevant potential confounders for this lympohocyte. Similar results held for analysis of CD45RO3, controlling for age (no other factor was selected for entry).

Table 3. Univariate analyses: means, t-tests and "effect sizes" (standardized mean differences) between each lymphocyte factor and "good/bad" house.

LYMPHOCYTE	MEAN LYMPHOCYTE BY "GOOD/BAD" HOUSE		t-test *p*-value	effect size
	Less Contaminated (n=20) Mean (sd)	More Contaminated (n=37) Mean (sd)		
CD4CD8 % CD4:CD8 ratio	1.8 (0.57)	1.6 (0.36)	0.042	0.54
CD45RO3CT Count of CD3+ cells expressing CD45RO	1.1 (0.63)	1.5 (0.55)	0.046	0.55
CD45RO3 % of CD3+ T cells expressing CD45RO	43.6 (16.4)	56.0 (14.8)	0.005	0.76
CD29HIGH % of CD3+ T cells expressing CD29 high	28.2 (8.5)	32.0 (6.9)	0.069	0.50
CD5 % of CD20+ cells expressing CD5	53.6 (10.0)	57.7 (8.2)	0.109	0.46

Table 4. Results of hierarchical entry multiple regression analyses: increment in R^2 in variance of lymphocyte subtype explained by molds and fungi before and after controlling for other factors. Age was always included as a control factor. Other control factors were selected via stepwise entry (p to enter of 0.15 and p to remove of 0.20) among furry/feathered pets, colds, dust mite antigens (der p 1 and der f 1), bedroom humidifier, log mean CO2 and log mean VOC.

DEPENDENT VARIABLE	INDEPENDENT VARIABLES ADDED TO MODEL			
	LOG MOLD AREA	LOG CFU GLYS	BEDROOM ERGOSTEROLS	LOG CFU GLYSUM + ERGOSTEROLS
Models:	$\_R^2$	$\_R^2$	$\_R^2$	$\_R^2$
CD4CD8				
IV(s) entered:				
alone	0.04	0.02	0.04	0.06
after AGE +	0.03	0.05$^+$	0.03	0.08$^+$
DER P 1 + DER F 1				
after AGE + COLDS + Log CO_2	0.05$^+$	0.03	0.02	0.04
CD45O3CT				
IV(s) entered:				
alone	<0.01	0.03	0.06$^+$	0.08
after AGE + COLDS	<0.01	0.02	0.07*	0.09$^+$
CD45RO3				
IV(s) entered:				
alone	<0.01	0.07$^+$	0.05$^+$	0.10
after AGE	<0.01	0.06$^+$	0.03	0.08$^+$
CD29HIGH				
IV(s) entered:				
alone	0.02	<0.01	0.06$^+$	0.06
after AGE[1]	<0.01	<0.01	0.02	0.02
CD5				
IV(s) entered:				
alone	<0.01	0.02	0.03	0.05
after AGE + PETS[1]	<0.01	<0.01	0.03	0.04
after AGE + PETS + HUMIDIF + LOG CO_2	<0.01	<0.01	0.02	0.03

* Significance of change in R^2 $p<0.05$

$^+$ Significance of change in R^2 $0.05<p<0.10$

[1] Humidifier entered as an additional control factor in other models, but results did not change.

4. CONCLUSIONS

Work undertaken in the extension to the contract leads to the following conclusions.

1. Moisture source strength calculated according to the methods described in Section 3.2 is significantly different in the good and bad houses and was one of the strongest predictors of measures of contamination level, including dust mite antigens and airborne viable particulates (colony forming units).

2. There is some indication that the method predicting air change (AIM-2 simulations) tends to underestimate air change at low predicted values and overestimate at high predicted values. This would have had an effect on the moisture source strength calculation, skewing the relationship with leakage area of the house and perhaps making it dependent on whether humidity readings were taken in periods of high or low driving forces.

3. The expansion of data on calculated moisture source strength strengthened the correlations with measures of biological contamination (mite antigens and mold colony forming units(CFUs))

4. Where measures of condensation potential are used as a combination variable (to relate air change, moisture source, indoor temperatures and weather), they were an apparently-strong predictor of changes in some lymphocyte measures, notably CD45O3CT.

5. More detailed analysis of the CO_2 monitoring showed significant elevations of CO_2 levels in the child's bedroom at night. The degree of elevation indicates that the ventilation provided to many of these bedrooms was inadequate to remove metabolic products. There were not the strong correlations expected between CO_2 increments and the reported number of occupants and whether or not the doors were kept open or closed.

6. The detailed statistical analysis of the mold and health data indicates the apparent association in lymphocyte subtype measured in the blood of the index children and mold growth factors, while the relationship was small, it remained after controlling for possible confounders including age of child, dust mite antigen levels, whether the child had a cold, CO2 levels and VOC levels.

Mark Lawton, P.Eng.
Building Science Specialist

Appendix A - Data Tables

		Moisture Source Analysis					Check
House ID No.	Sample Set	Average Air Change Rate	Calculated Moisture Source	Estimated Moisture Source	Ratio Calc./Est.	Difference Calc.-Est.	Ratio Night/Day Wiø
Units		ACH	kg/hr	kg/hr		kg/hr	
10	Bad	0.36	0.991	0.610	1.623	0.381	0.998
24	Bad	1.11	0.348	0.665	0.524	-0.317	1.021
28	Bad	1.14	1.200	0.669	1.794	0.531	1.020
30	Bad	2.03		0.560			
31	Bad	0.62	0.440	0.844	0.521	-0.404	1.001
34	Bad	0.42	0.670	0.663	1.011	0.008	1.024
43	Bad	0.67	0.779	0.788	0.989	-0.008	0.978
45	Bad	2.74	1.760	0.877	2.007	0.883	1.065
48	Bad	0.27	0.380	0.600	0.633	-0.220	1.049
55	Bad	1.10	0.977	0.471	2.075	0.506	1.030
86	Bad	2.90	1.214	0.940	1.292	0.274	1.053
96	Bad	0.69	0.605	0.702	0.862	-0.097	0.987
100	Bad	0.16	0.423	0.554	0.763	-0.131	1.019
102	Bad	0.67	0.819	0.823	0.995	-0.004	1.026
111	Bad	0.19	0.305	0.640	0.477	-0.335	0.977
115	Bad	0.42	0.601	0.494	1.217	0.107	1.026
117	Bad	0.45	0.542	0.763	0.711	-0.221	1.004
122	bad	1.33	0.779	0.663	1.176	0.117	0.915
132	Bad	1.08	0.659	0.579	1.138	0.080	1.076
134	Bad	1.60	2.432	0.815	2.986	1.617	1.072
136	Bad	1.14	1.050	0.571	1.839	0.479	1.013
150	Bad	0.48	1.040	0.533	1.950	0.507	0.984
154	Bad	0.45		1.046			
157	Bad	0.47		0.788			
167	Bad	0.60	0.621	0.694	0.895	-0.073	1.074
177	Bad	1.79	1.190	0.827	1.439	0.363	1.077
190	Bad	1.19	0.583	0.648	0.900	-0.065	1.081
197	Bad	0.43	0.447	0.673	0.664	-0.226	0.972
201	Bad	0.55	0.629	0.788	0.799	-0.159	1.094
206	Bad	1.45	0.376	0.567	0.664	-0.191	1.057
216	Bad	2.04	1.710	0.506	3.378	1.204	1.012
235	Bad	1.94	0.520	0.798	0.652	-0.278	0.859
247	Bad	0.72	1.180	0.856	1.378	0.324	1.069
250	Bad	0.23	0.110	0.488	0.226	-0.378	0.994
276	Bad			0.627			
332	Bad	1.89	0.691	0.642	1.077	0.049	1.026
377	Bad	1.02	1.093	0.467	2.342	0.626	1.041
395	Bad	0.79	0.736	0.513	1.436	0.224	1.039
396	Bad	1.99	1.860	0.842	2.210	1.018	1.069

			Moisture Source Analysis				Check
House ID No.	Sample Set	Average Air Change Rate	Calculated Moisture Source	Estimated Moisture Source	Ratio Calc./Est.	Difference Calc.-Est.	Ratio Night/Day Wiø
2	good	0.82	0.686	0.565	1.215	0.121	1.017
19	Good	0.32	0.195	0.933	0.209	-0.738	0.961
78	Good	0.30	0.267	0.917	0.291	-0.650	1.098
81	Good	1.00	0.984	0.581	1.693	0.403	1.051
109	Good	0.25	0.332	0.854	0.389	-0.522	0.973
138	Good	0.53	0.560	0.619	0.905	-0.059	1.037
215	Good	1.07	0.265	0.550	0.482	-0.285	0.933
226	Good	1.75	0.417	0.742	0.562	-0.325	0.973
232	Good	0.43	0.401	0.594	0.675	-0.193	0.877
267	Good	0.54	0.299	0.594	0.504	-0.295	1.021
274	Good	0.29	0.152	0.633	0.240	-0.481	0.921
352	Good	0.24	0.161	0.629	0.256	-0.468	0.955
356	Good	0.80	0.135	0.723	0.187	-0.588	1.032
409	Good	0.81	0.162	0.463	0.350	-0.301	1.013
414	Good	0.58	2.598	0.423	6.143	2.175	1.015
419	Good	0.32	0.308	0.596	0.517	-0.288	0.959
431	Good	0.80	0.628	0.860	0.730	-0.232	1.041
432	Good	0.55	0.365	0.854	0.427	-0.489	1.023
434	Good	1.02	0.780	0.438	1.783	0.343	1.022
447	Good	0.76	0.555	0.804	0.690	-0.249	0.959
Bad	N	38	35	39	35	35	34
	Mean	1.03	0.850	0.682	1.276	0.177	1.021
	Std. Dev	0.71	0.497	0.142	0.722	0.472	0.048
Good	N	20	20	20	20	20	20
	Mean	0.66	0.513	0.669	0.912	-0.156	0.994
	Std. Dev.	0.37	0.543	0.158	1.312	0.625	0.052
	TTest (p	0.03	0.02	0.75	0.19	0.03	0.97

		Predicted Condensation on Windows					
House ID No.	Sample Set	No. of periods with condensation	Longest condensation period	Total hours of condensation	No. of periods likely to promote mold growth	Cond. Related Mold Growth Potential Score	Other Mold Growth
Units		no.	hrs	hrs	no	wks * cm2	cm2
10	Bad	180	22	558	21	1761	22144
24	Bad	15	10	44	2	3	0
28	Bad	107	40	478	16	2986	3050
30	Bad	0	0	0	0	0	1279
31	Bad	5	7	20	1	9	3000
34	Bad	232	47	1107	25	11850	15
43	Bad	27	12	76	4	286	2
45	Bad	6	10	29	2	143	1300
48	Bad	6	10	29	2	27	11000
55	Bad	44	21	138	5	646	12330
86	Bad	3	7	13	1	0	320
96	Bad	5	7	14	1	100	100
100	Bad	101	22	305	13	1653	100
102	Bad	24	14	77	4	309	270
111	Bad	20	10	64	4	168	184748
115	Bad	81	21	254	18	985	60
117	Bad	69	21	254	10	1200	16700
122	bad	20	23	129	5	0	0
132	Bad	4	7	12	1	86	850
134	Bad	195	240	3543	15	429	320
136	Bad	2	7	12	1	29	300
150	Bad	94	21	295	12	12806	19600
154	Bad	262	63	1559	36	1286	300
157	Bad	82	21	244	10	6514	2300
167	Bad	26	20	99	4	198	500
177	Bad	14	10	43	4	571	2700
190	Bad	3	3	6	0	0	200
197	Bad	118	21	343	12	3177	500
201	Bad	26	20	100	4	400	16700
206	Bad	0	0	0	0	0	37250
216	Bad	85	25	362	13	929	400
235	Bad	2	7	12	1	0	0
247	Bad	32	21	113	5	494	300
250	Bad	2	7	10	0	0	0
276	Bad					0	450
332	Bad	0	0	0	0	0	1952
377	Bad	8	10	32	3	171	4650
395	Bad	36	14	88	4	464	2500
396	Bad	14	10	48	4	2171	11710

		Predicted Condensation on Windows					
House ID No.	Sample Set	No. of periods with condensation	Longest condensation period	Total hours of condensation	No. of periods likely to promote mold growth	Cond. Related Mold Growth Potential Score	Other Mold Growth
2	good	3	6	10	0	4	600
19	Good	3	7	11	0	0	20
78	Good	2	6	9	0	3	0
81	Good	2	7	11	1	0	150
109	Good	10	10	36	3	643	1
138	Good	61	21	201	13	8844	0
215	Good	0	0	0	0	0	70
226	Good	2	7	11	1	0	500
232	Good	4	6	17	1	129	5000
267	Good	2	5	8	0	0	180
274	Good	0	0	0	0	0	0
352	Good	2	5	8	1	28	0
356	Good	14	30	129	5	2696	9125
409	Good	1	1	1	0	4526	0
414	Good	266	73	2095	42	4263	110
419	Good	54	21	175	6	0	0
431	Good	7	10	27	2	0	0
432	Good	2	7	12	1	57	200
434	Good	30	20	110	4	0	80
447	Good	0	0	0	0	0	600
Bad	N	38	38	38	38	39	39
	Mean	51.3	21.9	277	6.92	1329	9228
	Std. Dev	68.0	38.5	627	8.06	2876	29960
Good	N	20	20	20	20	20	20
	Mean	23.3	12.1	144	4.00	1060	832
	Std. Dev.	59.8	16.4	463	9.48	2318	2242
	TTest (p	0.13	0.28	0.41	0.22	0.72	0.22

		Analysis of CO2 Results									
House ID No.	Sample Set	Mean CO2 (from Phase II)	Nightime CO2 Level	Daytime CO2 Level	Ratio Night/Day CO2 Level	Average CO2 Low Point	NightAvg minus LowPtAvg	DayAvg minus LowPtAvg	CO2 Ratio Night/Day minus LwPtAvg	Nightime Occ'y	Nightime Door Open/ Close
Units		ppm	ppm	ppm		ppm	ppm	ppm		no of people	0=open
10	Bad	1512	1832	1137	1.61	700	1132	437	2.59	2	1
24	Bad	702	831	547	1.52	500	331	47	7.06	1	0
28	Bad	636	603	624	0.97	550	53	74	0.71	1	0
30	Bad	600	724	473	1.53	300	424	173	2.46	1	1
31	Bad	1144	1384	898	1.54	700	684	198	3.44	2	0
34	Bad	1499	1698	1218	1.39	1100	598	118	5.07	1	1
43	Bad	722	988	543	1.82	500	488	43	11.39	1	1
45	Bad	719	799	600	1.33	500	299	100	3.00	2	0
48	Bad	1000	1066	855	1.25	700	366	155	2.36	1	1
55	Bad	689	755	608	1.24	600	155	8	18.54	1	0
86	Bad	570	611	520	1.18	400	211	120	1.76	1	0
96	Bad	710	766	651	1.18	500	266	151	1.77	0	0
100	Bad	955	1212	712	1.70	500	712	212	3.35	1	1
102	Bad	647	743	582	1.28	500	243	82	2.96	1	0
111	Bad	949	1055	806	1.31	600	455	206	2.21	1	0
115	Bad	646	688	595	1.16	500	188	95	1.99	1	
117	Bad	1001	1360	661	2.06	400	960	261	3.68	1	1
122	bad	593	573	566	1.01	400	173	166	1.04	2	0
132	Bad	852	969	648	1.50	500	469	148	3.17	1	0
134	Bad	920	1310	579	2.26	400	910	179	5.09	3	0
136	Bad	669	707	634	1.11	500	207	134	1.54	1	0
150	Bad	874	1218	614	1.98	500	718	114	6.30	1	1
154	Bad	754	743	689	1.08	600	143	89	1.62	1	0
157	Bad	717									
167	Bad	995	1032	910	1.13	600	432	310	1.39	1	0
177	Bad	950	1105	848	1.30	600	505	248	2.04	1	1
190	Bad	579	708	452	1.57	400	308	52	5.88	1	1
197	Bad	1163	1202	1007	1.19	700	502	307	1.63	2	0
201	Bad	756	951	521	1.82	400	551	121	4.53	1	1
206	Bad	578	609	520	1.17	400	209	120	1.75	1	1
216	Bad	779	933	613	1.52	500	433	113	3.83	1	0
235	Bad	540	534	521	1.02	400	134	121	1.11	3	0
247	Bad	1032	1340	737	1.82	500	840	237	3.54	1	1
250	Bad	629	688	539	1.28	500	188	39	4.83	1	0
276	Bad	543	574	477	1.20	300	274	177	1.55	1	0
332	Bad	587	615	523	1.18	500	115	23	5.10	1	1
377	Bad	679	754	621	1.21	500	254	121	2.09	1	1
395	Bad	1041	1172	868	1.35	700	472	168	2.80	1	0
396	Bad	837	1006	644	1.56	500	506	144	3.51	2	0

			Analysis of CO2 Results								
House ID No.	Sample Set	Mean CO2 (from Phase II)	Nightime CO2 Level	Daytime CO2 Level	Ratio Night/Day CO2 Level	Average CO2 Low Point	NightAvg minus LowPtAvg	DayAvg minus LowPtAvg	CO2 Ratio Night/Day minus LwPtAvg	Nightime Occ'y	Nightime Door Open/ Close
2	good	763	985	644	1.53	400	585	244	2.40	1	1
19	Good	987	1163	836	1.39	600	563	236	2.39	1	0
78	Good	796	951	628	1.51	550	401	78	5.12	1	1
81	Good	422	511	330	1.55	300	211	30	7.04	2	0
109	Good	1157	1302	965	1.35	800	502	165	3.05	2	0
138	Good	2317	3121	1704	1.83	1500	1621	204	7.95	1	0
215	Good	648	765	555	1.38	500	265	55	4.86	1	0
226	Good	900	1088	658	1.65	400	688	258	2.67	1	1
232	Good	718	814	587	1.39	500	314	87	3.61	1	0
267	Good	1102	1308	900	1.45	500	808	400	2.02	1	0
274	Good	741	658	681	0.97	600	58	81	0.71		
352	Good	1215	1569	864	1.82	650	919	214	4.30	2	0
356	Good	737	834	575	1.45	400	434	175	2.48	1	1
409	Good	500	496	518	0.96	400	96	118	0.82	1	0
414	Good	831	777	509	1.53	400	377	109	3.48	1	0
419	Good	782	1038	467	2.22	300	738	167	4.40	1	0
431	Good	682	856	520	1.65	400	456	120	3.81	2	1
432	Good	904	1082	671	1.61	400	682	271	2.52	1	0
434	Good	675	751	579	1.30	400	351	179	1.96	1	0
447	Good	980	1200	740	1.62	600	600	140	4.28	2	0
Bad	N	39	38	38	38	38	38	38	38		37
	Mean	815	944	673	1.40	525	419	148	3.65		0.392
	Std. Dev	239	318	181	0.31	142	257	87	3.22		0.485
Good	N	20	20	20	20	20	20	20	20		19
	Mean	893	1064	696	1.51	530	534	166	3.49		0.237
	Std. Dev.	391	556	284	0.28	260	343	89	1.83		0.402
	TTest (p	0.34	0.30	0.70	0.21	0.92	0.16	0.44	0.84		0.24

Appendix B - Statistical Analysis of Health Data

Statistical Analysis of Health Data

by Corrine Dulberg, Ph.D.

The study objectives were:

1. To examine differences between less and more contaminated houses with respect to selected environmental characteristics, characteristics of index children, and lymphocyte immunophenotypes.

2. To examine differences in lymphocytes between more and less contaminated houses, before and after controlling for potentially confounding factors.

3. To determine whether levels of lymphocytes of the index children could be explained by relationships with other measures of fungal contamination, before and after controlling for potentially confounding factors.

ANALYSES

Analyses were run using SPSS/PC+ Version 4.0.1 and BMDP/PC Version 7.01. Prior to running the analyses, continuous variables were examined for non-normality using the Kolmorgorov-Smirnov test. Log (base 10) transformations were used, as necessary. Because of the high proportion (42%) of houses in the sample with non-detectable levels of ergosterol, no transformation was successful in normalizing this variable; therefore, ergosterol was dichotomized into measurable or not.

To avoid listwise deletion of cases in multivariate analyses, missing values of independent variables were replaced as follows: the five houses with information missing on pets were treated as not having pets; for the ten houses with missing information on derfs and derps, missing values for each variable

were replaced with the mean from the more/less contaminated house group to which the case belonged. Missing values were equally distributed between the home categories.

While levels of statistical significance are provided throughout, given the very large number of analyses conducted for such a small sample size, the overall error rate is far beyond the conventional 0.05 level. Because these analyses are exploratory in nature, alpha levels were not corrected for multiple comparisons. Results of inferential statistics should therefore only be used as a guide in evaluating the importance of various factors.

Objective 1. In the analyses performed to examine differences between less and more contaminated houses on selected variables, independent groups t-tests or chi squared tests were used, depending upon whether the variable was continuous or dichotomous. For these t-tests (alpha=0.05 2-tailed), 59 cases provided sufficient power, i.e. 80%, to detect a large effect size of 0.80. In addition to t-tests on lymphocyte factors, point biserial correlations were calculated to provide measures of the magnitude of associations between fungal contamination (i.e. good/bad house) and each lymphocyte measure.

Selection of potential confounding factors. The independent variables considered as factors to be controlled in multivariate analyses were chosen primarily on substantive grounds. These were factors that varied between good/bad houses and which could themselves be associated with levels of lymphocytes: age of index child, presence (no/yes) of furry/feathered pets in the house, whether or not the child had a cold at the time of the health survey, log derfs, log derps and presence of a humidifier in the index child's bedroom.

Given the limited available sample size, it was necessary to try to reduce the total number of factors that would be controlled in multivariate analyses. To this end, backwards logistic regression, with good/bad house as the dependent measure, was used to select key variables among the six potential control factors. Liberal values of p to enter and remove variables from the model ($p<.10$ and $p>.15$,

respectively) were used to try to ensure that all possible factors that should be controlled would be retained.

The six variables were examined in two logistic regression models. The first model tested five variables: age of index child, pets, whether or not the child had a cold, log derfs and log derps. The second model tested all six independent variables. Two variables remained in the first model: age and log derfs. Age, log derfs and presence of humidifier remained in the second model. Both sets of potential confounders were used in multivariate analyses.

Objective 2. The second objective addressed the question of whether the results of the univariate comparisons of lymphocytes between good/bad houses would change when controlling for potential confounders. The analytic approach used was hierarchical entry logistic regression, with good/bad as the outcome measure. Each lymphocyte factor was added to the model before and after both sets of control variables. Improvement in fit of the model from the addition of the lymphocyte and the overall goodness of fit of the model including the lymphocyte factor were examined.

Selection of components of fungal contamination. Houses had been classified into the categories of more or less contaminated by several criteria. The question was whether one or more of the objective fungal contamination measures was a critical component of the classification. To this end, stepwise discriminant function analysis was run, using good/bad house as the grouping variable and lglysum, ergosterol and log of total surface mold area (excluding attic mold) as the three predictors.

Two of the three variables entered the discriminant function: ergosterol and log glysum. Most of the variation in good/bad house was accounted for by the dichotomous variable, ergosterol (Wilks' Lambda=0.30). Lglysum, which entered at the second step in the model, improved prediction by a significant amount (Wilks' Lambda in the full model was 0.20, canonical correlation=0.90, c^2=91.2, df=2, p<.001).

The discriminant function containing these two factors was successful in accurately predicting 93% of the houses. All 20 houses in the less contaminated group were accurately classified; four of the 39 in the more contaminated group were misclassified. Three of these were among five "bad" houses that had non-detectable levels of ergosterol.

Objective 3. The first two objectives focused on the relationship between lymphocytes and level of fungal contamination defined by "good/bad" house. The third objective was to examine the relationship between lymphocytes and the two key criteria of fungal contamination, glysum and ergosterol (dichotomized), which formed the basis of the original good/bad dichotomy.

For this final set of analyses, hierarchical multiple regression was used to determine whether each lymphocyte was related to the two measures of contamination, before and after controlling for the two sets of potential confounders. For these analyses, each lymphocyte factor was the dependent measure; the independent variables were lglysum, ergosterol or lglysum plus ergosterol. Lymphocytes were used as the dependent measures for two reasons: 1) to determine whether objective measures of fungal contamination could be used to predict level of the lymphocytes; and 2) because this made more sense, given the plausible causal pathway for the relationship between lymphocyte and fungal contamination.

A total of nine models were examined for each lymphocyte: lglysum, ergosterol and lglysum plus ergosterol were entered as the sole variable(s) in the model as well as after each of the two sets of confounders. For each model, residual statistics (including scatterplots of predicted values by standardized residuals, normal probability plots of the standardized residuals, and Cook's distance) were examined to verify that assumptions of the test were met and that there was no influential outlier.

The primary statistic of interest in these analyses was the increment in R^2, or the unique contribution to the variance in the lymphocyte made by inclusion of the measure(s) of fungal contamination. For the multiple regression analyses, there was approximately 80% power (alpha=0.05) to detect an increment

in R^2 of 0.12 attributable to the addition of one variable after two or three variables were already in the model; there was 80% power to detect an increment in R^2 of 0.15 attributable to the addition of two variables to the model. (Borenstein, M and Cohen, J. Statistical Power Analysis: A computer Program. Hillsdale, New Jersey: Lawence Erlbaum Associates, Inc., 1988.)

RESULTS

Objective 1. Tables 1 through 3 present descriptive statistics of selected environmental characteristics, characteristics of index children, and lymphocyte characteristics between the less and more contaminated houses.

Table 1 provides descriptive statistics, results of t-tests and the point biserial correlations between each of the six selected lymphocyte factors and good/bad houses. Each lymphocyte had at least a small correlation with good/bad house (r's > 0.20) with the range from 0.22 (CD5) to 0.37 (CD45RO3), and only four of the six correlations (and t-tests) reached the conventional criterion of $p<0.05$.

Objective 2. Table 2 presents the results of the hierarchical logistic regression analyses with good/bad house as the dependent variable. When examining results, keep in mind that the *p*-value for the improvement chi squared has the conventional interpretation of the lower the value the better, but that the interpretation for the goodness of fit chi squared *p*-value, is completely opposite: the higher the *p*, the better the fit between observed and expected frequencies.

The *p*-values for the improvement in the goodness of fit chi squared tests with only lymphocytes in the models were completely consistent with the point biserial correlations. Among the six models containing a single lymphocyte as the independent variable, the best fitting model contained CD45RO3 (*p*-value for the Hosmer-Lemeshow goodness of fit test = 0.946).

For CD45O3CT and CD45RO3, the addition of the lymphocyte after age and log derfs significantly (p <0.05) improved the fit of the model, but CD4CD8 was only borderline. For the other three lymphocytes, control of age and log derfs essentially eliminated the contribution of the lymphocytes to the fit of the models.

With humidifier included in the set of control factors, CD45O3CT and CD45RO3 still significantly improved the fit of the model (p <0.05), but CD4CD8 and CD5 were borderline. Goodness of fit of the full models was acceptable to good for most of the models, but the drastic reduction of goodness of fit for CD45RO3 after inclusion of control variables is notable.

Objective 3. Table 3 presents the results of the hierarchical entry multiple regression analyses. In each model, the dependent measures were the six measures of lymphocytes. In the first set of models for each lymphocyte, lglysum was entered alone, then after age and log derfs, and finally, after age, log derfs plus humidifier. In the second set of analyses, ergost2 was entered before and after the two groups of control factors. Finally, both lglysum and ergost2 two were entered before and after the control factors. The table provides the R for the full model, and the increment in R^2 (along with the p-value of the F for change) resulting from the addition of the fungal measure(s).

Not surprisingly, given the very strong correlation between good/bad and ergosterol, ergosterol was more strongly associated than lglysum with most lymphocytes. Surprisingly, no general statement can be made about the impact of controlling age and log derfs or age, log derfs and humidifiers. Looking within each cell of the table, one can see that the addition of more control factors usually, but not always, resulted in a decrease in the unique proportion of variance in the dependent measure explained by the fungal measure(s). Although the full models had large multiple correlations (R) between lymphocytes and the linear combinations of the factors in the models, it was only with CD45O3CT that the additional of the fungal measure(s) resulted in a significant increase in R^2.

Table 1. Univariate analyses: means, t-tests and point biserial correlations between each lymphocyte factor and "good/bad" house.

LYMPHOCYTE	MEAN LYMPHOCYTE BY "GOOD/BAD" HOUSE		t-test	point biserial
	Less Contaminated	More Contaminated	*p*-value	corr.
CD4CD8 % CD4:CD8 ratio	1.81 (n=20)	1.56 (n=37)	0.042	-0.27
CD45RO3CT Count of CD3+ cells expressing CD45RO	1.14 (n=20)	1.47 (n=36)	0.046	0.27
CD45RO3 % of CD3+ T cells expressing CD45RO	43.55 (n=20)	55.95 (n=37)	0.005	0.37
CD29HIGH % of CD3+ T cells expressing CD29 high	28.15 (n=20)	32.00 (n=37)	0.069	0.24
CD5 % of CD20+ cells expressing CD5	53.56 (n=18)	57.70 (n=37)	0.109	0.22
RANROP % of CD3+ T cells expressing RA-RO+	15.85 (n=20)	20.19 (n=37)	0.047	0.26

Table 2. Results of hierarchical logistic regression analyses predicting good/bad house. Each lymphocyte included in model as sole variable or added to model after two sets of control factors. Table provides *p*-values for: 1) the chi squared test for improvement in goodness of fit adding each lymphocyte factor; and 2) the Hosmer-Lemeshow goodness of fit chi squared test for the full model.

LYMPHOCYTE	LYMPHOCYTE ADDED TO MODEL CONTAINING:					
	-- no other variable		AGE + DERFS		AGE + DERFS + HUMIDIFIER	
	Improvemnt c^2	c^2 Full Model	Improvemnt c^2	c^2 Full Model	Improvemnt c^2	c^2 Full Model
CD4CD8	0.041	0.226	0.073	0.844	0.057	0.359
CD45RO3CT	0.038	0.041	0.007	0.222	0.016	0.262
CD45RO3	0.005	0.946	0.013	0.554	0.024	0.105
CD29HIGH	0.064	0.274	0.318	*na*	0.133	0.467
CD5	0.102	0.043	0.232	*na*	0.066	0.072
RANROP	0.043	0.098	0.233	*na*	0.133	0.170

na lymphocyte variable did not enter, hence no value for goodness of fit of model containing it

Table 3. Results of Hierarchical Entry Multiple Regression Analyses:

Dependent variable = lymphoctye
Independent Variables:
models 1,2,3 - LGLYSUM (alone), ERGOST2 (alone), LGLYSUM + ERGOST2
models 4,5,6 - fungal measures entered after AGE + DERPS
models 7,8,9 - fungal measures entered after AGE + DERPS + HUMIDIFIERS

DEPENDENT VARIABLE	INDEPENDENT VARIABLES ADDED TO MODEL								
	LGLYSUM			ERGOST2			LGLYSUM + ERGOST2		
Models	R full model	ΔR^2	Sig of change	R full model	ΔR^2	Sig of change	R full model	ΔR^2	Sig of change
CD4CD8									
IV(s) entered:									
alone	0.16	.02	(.242)	0.21	.04	(0.122)	0.25	.06	(0.178)
after AGE + DERFS	0.19	.02	(.336)	0.22	.03	(0.208)	0.25	.05	(0.281)
after AGE + DERFS + +HUMIDIFIER	0.19	.02	(.348)	0.22	.03	(0.221)	0.26	.05	(0.300)
CD45O3CT									
IV(s) entered:									
alone	0.16	.03	(0.242)	0.24	.06	(0.072)	0.28	.08	(0.124)
after AGE + DERFS	0.20	.04	(0.164)	0.30	.08	(0.033)	0.35	.12	(0.039)
after AGE + DERFS + +HUMIDIFIER	0.25	.03	(0.202)	0.36	.08	(0.035)	0.35	.11	(0.048)
CD45RO3									
IV(s) entered:									
alone	0.26	.07	(.055)	0.22	.05	(0.096)	0.32	.10	(0.051)
after AGE + DERFS	0.30	.04	(.115)	0.26	.02	(0.244)	0.34	.07	(0.142)
after AGE + DERFS + +HUMIDIFIER	0.35	.04	(.125)	0.32	.02	(0.284)	0.38	.06	(0.169)
CD29HIGH									
IV(s) entered:									
alone	0.08	.01	(0.554)	0.24	.06	(0.073)	0.24	.06	(0.185)
after AGE + DERFS	0.41	<.01	(0.692)	0.43	.02	(0.241)	0.44	.02	(0.465)
after AGE + DERFS + +HUMIDIFIER	0.47	<.01	(0.625)	0.50	.03	(0.175)	0.50	.03	(0.354)
CD5									
IV(s) entered:									
alone	0.14	.02	(0.321)	0.18	.03	(0.173)	0.22	.05	(0.266)
after AGE + DERFS	0.21	.02	(0.331)	0.22	.02	(0.252)	0.26	.05	(0.300)
after AGE + DERFS + +HUMIDIFIER	0.34	.02	(0.286)	0.36	.03	(0.198)	0.39	.05	(0.223)
RANROP									
IV(s) entered:									
alone	0.11	.01	(0.421)	0.22	.05	(0.101)	0.24	.06	(0.212)
after AGE + DERFS	0.35	<.01	(0.839)	0.36	.01	(0.432)	0.36	.01	(0.720)
after AGE + DERFS + +HUMIDIFIER	0.36	<.01	(0.813)	0.38	.01	(0.398)	0.38	.01	(0.681)