

Neurobehavioral and pulmonary impairment in 105 adults with indoor exposure to molds compared to 100 exposed to chemicals

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Abstract

Patients exposed at home to molds and mycotoxins and those exposed to chemicals (CE) have many similar symptoms of eye, nose, and throat irritation and poor memory, concentration, and other neurobehavioral dysfunctions. To compare the neurobehavioral and pulmonary impairments associated with indoor exposures to mold and to chemicals, 105 consecutive adults exposed to molds (ME) indoors at home and 100 patients exposed to other chemicals were compared to 202 community referents without mold or chemical exposure. To assess brain functions, we measured 26 neurobehavioral functions. Medical and exposure histories, mood states score, and symptoms frequencies were obtained. Vital capacity and flows were measured by spirometry. Groups were compared by analysis of variance (ANOVA) after adjusting for age, educational attainment, and sex, by calculating predicted values ($\text{observed/predicted} \times 100 = \% \text{ predicted}$). And $p < .05$ indicated statistical significance for total abnormalities, and test scores that were outside the confidence limits of the mean of the percentage predicted. People exposed to mold had a total of 6.1 abnormalities and those exposed to chemicals had 7.1 compared to 1.2 abnormalities in referents. Compared to referents, the exposed groups had balance decreased, longer reaction times, and blink reflex latencies lengthened. Also, color discrimination errors were increased and visual field performances and grip strengths were reduced. The cognitive and memory performance measures were abnormal in both exposed groups. Culture Fair scores, digit symbol substitution, immediate and delayed verbal recall, picture completion, and information were reduced. Times for peg-placement and trail making A and B were increased. One difference was that chemically exposed patients had excess fingertip number writing errors, but the mold-exposed did not. Mood State scores and symptom frequencies were greater in both exposed groups than in referents. Vital capacities were reduced in both groups. Neurobehavioral and pulmonary impairments associated with exposures to indoor molds and mycotoxins were not different from those with various chemical exposures.

Keywords

Balance, visual field performance, memory, trichothecene-mycotoxin, neuropsychological impairment, neurophysiological tests, cognitive function, memory deficits

Introduction

People's adverse health effects were attributed to indoor air after the 1973 energy crisis increased air recycling and decreased air leaks from buildings (Dalley et al., 1981; Kress, 1990; Small and Borus, 1983). Mold disease emerged in the 1990s with infantile pulmonary hemosiderosis in Cleveland, Ohio (Centers for Disease Control and Prevention [CDCP], 1994; Etzel et al., 1998; Montana et al., 1997). News media, insurance companies, courts, physicians, and

government agencies have doubts about indoor air disorders attributing to molds. Literally, people's descriptions of sickness related to musty odors and

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black mold on walls were not believed (Hardin et al., 2003; Institute of Medicine, 2004; Johanning et al., 1996; Mazur and Kim, 2006; Storey et al., 2004).

Disbelief took common forms. People were labeled variously as hysterical (Small and Borus, 1983), having crowd phenomena (Landrigan and Miller, 1983; Hefez, 1985) and somatization reactions (Hardin et al., 2003). Epidemiological studies (Dales, 1991; Burge et al., 1987; Andersson, 1997; Kilburn 2002, 2003; Menzies et al., 1993) showed flu-like symptoms, eye and respiratory tract irritation, chronic fatigue, skin irritations, joint pain, dizziness and impaired balance, and inability to co-process or to remember frequently used information. Initially, symptoms diminished during days out of the building, later they persisted (Menzies et al., 1993) even after air turnovers were increased (Burge et al., 1987).

Ten years ago, infant deaths from pulmonary hemorrhage associated with molds such as *Stachybotrys chartarum* in excessively humid homes were differentiated from previous indoor air problems (CDCP, 1994; Etzel et al., 1998; Montana et al., 1997). They appear to have heralded mold disorders in children and adults in the United States. Contributors to the mold syndrome are *Stachybotrys* species: *Aspergillus/Penicillium* species, *Cladosporium*, *Fusarium*, *Actinomyces* (*Nocardia*, *Streptomyces*, especially *californicus*) and bacteria (Mirocha et al., 1977; Bennett and Klich, 2003).

Moisture in poorly ventilated walls, attics, and crawl spaces encourages fungal and bacterial growth particularly on drywall (gypsum board) with paper (cellulose) on both sides, which nourishes fungi. Water enters the walls from leaks of pipes, refrigerator icemaker tubes, inadequately sealed external walls and roofs, and from condensation in air conditioning ducts (Kilburn, 2003; Vojdani et al., 2003). Indoor mold sickness was recognized co-temporally with the destruction of Caribbean coral reefs and growth of *Aspergillus showaii* (Shinn, 2000) and the doubling or tripling of asthma prevalence in the United States (Burge and Roger, 2000; Burr, 1993; Howitt et al., 1998) and in several Caribbean Islands suggesting an association.

The 105 symptomatic patients from moldy homes were evaluated interspersed with 100 patients exposed to chemicals (CE) without asymptomatic unexposed (UE) control subjects. We compared each patient's scores to their predicted values for each test expressed as percentage of predicted and compared them to the 202 referent subjects (Kilburn et al.,

1998a,b; used to derive the prediction equations), which adjusted for age, sex, educational attainment in years, and other factors with statistically significant coefficients for performance of 26 neurobehavioral tests. Spirometric measurements of pulmonary function were compared similarly.

Methods

Adults, 69 women and 36 men, exposed to molds (ME) in homes in California, Arizona, and Texas were a self-selected consecutive case series, studied from 2001 to 2003. The matched 53 women and 47 men were CE and so were all other patients seen during this period. It would be ideal to measure mycotoxins and other adverse factors in indoor air, but methods such as for mold toxins, enzymes, and monoclonal antibodies and DNA fluorescence after its augmental by the polymerase chain reaction (PCR) are under development. All mold patients' exposures were in homes with mold growth observed on walls and/or floors. Surface samples lifted on transparent tape helped identify molds microscopically. Cultures of indoor air samples and scraping frequently grew *Stachybotrys chartarum*, *Aspergillus*, *Penicillium*, *Chaetomium*, *Alternaria*, *Fusarium*, and *Rhizopus*. For most homes that were sampled, concentrations of mold spores in indoor air samples exceeded those in outdoor air by multiples of 4 or more.

The 100 consecutive CE patients (53 women and 47 men) tested concurrently had 19 exposed to hydrogen sulfide, 13 to diesel exhaust, 9 to formaldehyde and indoor air, 8 to organophosphate insecticides, 7 to glutaraldehyde, 7 to cleaning chemicals, 4 to polychlorinated biphenyls, 4 to carbon monoxide, 3 to chlorine, 3 to chlordane, and 27 to 11 other chemicals.

The 105 mold patients (69 women and 36 men), and the 100 CE patients (53 women and 47 men), and 202 (107 women and 95 men) UE people were compared by analysis of variance (ANOVA) after every observed value was divided by its predicted value. Each subject-observed values for each test was divided by the predicted value, multiplied by 100, and expressed as percentage predicted that adjusted each measurement for gender, height, weight, and education (highest school grade completed). Kindergarten through 12th grade equals 12 years, a bachelor's degree equals 16 years, and a doctoral degree equals 20 years.

The 202 UE subjects group's data were pooled and distributions plotted, and numbers transformed when that improved symmetry and then regression

equations were developed to predict each persons score for each test. The 202 UE subjects were drawn at random from voter registration rolls. They were interviewed to exclude occupational chemical exposures and medical or neurological diseases and 105 women and 95 men measured to develop the prediction equations (Kilburn et al., 1998a,b). The UE subjects were reimbursed for their time.

All subjects in the three groups gave informed consent to the protocol approved by the Human Studies Research Committee of the University of Southern California, Keck School of Medicine.

Questionnaires were checked by computer-guided reading, so subjects rectified their omissions as they completed them. They rated the frequencies of 35 common health complaints (Kilburn et al., 1987) from 1 for never to 11 for daily. They answered the American Rheumatism Association lupus erythematosus questions (Levin et al., 1984); a standard respiratory questionnaire (Ferris, 1978); and gave histories of occupational and other exposures to chemicals, pesticides and herbicides, tobacco, alcohol, and drug use (prescription and illicit). Histories recorded unconsciousness, anesthesia, head trauma, and medical and neurological psychiatric illnesses (Kilburn, 1994). Questionnaires had been tested in groups of people exposed to formaldehyde (Kilburn et al., 1987), thermolysis products of PCBs (Kilburn et al., 1989), to toluene-rich chemical waste (Kilburn and Warshaw, 1995), and subjects without (known) chemical exposure (Kilburn and Warshaw, 1995; Kilburn et al., 1998a,b).

Neurophysiological tests

Simple reaction time (SRT) and visual two choice reaction time (CRT) were timed from appearance to cancellation of the 10-cm block A (simple) and A and S (choice); (Miller et al., 1989). The lowest median score of the last seven in each of the two trials of 20 was accepted for SRT and for CRT.

Balance was measured at body position by tracking a sound-generating stylus on a headband with two microphones mounted in the plane of the head while standing erect with feet together. Data were computer processed for mean speed of sway in cm/sec (Kilburn and Warshaw, 1994). Then the minimal values of the three consecutive 20-sec trials with eyes open alternating with three with eyes closed were recorded.

Surface electromyographic electrodes (EMG) recorded blink R-1 from lateral orbicularis oculi muscles after tapping the right and left supraorbital

notches with a light hammer (Kilburn et al., 1998a,b; Shahani and Young, 1972) that triggered a computer. Ten R-1's were averaged for each side and failures were recorded. Color confusion index was measured with the desaturated Lanthony 15 hue test under constant illumination (Lanthony, 1978) and scored by the method of Bowman (1982). Hearing was measured with standard audiometers (model ML-Am Microaudiometrics, So. Daytona, FL, USA) at stepped frequencies of 500 to 8000 Hertz. Both ears' deficits were the hearing (loss) scores.

Visual field thresholds were mapped with a (Med Lab Technologies) computerized perimeter for the central 30° of each eye. Performance was the sum (in decibels) of the thresholds for seeing 80 points. Neurological examination assessed cranial nerve function, muscle movements, strength, and cerebellar signs.

Neuropsychological tests

Immediate and 30-min delay verbal recall was measured with two stories from Wechsler's Memory Scale, revised (Wechsler, 1945, and 1987). Culture Fair tested non-verbal non-arithmetical intelligence with four sets of designs (Cattell, 1951; Cattell et al., 1941). It resembles Raven's progressive matrices (Raven et al., 1988). The 46-word multiple choice vocabulary test was from the multidimensional aptitude battery (Jackson, 1985). Digit symbol substitution from the Wechsler Adult Intelligence Scale-revised (WAIS-R; Wechsler, 1981, 1987) tested attention and integrative capacity. Information, picture completion and similarities, from the WAIS-R, tested long-term (embedded or hold) memory. Time needed to place 25 pegs in the Lafayette slotted pegboard and to make trails A and B assessed dexterity, coordination, and decision making. These tests and fingertip number writing errors that measured peripheral sensory discrimination were from the Halstead-Reitan battery (Reitan, 1958, 1966).

Responses to 65 terms describing feelings for the preceding week were assessed by the Profile of Mood States (POM) (1971/1981). The Limbic checklist assessed somatic and functional responses (Teicher et al., 1993). Recall of Rey's 15 figures assessed visual memory and gave clues as to possible malingering (Rey, 1964).

Expiratory flows and vital capacities were measured by blowing rapidly (exhaling) a full inspiration into a volume displacement (Ohio) spirometer while standing (using a nose clip). Forced expirations were

repeated until two agreed within 5% (ATS Statement, 1987). Records were traced into a computer and compared to predicted values adjusted for height, age, gender, and years of cigarette smoking (Miller et al., 1986). Alcohol and carbon monoxide were measured in air expired after a 20-second breath hold using appropriate fuel cell analyzers.

Statistical analysis

Scores and computed data were entered into an IBM compatible microcomputer. Stepwise linear regression modeling of each measurement in 293 symmetrically distributed subjects had produced prediction equations for each test that adjusted for differences in age, education, gender, height, and weight, using Strata Statistical Software (Strata Corporation, College Station, TX). Then the observed *measurements were compared to individual predicted values and expressed as percentage predicted* (Kilburn et al., 1998a). The two exposed groups' mean values, as percentage predicted were compared to the control group mean values by ANOVA. Family income, hours of general anesthesia, POMS score, and depression score were excluded because of insignificant coefficients. Statistical significance was defined as $p < .05$. Abnormalities for the patient's and referent's were counted (Table 1) valuing bilateral tests 0.5 per side, except that hearing and visual field performance counted 1 per side and balance 1 for eyes open and 1 for eyes closed.

Results

Exposure

The 105 patients had observed black mold growing on indoor walls and smelled musty odors that were enhanced when electrical switch and socket plates were removed and air-conditioning ducts that accessed inner walls were opened. Microscopy showed mold on surface imprint tapes. Usually indoor air grew more colony units of mold genera than outdoor air and grew bacteria including ones producing endotoxins.

The 100 CE patients and the 202 UE subjects had not observed mold in their homes and denied mold exposure.

The 69 ME women and 36 ME men and the 53 CE women and 47 CE men were not significantly older than the 202 UE subjects (Table 1), but both groups had more years of education, mean 14.7 and 13.6 than 12.9 in the UE group. Comparisons of function were

as percentage predicted (% pred.) to the UE group which adjusted for these differences with statistically significant ones described. More years of education would raise scores acting against decreases due to either exposure. Both exposed groups mean simple reaction times were significantly prolonged compared to UE subjects, % pred 103.6, 105.8 versus 99.9 and ($p < .0001$). CRT was 102.2 and 104.1 versus 100.0 ($p < .0001$). Balance, measured as sway speeds were faster, 141.3 and 195.0 versus 100.2 for eyes open ($p < .0001$) and 174.2 and 216.3 versus 103.1 eyes closed ($p < .0001$) and extremely abnormal. Blink reflex latencies, R-1, were delayed, 113.4 and 112.3 versus 99.4 on the right ($p < .0001$) ($p < .054$) and 111.9 and 109.9 versus 96.4 on the left ($p < .0001$).

Color discrimination errors were increased (however observed values decreased compared to predicted ones because they were the reciprocals of quadratic power), right eye 70.3 and 74.6 versus 102.6 ($p < .0001$) and left eye 73.9 and 62.1 versus 102.6 ($p < .0001$). Visual field performances were significantly reduced in both eyes, right 91.7 and 86.9 versus 100.0 ($p < .0016$) and left 92.0 and 88.8 versus 101.1 ($p < .0009$).

Grip strengths were significantly weaker, right 91.3 and 87.9 versus 99.3 in referents and left 87.0 and 83.8 versus 99.1 in referents ($p < .0001$). Hearing losses were not significantly different in either exposed group compared to the UE subjects.

Problem solving tests showed significantly lower scores for Culture Fair 92.9 and 89.9 versus 101.2 ($p < .0006$), digit symbol substitution 91.3 and 90.8 versus 101.5 ($p < .0001$). Vocabulary was 87.7 and 81.6 versus 99.2 ($p < .002$) ($p < .0001$). Immediate verbal recall (of stories) was significantly lower at 81.0 and 76.0 versus 99.8 ($p < .0001$) and decreased after 30 min at 71.4 and 63.9 versus 99.9 ($p < .0001$).

Times needed for peg placement were 92.9 and 88.8 versus 101.8 ($p < .001$), trail making A 103.6 and 106.6 versus 100.3 ($p < .0006$) and trail making B 103.1 and 104.5 versus 100.4 ($p < .004$) were significantly prolonged, showing perceptual motor slowing. Fingertip number writing errors did not differ from UE subjects, but were significantly greater in CE people. Information 82.5 and 81.9 versus 101.5 ($p < .0001$) and picture completion 81.2 and 79.8 versus 99.3 ($p < .0001$), differed from UE subjects, but not similarities 93.1 and 89.4 versus 98.1 ($p < .246$ and $p < .092$). These three tests are usually unaffected by chemical exposures or are last to show impairment (Ryan et al., 1987).

Table 1. Neurobehavioral function for mold exposed (ME) subjects (105) and chemically exposed (CE) subjects (100) compared to 202 referent subjects as percent of predicted, means and standard deviations (SD), *p* values by analysis of variance

	A 105 Mold Mean \pm SD	B 100 Chemical Mean \pm SD	C 202 Referent Mean \pm SD	<i>p</i> Values A vs C	<i>p</i> Values B vs C
Age (years)	46.5 \pm 13.3	46.5 \pm 10.3	47.2 \pm 20.2	.621	.98
Educational level (years)	14.7 \pm 2.6	13.6 \pm 2.8	12.9 \pm 2.3	.0001	.014
Physiological tests					
Simple reaction time (ms)	103.6 \pm 6.0	105.8 \pm 6.5	99.9 \pm 3.7	.0001	.0001
Choice reaction time (ms)	102.2 \pm 4.0	104.1 \pm 4.1	100.0 \pm 2.5	.0001	.0001
Balance sway speed (cm/sec)					
Eyes open	141.3 \pm 60.9	195.0 \pm 193.5	100.2 \pm 20.0	.0001	.0001
Eyes closed	174.2 \pm 93.5	216.3 \pm 184.1	103.1 \pm 26.8	.0001	.0001
Blink reflex latency R-I (ms)					
Right	113.4 \pm 13.0	112.3 \pm 12.2	99.4 \pm 14.6	.0001	.054
Left	111.9 \pm 15.2	109.9 \pm 10.2	96.4 \pm 13.2	.0001	.0001
Hearing losses					
Right	98.1 \pm 36.5	98.3 \pm 30.3	101.5 \pm 24.5	.673	.454
Left	99.4 \pm 35.2	97.4 \pm 25.0	99.3 \pm 21.7	.533	.606
Color discrimination errors					
Right	70.3 \pm 43.3	74.6 \pm 49.2	102.6 \pm 51.1	.0001	.0001
Left	73.9 \pm 46.1	62.1 \pm 46.1	102.6 \pm 51.1	.0001	.0001
Visual field performance					
Right	91.7 \pm 23.2	86.9 \pm 21.7	100.0 \pm 22.7	.0016	.0002
Left	92.0 \pm 24.5	88.8 \pm 23.7	101.1 \pm 21.6	.0009	.0007
Grip strength					
Right	91.3 \pm 18.7	87.9 \pm 23.2	99.3 \pm 17.5	.0003	.0001
Left	87.0 \pm 20.8	83.8 \pm 24.7	99.1 \pm 17.5	.0001	.0001
Psychological tests					
Culture fair	92.9 \pm 22.6	89.9 \pm 23.6	101.2 \pm 20.0	.0006	.0001
Digit symbol	91.3 \pm 11.4	90.8 \pm 14.3	101.5 \pm 9.2	.0001	.0001
Vocabulary	87.7 \pm 31.9	81.6 \pm 31.2	99.2 \pm 30.8	.002	.0001
Verbal recall					
Immediate	81.0 \pm 24.2	76.0 \pm 28.1	99.8 \pm 31.1	.0001	.0001
Delayed	71.4 \pm 36.4	63.9 \pm 34.5	99.9 \pm 41.3	.0001	.0001
Pegboard	92.9 \pm 15.2	88.8 \pm 16.0	101.8 \pm 25.7	.001	.0001
Trails A	103.6 \pm 8.1	106.6 \pm 9.5	100.3 \pm 8.3	.0006	.0001
Trails B	103.1 \pm 9.0	104.5 \pm 8.7	100.4 \pm 7.5	.004	.0001
Finger writing errors					
Right	98.9 \pm 7.6	102.7 \pm 8.5	100.0 \pm 7.4	.233	.020
Left	99.7 \pm 9.6	103.5 \pm 9.0	100.0 \pm 7.8	.825	.004
Information	82.5 \pm 34.9	81.9 \pm 35.6	101.5 \pm 39.4	.0001	.0001
Picture completion	81.2 \pm 39.0	79.8 \pm 35.6	99.3 \pm 32.2	.0001	.0001
Similarities	93.1 \pm 32.0	89.4 \pm 41.5	98.1 \pm 41.2	.246	.092
Total abnormalities	9.8 \pm 4.9	10.2 \pm 5.6	2.3 \pm 2.4	.0001	.0001

CE subjects' impairments matched those of the ME except that finger tip number writing errors were abnormal only in CE people Table 1. One test in 26 could be different by chance alone.

Twelve of 14 physiological functions and 10 of 13 psychological tests, a total of 22 of 26 tests were abnormal in ME compared to 23 abnormalities in CE people.

The ME group averaged 9.8 abnormal tests Figure 1A compared to 10.2 in CE people ($p < .0001$), Figure 1C and 2.3 in the referents ($p < .0001$), Figure 1B.

ME subjects' mood states (POMS scores, the sum of 5 adverse moods minus vigor) were slightly lower than the CE, but nearly twice the UE group. Limbic system complaints like memory flashbacks, unusual

behavior, staring, twitching, and sensory hallucinations were frequent, did not differ between ME to CE, but were much higher than unexposed. Frequencies of 35 symptoms averaged 4.8 ± 1.2 (standard deviation) in ME and 5.2 ± 2.0 in CE. Both were significantly different from referents 2.6 ± 1.1 ($p < .0001$; Table 2).

Vital capacity and forced expiration volume in 1 second (FEV_1) were significantly reduced in ME (Table 3). CE subjects had reduced vital capacities with reductions of FEV_1 that were almost significant ($p < .125$). Vital capacity as percentage predicted was decreased more than FEV_1 in both exposed groups.

Fifty-two percent of ME people had symptoms of peripheral neuropathy compared to 14% of CE and 2% of referents. Frequent neurological signs in ME-exposed subjects exceeded those for CE-exposed subjects were past pointing in 75%, ataxia in 49%, slow alternating movements in 32%, dysmetria in 25%, and tremors in 18%. And 30% had abnormal cerebellar tests.

Discussion

The 105 people exposed to molds and mycotoxins at home were impaired to a similar extent as the 100 CE people. Exposures were associated with slowed reaction times and impairment in balance, color discrimination, visual field performance, and grip strength, but not decreased hearing. Verbal recall was decreased. Perceptual motor functions were impaired for peg placement and trail making. Problem solving was reduced in digit symbol substitution and Culture Fair. Even functions considered refractory to chemical damage like vocabulary, information, and picture completion were impaired, but classification, finding similarities was not impaired. Abnormal balance was associated with abnormal neurological tests of cerebellar function. This finding confirmed and extended our previous studies (Northup and Kilburn, 1977; Kilburn, 2002, 2003) and those of others (Baldo et al., 2002; Gordon et al., 2004). Small airways obstruction had reduced vital capacity, but FEV_1/FEV ratio increased, which is characteristic of small airways disease rather than asthma (Northup and Kilburn, 1977; Miller et al., 1986).

Strengths and limitations

Finding that 105 consecutive symptomatic ME patients had neurobehavioral abnormalities that matched 100 CE patients, confirms previous findings

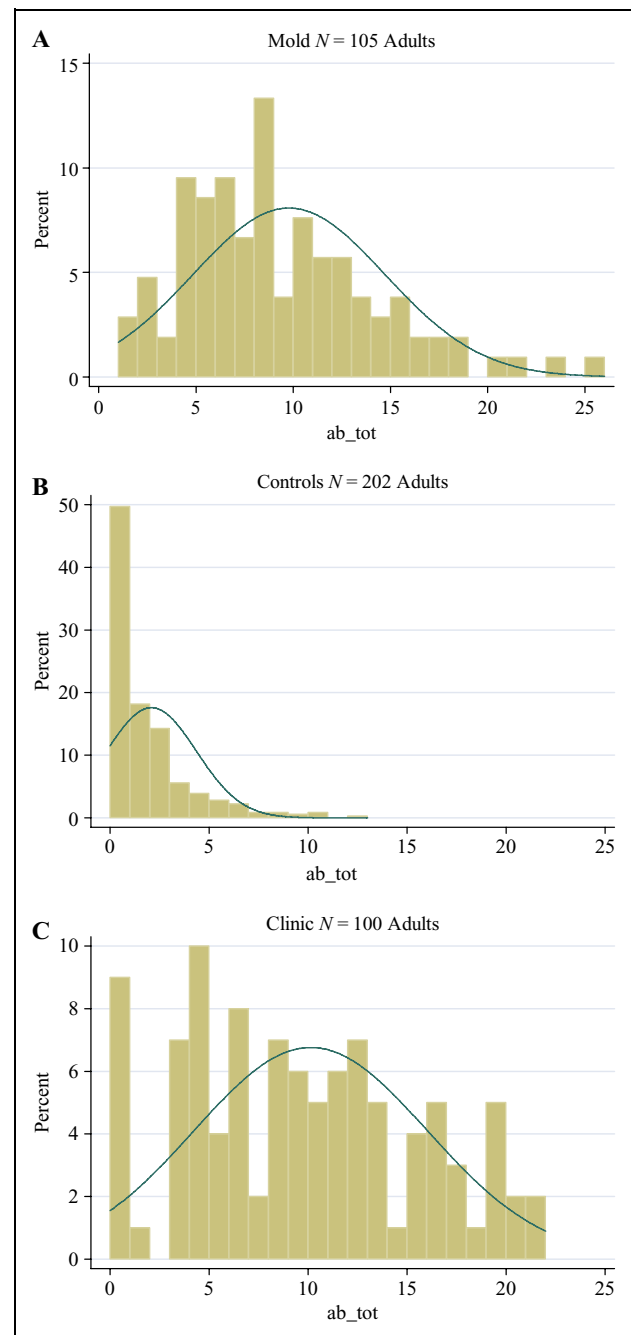


Figure 1. (a) The frequency of total abnormalities of 105 mold exposed (ME) patients showed a symmetrical distribution around the mean of 9.8. (b) The frequency of total abnormalities for 202 referent subjects was skewed sharply left from the mean of 2.3. (c) The frequency of total abnormalities for 100 chemically exposed (CE) patients was symmetrically distributed around the mean of 10.2 and resembles that of the mold-exposed (ME) group.

that mold exposure adversely affects the human brain. Opportunities for bias are reduced by large numbers and individuals appearing randomly, so groups were

Table 2. Profile of mood states for 108 mold exposed (ME) and 100 chemically exposed (CE) compared to 202 unexposed (UE) subjects (POMS score is the sum of tension, depression, anger, fatigue, and confusion minus vigor)

POMS	A 108 mold Mean \pm SD	B 100 chemical Mean \pm SD	C 202 referent Mean \pm SD	<i>p</i> values A vs C	<i>p</i> values B vs C
Score	69.2 \pm 37.9	83.1 \pm 43.4	22.1 \pm 25.0	.0001	.0001
Tension	17.3 \pm 8.3	19.8 \pm 8.0	8.9 \pm 4.6	.0001	.0001
Depression	16.1 \pm 12.9	21.9 \pm 15.2	7.9 \pm 7.1	.0001	.0001
Anger	13.3 \pm 10.1	15.5 \pm 9.8	7.7 \pm 6.5	.0003	.0003
Fatigue	16.8 \pm 7.9	18.3 \pm 7.0	8.3 \pm 5.6	.0001	.0001
Vigor	10.8 \pm 6.9	10.0 \pm 6.5	17.0 \pm 6.2	.0001	.0001
Confusion	13.9 \pm 6.8	16.3 \pm 6.3	6.4 \pm 3.7	.0001	.0001
Symptom frequency	4.8 \pm 1.2	5.2 \pm 2.0	2.6 \pm 1.1	.0001	.0001

SD, standard deviation.

Table 3. Pulmonary function for 108 mold exposed (ME) subjects and 100 chemically exposed (CE) compared to 202 referent subjects as percentage of predicted, means and standard deviations (SD), *p* values by analysis of variance

	A 105 mold Mean \pm SD	B 100 chemical Mean \pm SD	C 202 referent Mean \pm SD	<i>p</i> values A vs C	<i>p</i> values B vs C
FVC	91.2 \pm 12.6	94.8 \pm 16.1	101.6 \pm 15.1	.0001	.0005
FEV ₁	87.8 \pm 14.0	90.4 \pm 18.7	93.6 \pm 15.8	.0008	.125
FEF ₂₅₋₇₅	94.1 \pm 29.1	102.6 \pm 37.4	88.1 \pm 35.0	.191	.001
FEF ₇₅₋₈₅	90.2 \pm 47.4	100.1 \pm 53.8	78.1 \pm 52.7	.099	.001
FEV ₁ /FVC	77.4 \pm 7.5	76.5 \pm 8.9	72.8 \pm 9.5	.0001	.001

FEV₁, forced expiration volume in 1 second, FVC = forced vital capacity, FEF 25-75 = forced expiratory flow 25% to 75%, FEF 75-85 = forced expiratory flow 75% to 85%

aggregated after being tested. Asymptomatic-exposed individuals were not included as they were not motivated to seek testing.

Standardized testing and data handling ensured comparability. There was no way to quantify neither mold/mycotoxin exposures nor chemical exposures. Methods were not available to assay dozens of patients' homes for toxins from molds or other chemicals (Brasel et al., 2005; Straus, 2009). Many homes had had interventions to reduce exposure, especially to mold before the residents' impairment was verified by testing. When assays become available it would be more practical to measure people exposed in schools or office buildings where many people shared exposures rather than in homes (Brasel et al., 2005).

People's ages, wellness, and hours per day of exposure vary more in a home-based group than in a workforce. Self-selection (bias) may increase reporting of symptoms but has not affected neuro-behavioral measurements (Kilburn 1997a,b, 1999; Kilburn and Warshaw, 1993) nor have such patients

manipulated tests or malingered in previous studies (Kilburn, 2000a,b). Clinical judgment favored molds and mycotoxins as primary causes although homes, schools and offices contain other toxic chemicals (Dally et al., 1981; Storey et al., 2004).

People's mold exposure impairments resembled those associated with formaldehyde and the indoor air syndrome, including lengthened blink reflex latency (Kilburn, 2000a). Prolonged blink reflex latency has been associated with exposure to chlorinated solvents (Kilburn, 2000b; Kilburn and Warshaw, 1993) including polychlorinated biphenyls (Kilburn et al., 1989) chlorine (Kilburn, 2000c), and to arsenic (Kilburn, 1997a) and occurs from other chemicals (Kilburn, 1997b).

Bioassays for toxic activity on cells or enzymes as models to assay trichothecene activity have been explored to characterize air samples and particles (Brasal et al., 2005; Gorny et al., 2002; Johanning et al., 2002; McLaughlin et al., 1977; Savilathi et al., 2000; Smoragiewicz et al., 1993) from a Montreal office building assayed by thin layer

chromatography (TLC) using 4- (p-nitrobenzyl) pyridine to identify the 12,12-epoxy group of trichothecenes (Smoragiewicz et al., 1993). Methanol extracts of water-damaged building materials and gypsum board were 200 times as toxic as board extracts that were not water damaged for feline skin and lung cells. Another problem is that spores and fragments of three molds (*Aspergillus versicolor*, *Penicillium melinii* and *Cladosporium cladosporioides*) share antigens and immunological reactivity (Gorny et al., 2002). Inhibition of protein synthesis using firefly luciferase translation in rabbit reticulocytes has been correlated with fungal counts in indoor air (Yike et al., 1999) and attributed to trichothecenes. Also trichothecenes were identified in ultra-small mold particles (Brasel et al., 2005).

Trichothecenes were identified as toxic agents for swine kidney cell cultures by MTT cleavage using 3- (4,5-dimethylthiazol-2-yl) -2,5 diphenyltetrazolium bromide and characterized chemically by high pressure liquid chromatography with a diode array detector (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS; Yike et al., 1999). A chymotrypsin-like serin proteinase from *Stachybotrys chartarum* has been isolated and purified from the lung of an infant with pulmonary hemosiderosis. It cleaved lung proteinase inhibitors, bioactive peptides, and collagen, suggesting it could destroy lung tissue (Kordula et al., 2002). Most recently, an Elisa assay, was specific for trichothecenes on tiny mold fragments caught in ultra-fine filters (Brasal et al., 2005; Straus, 2009).

The 105 patient study confirmed the abnormalities in balance, reaction time, recall, memory, and trail-making in 20 previously evaluated patients exposed to *Stachybotrys atra* in one building (Kilburn, 2002) and people exposed to various molds and mycotoxins in homes and schools (Kilburn, 2003).

We postulate that inhaled mycotoxins adsorbed on spores and hyphae from mold growing indoors cause brain impairment. Trichothecenes and satratoxin are epoxides that covalently adduct DNA, RNA, protein, and microtubules of nerve axons (Hayes and Campbell, 1986; McLaughlin et al., 1977) and impair protein synthesis to damage lung, brain, and the immune system. Molds: *Penicillium* and *Streptomyces* make penicillin, aminoglycosides, and other therapeutic antibiotics (Waksman, 1967), ergot alkaloids, coumadins, adriamycin C, used to treat cancer and Aflatoxin B1 and others that cause liver cell cancer in people exposed to hepatitis B (Ross et al., 1992; Squire, 1981). And Ochratoxin A may cause cancer of the human kidney (Creppy, 1999).

Since ancient times, asthma, cancer, wasting, hemorrhage, inanition, and death have been linked to mold exposures (Rall and Schleifer, 1985). The current wave of sickness from molds has developed since World War II, and paralleled in time the construction of the interior walls of homes shifted to plasterboard (gypsum sandwiched between paper-cellulose layers) from wood or metal lath, plaster with a lime coat. When inner walls became damp from inadequate venting of moisture of from (Hintekka, 1977) leaky walls, roofs, or plumbing mold grew on paper cellulose and gypsum (Nielsen et al., 1998). Drying after being wet appears to stress molds to produce spores and toxins such as trichothecenes and satratoxins that escape into living and working spaces. Recently, these toxins have been measured in serum from people exposed in moldy homes. Plaster is strongly alkaline and discourages the growth of toxigenic molds. The PCR has been used to amplify fungal DNA and show mold exposure in human tissue and in urine (Hooper et al., 2009).

There are no antidotes for mold toxins (Rall and Schleifer, 1985). Methoxymethane (dimethylether) removes aflatoxins from peanuts (Aibara and Yano, 1977), but no evidence has been found that it applies to the removal of satratoxins or trichothecenes or to other agents. Agents developed to lessen mold exposure must not be toxic. Cholestyramine, an oral cholesterol lowering agent has been administered to lower trichothecene burdens in the gut (Shoemaker and House, 2005).

Prevention

Avoiding brain damage from molds and mycotoxins requires primary prevention. Molds cause neurological and respiratory damage and illnesses that appear to progress and become irreversible and so avoidance of exposure is imperative. First, the building must be designed to prevent moisture within walls. This means preventing intrusion from roofs (rain and snow) condensation (including within air-conditioning ducts, and plumbing leaks (supply, icemakers and sewers). Facilitate ventilation of walls and roofs so they breathe. Second, construction materials that do not aid the growth of *Stachybotrys* and other toxic molds should be used. Strongly alkaline building materials for repair and new construction such as lime, plaster, and concrete deter mold growth.

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insurance and by several law firms ascertaining whether to undertake litigation. All subjects gave informed consent after explanation of the protocol that was approved by the Human Studies Research Committee of the University of Southern California, Keck School of Medicine, USA.

Declaration of Conflicting Interests

Dr Kilburn provides expert testimony for plaintiffs and defendants.

References

- Aibara K, Yano N (1977) New approach to aflatoxin removal. In: Rodricks JV, Hesseltine CW, Mehlman MA (eds.) *Mycotoxins in Human and Animal Health*. Park Forest South, IL: Pathotox Publishers, Inc, p.151–161.
- Andersson MA, Nikulin M, K ljalg U, et al. (1997) Bacteria, molds and toxins in water-damaged building materials. *Applied and Environmental Microbiology* 63: 387–393.
- ATS Statement (1987) Standardization of spirometry–1987 update. *American Review Respiratory Disease* 136: 1285–1298.
- Baldo JV, Ahmad L, and Ruff R (2002) Neuropsychological performance of patients following mold exposure. *Applied Neuropsychology* 9: 193–202.
- Bennett JW, Klich M (2003) Mycotoxins. *Clinical Microbiology Reviews* 16: 497–516.
- Bowman BJ (1982) A method for quantitative scoring of the Farnsworth panel D-15. *Acta Ophthalmologica* 60: 907–916.
- Brasel TL, Douglas DR, Wilson SC, and Straus DC (2005) Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia. *Applied and Environmental Microbiology* 71: 114–122.
- Burge HA, Rogers CA (2000) Outdoor allergens. *Environmental Health Perspectives* 108: 653–659.
- Burge S, Hedge A, Wilson S, Bass JH, and Robertson A (1987) Sick building syndrome: a study of 4373 office workers. *The Annals of Occupational Hygiene* 31: 493–504.
- Burr ML (1993) Epidemiology of asthma. *Mono Allergy* 3: 80–124.
- Cattell RB (1951) Classical and standard score IQ standardization of the IPAT: culture free intelligence scale 2. *Journal of Consulting Psychology* 15: 154–159.
- Cattell RB, Feingold SN, and Sarason SB (1941) A culture free intelligence test II evaluation of cultural influences on test performance. *Journal of Educational Psychology* 32: 81–100.
- Centers for Disease Control and Prevention (CDCP) (1994) Acute pulmonary hemorrhage hemosiderosis among infants–Cleveland, January 2003–November 1994. *MMWR. Morbidity and Mortality Weekly Report* 43: 881–883.
- Creppy EE (1999) Human ochratoxicosis. *Journal of Toxicology–Toxin Reviews* 18: 277–293.
- Dales RE, Burnett R, and Zwanenburg H (1991) Adverse health effects among adults exposed to home dampness and mold. *American Review Respiratory Disease* 143: 505–509.
- Dally KA, Hanrahan LP, Woodbury MA, and Kanareck MS (1981) Formaldehyde exposure in nonoccupational environments. *Archives of Environmental Health* 36: 277–284.
- Etzel RA, Montana E, Sorenson WG, Kullman GJ, Allen TM, and Dearborn DG (1998). Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. *Archives of Environmental Health* 152: 757–762.
- Ferris BG, Jr (1978) Epidemiology standardization project. *American Review Respiratory Disease* 118: 7–54.
- Gordon WA, Cantor JB, Johanning EL, et al. (2004) Cognitive impairment associated with toxigenic fungal exposure: a replication and extension of previous findings. *Applied Neuropsychology* 11: 65–74.
- Gorny RL, Reponen T, Willeke K, et al. (2002) Fungal fragments as indoor air biocontaminants. *Applied and Environmental Microbiology* 68: 3522–3531.
- Hardin BD, Kelman BJ, and Saxon A (2003) Adverse human health effects associated with molds in the indoor environment. *Journal of Occupational and Environmental Medicine* 45: 470–478.
- Hayes JR, Campbell TC (1986) Food additives and contaminants. In: Klaassen CD, Amdur MO, Doull J (eds.) *Casarett and Doull's Toxicology. The Basic Science of Poisons*. 3rd ed. New York: Macmillan, p.771–800.
- Hefez A (1985) The role of the press and the medical community in the epidemic of “mysterious gas poisoning” in the Jordan West bank. *American Journal of Psychiatry* 142: 833–837.
- Hintikka EL (1977) Human stachybotryotoxicosis. In: Wyllie TD and Morehouse LG (eds.) *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses*. New York: Marcel Dekker, Inc. Vol. 3, 87–89.
- Hooper DG, Bolton VE, Guilford FT, Straus DC, (2009) Mycotoxin detection in human samples from patients exposed to environmental molds. *International J Molecular Science* 10:1465–1475.
- Howitt ME, Niabu R, and Roach TC (1998) Prevalence of childhood asthma in Barbados. *American Journal of Respiratory and Critical Care Medicine* 157: A624.

- Institute of Medicine (IOM) (2004). *Damp Indoor Spaces and Health*. Washington, DC: National Academy of Sciences.
- Jackson DN (1985) *Multidimensional Aptitude Battery*. Port Huron, MI: Sigma Assessments Systems, Inc.
- Johanning E, Gareis M, Nielsen K, Dietrich R, and Märklbauer E (2002) Airborne mycotoxin sampling and screening analysis. *Proceeding Indoor Air Albany*. New York: Albany Medical College.
- Johanning E, Morey P, Jarvis B, Biagini R, Hull D, and Landsbergis P (1996) Health and immunology study following exposure to toxigenic fungi *Stachybotrys chartarum* in a water-damaged office environment. *International Archives of Occupational and Environmental Health* 68: 207–218.
- Kilburn KH (1994) Neurobehavioral impairment and seizures from formaldehyde. *Archives of Environmental Health* 49: 37–44.
- Kilburn KH (1997a) Neurobehavioral impairment from long-term residential arsenic exposure. In: Abernathy CO, Calderon RL, Chappell WR (eds.) *Arsenic. Exposure and Health Effects*. New York: Chapman and Hall, p.159–175.
- Kilburn KH (1997b) Exposure to reduced sulfur gases impairs neurobehavioral function. *Southern Medical Journal* 90: 997–1006.
- Kilburn KH (1999) Evidence for chronic neurobehavioral impairment from chlorpyrifos. *Environmental Epidemiology & Toxicology* 1: 153–162.
- Kilburn KH (2000a) Visual and neurobehavioral impairment associated with polychlorinated biphenyls. *Toxicology* 21: 489–500.
- Kilburn KH (2000b) Indoor air effects after building renovation and in manufactured homes. *American Journal of Medical Science* 320: 249–254.
- Kilburn KH (2000c) Chlorine-induced damage documented by neurophysiological, neuropsychological and pulmonary testing. *Archives of Environmental Health* 55: 31–37.
- Kilburn KH (2002) Inhalation of mould and mycotoxins. *European Journal of Oncology* 7: 197–202.
- Kilburn KH (2003) Indoor mold exposures associated with neurobehavioral and pulmonary impairment: a preliminary report. *Archives of Environmental Health* 58: 400–412.
- Kilburn KH, Thornton JC, and Hanscom B (1998a) Population based prediction equations for neurobehavioral tests. *Archives of Environmental Health* 53: 257–263.
- Kilburn KH, Thornton JC, and Hanscom B (1998b) A field method for blink reflex latency R-1 (BRL R-1) and prediction equations for adults and children. *Electromyography and Clinical Neurophysiology* 38: 25–31.
- Kilburn KH, Warshaw RH (1993) Effects on neurobehavioral performance of chronic exposure to chemically contaminated well water. *Toxicology and Industrial Health* 9: 391–404.
- Kilburn KH, Warshaw RH, and Hanscom B (1994) Balance measured by head (and trunk) tracking and a force platform in chemically (PCB and TCE) exposed and referent subjects. *Occupational and Environmental Medicine* 51: 381–385.
- Kilburn KH, Warshaw RH (1995) Neurotoxic effects from residential exposure to chemicals from an oil reprocessing facility and superfund site. *Neurotoxicology and Teratology* 17: 89–102.
- Kilburn KH, Warshaw RH, and Shields MG (1989) Neurobehavioral dysfunction in firemen exposed to polychlorinated biphenyls (PCBs): possible improvement after detoxification. *Archives of Environmental Health* 44: 345–350.
- Kilburn KH, Warshaw R, and Thornton JC (1987) Formaldehyde impairs memory, equilibrium, and dexterity in histology technicians: effects which persist for days after exposure. *Archives of Environmental Health* 42: 117–120.
- Kordula T, Banbula A, Macomson J, and Travis J (2002) Isolation and properties of stachyrase A, a chymotrypsin-like serine proteinase from *Stachybotrys chartarum*. *Infection and Immunity* 70: 419–421.
- Kreiss K (1990) The sick building syndrome: where is the epidemiologic basis? *American Journal of Public Health* 80: 1172–1173.
- Landrigan PJ, Miller B (1983) The Arjenyattah epidemic. Home interview data and toxicological aspects. *Lancet* 2: 1474–1476.
- Lanthony P (1978) The desaturated panel D-15. *Documenta Ophthalmologica* 46: 185–189.
- Levin RE, Weinstein A, Peterson M, Testa MA, and Rothfield NF (1984) A comparison of the sensitivity of the 1971 and 1982 American Rheumatism Association criteria for the classification of lupus erythematosus. *Arthritis and Rheumatism* 27: 530–538.
- Mazur LJ, Kim J (2006) Spectrum of non-infection health effects from molds. *Pediatrics* 118: 1909–1926.
- McLaughlin CS, Vaughan MH, Campbell IM, Wei CM, Staffor ME, and Hansen BS (1977) Inhibition of protein synthesis by trichothecenes. In: Rodricks JV, Hesseltine CW, and Mehlman MA (eds.) *Mycotoxins in Human and Animal Health*. Park Forest South, IL: Pathotox Publishers, Inc, p.263–273.

- McNair DM, Lorr M, Droppleman LF, EITS Manual for Profile of Mood States. (1971/1981) San Diego, CA: Educational and Industrial Testing Service.
- Menzies R, Tamblyn R, Farant JP, Hanley J, Nunes F, and Tamblyn R (1993) The effect of varying levels of outdoor air supply on the symptoms of sick building syndrome. *The New England Journal of Medicine* 328: 821–827.
- Miller JA, Cohen GS, Warshaw R, Thornton JC, and Kilburn KH (1989) Choice (CRT) and simple reaction times (SRT) compared in laboratory technicians: factors influencing reaction times and a predictive model. *American Journal of Industrial Medicine* 15: 687–697.
- Miller A, Thornton JC, Warshaw R, Bernstein J, Selikoff IJ, and Teirstein AS (1986) Mean and instantaneous expiratory flows, FVC and FEV₁: prediction equations from a probability sample of Michigan, a large industrial state. *Bulletin Européen de Physiopathologie Respiratoire* 22: 589–597.
- Mirocha CJ, Pathre SV, and Christensen CM (1977). Chemistry of Fusarium and Stachybotrys mycotoxins. In: Wyllie TD and Morehouse LG (eds.) *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses*. New York: Marcel Dekker, Inc. Vol. 1, p.365–420.
- Montana EA, Etzel RA, Tallen E, Horgan, and Dearborn DG (1997) Hemosiderosis in a Cleveland Community with pediatric idiopathic pulmonary hemorrhages. *Pediatrics* 99: 1–8.
- Nielsen KF, Hansen MO, Larsen TO, and Tharne U (1998) Production of mycotoxins on artificially inoculated building materials. *International Biodeterioration & Biodegradation* 42: 1–7.
- Northup SC, Kilburn KH (1977) The role of mycotoxins in human pulmonary disease. In: Wyllie TD and Morehouse LG (eds.) *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses*. New York: Marcel Dekker, Inc., Vol. 3, p.91–108.
- Rall TW, Schleifer LS (1985) Oxytocin, prostaglandins, ergot alkaloids and other drugs; tocolytic agents. In: Gilman AG, Goodman LS, Rall TW, and Murad F (eds.) *The Pharmacological Basis of Therapeutics*. 7th ed. New York: Macmillan Publishing Company, p.926–945.
- Raven JC, Court JH, and Raven J (1988) *Standard Progressive Matrices*. Great Britain: Oxford Psychologists Press.
- Reitan RM (1958) Validity of the trail-making test as an indicator of organic brain damage. *Perceptual & Motor Skills* 8: 271–276.
- Reitan RM (1966) A research program on the psychological effects of brain lesions in human beings. In: NR Ellis (ed.) *International Review of Research in Mental Retardation*. New York: Academic Press, p.153–216.
- Rey A (1964) *L'examen Clinique en Psychologie*. Paris: Presses Universitaires de France.
- Ross RK, Yuan JM, Yu MC, et al. (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet* 339: 1413–1414.
- Ryan CM, Morrow LA, Bromet EJ, and Parkinson DK (1987) Assessment of neurobehavioral dysfunction in the workplace: normative data from the Pittsburg Occupational Exposure Test Battery. *Journal of Clinical and Experimental Neuropsychology* 9: 665–679.
- Savilahti R, Uitti J, Laippala P, Husman T, and Roto P (2000) Respiratory morbidity among children following renovation of water-damaged school. *Archives of Environmental Health* 55: 405–410.
- Shahani BT, Young RR (1972) Human orbicularis oculi reflexes. *Neurology* 22: 149–154.
- Shinn E (2000) African dust and the demise of Caribbean coral reefs. *Geophysical Research Letters* 27: 3029–3032.
- Shoemaker RC, House DE (2005) A time-series study of sick building syndrome: chronic biotoxin associated illness from exposure to a water damaged building. *Neurotoxicology and Teratology* 27: 29–46.
- Small GW, Borus JF (1983) Outbreak of illness in a school chorus. Toxic poisoning or hysteria? *The New England Journal of Medicine* 308: 632–635.
- Smoragiewicz W, Cossette B, Boutard A, and Krzystyniak K (1993) Trichothecene mycotoxins in the dust of ventilation systems in office buildings. *International Archives of Occupational and Environmental Health* 65: 113–117.
- Squire RA (1981) Ranking animal carcinogens: a proposed regulation approach. *Science* 214: 87–880.
- Storey E, Dangman K, Schenck P, et al. (2004) *Guidance for Clinicians on the Recognition and Management of Health Effects Related to Mold Exposure and Moisture Indoors*. Farmington, CT: University of Connecticut Health Center, p.1–206.
- Straus DC (2009) Estimation of human exposure to stachybotrys and its mycotoxin. *Toxicology and Industrial Health* 25 (in press).
- Teicher MH, Glod CA, Surrey J, and Swett C, Jr (1993) Early childhood abuse and limbic system ratings in adult psychiatric outpatients. *The Journal of Neuropsychiatry and Clinical Neurosciences* 5: 301–306.
- Vojdani A, Campbell AW, Kashanian A, and Vojdani E (2003) Antibodies against molds and mycotoxins following exposure to toxigenic fungi in a water damaged building. *Archives of Environmental Health* 58: 324–336.

- Waksman SA (1967) Antibiotics of actinomycetes that have found important practical applications. In: Waksman SA (ed.) *The Actinomycetes: A Summary of Current Knowledge*. New York: Ronald Press Company, p.208–226.
- Wechsler D (1981) *Adult Intelligence Scale Manual-Revised*. New York: The Psychological Corporation, p.7–156.
- Wechsler D (1945) A standardized memory scale for clinical use. *The Journal of Psychology* 19:87–95 (WMS-revised 1987, Psychological Corporation 1987 (p. 1–20)).
- Yike I, Allan T, Sorenson WG, and Dearborn DG (1999) Highly sensitive protein translation assay for trichothecene toxicity in airborne particulates: comparison with cytotoxicity assays. *Applied and Environmental Microbiology* 65: 88–94.

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