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Incorporating Environmental Health in Clinical Medicine

Guest Editors: Stephen J. Genuis, Margaret Sears, Gerry Schwalfenberg, Janette Hope, and Robin Bernhoft

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Editorial Board

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Editorial

Incorporating Environmental Health in Clinical Medicine

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What are the objectives of 21st century healthcare? Five fundamental pillars and presuppositions appear to underscore the provision of clinical healthcare, whether conventional or alternative:

(i) Healthcare should genuinely assist individuals
   (a) to prevent illness through health education;
   (b) to overcome and recover from disease and suffering when possible;
   (c) to receive compassionate and ongoing care when cure is no longer a reality.

(ii) Healthcare should be based on credible scientific evidence rather than ideology, anecdote, or corporate science.

(iii) Healthcare professionals should be competent, compassionate, and accountable for the care they provide.

(iv) Outcome measures of healthcare approaches should be in place to determine evidence-based success and to protect the public interest.

(v) Ongoing research to study proposed interventions is in the public interest and is required for the advancement of clinical care.

While providers of clinical care, whatever the stripe, may share the same vision and objectives, twenty-first century healthcare is increasingly fragmented. We now have medicine with myriad descriptors: conventional, alternative, naturopathic, holistic, ayurvedic, traditional Chinese, osteopathic, integrative, restorative, functional, homeopathic, and so on. The potpourri of healthcare disciplines and labels can be confusing and it is challenging to recount the ideology and practices peculiar to each faction. Each discipline brings something unique to the table and proponents often advance their particular interpretation of science and their interventions as the best way to deliver health and healing to the suffering masses.

Yet, despite noble intentions and sincere aspirations by clinical practitioners from the differing groups, there is also escalating derision between assorted disciplines. Conventional and nonallopathic factions often accuse each other of lacking scientific credibility, of being deficient in evidence for various interventions, and of failing the public interest. All the while, rates of chronic mental and physical illness in both adults and children continue to escalate and to incapacitate so many suffering people [1, 2].

So with the ongoing dispersion of medical approaches, the rancour between competing ideologies, and the challenging state of health in much of the world's population, why do we need yet another branch of clinical medicine and why does the Journal of Environmental and Public Health publish a special issue to introduce and advance the clinical field of "Environmental Medicine"? We endeavour to answer such questions in this publication.

At the outset of the issue, S. Genuis aims to provide historical and contemporary evidence for the clinical field of environmental medicine as the preferred scientific approach to healthcare in an introductory piece entitled "What's out there making us sick?" An eclectic collection of papers follow that explore varied aspects of this emerging discipline and that attempt to bridge the gap between established research

in relation to environmental health science and determinants of disease, and the day-to-day patient encounters in clinical practice. In selecting articles, we have endeavoured to be relevant and contemporary by remaining attuned to modern trends as well as providing a scholarly forum for the expression of novel and controversial developments, presented to standards of peer-reviewed scientific publication. We believe that presenting ideas and proposed clinical strategies for scrutiny and debate is healthy.

For example, we have a clinically relevant research article by D. Kennedy et al. reporting on the scientific efficacy of a widely popular detoxification strategy called "Ionic footbaths," we present a manuscript exploring the clinical usefulness of sweating as a means to eliminate accrued toxicants, as well as providing a practical paper by D. Colson on the safe removal of dental amalgam. Furthermore, we have accepted a provocative review piece by a number of scientists on research relating to a phenomenon they call "Earthing" as well as a paper that introduces an unconventional yet apparently successful clinical approach to assisting patients with bone compromise. We are not promoting or endorsing any specific clinical therapy or intervention. Rather, we believe that scientific censorship is dangerous and that the broad spectrum of therapies should be critically assessed equally and evaluated based on scientific merit, not on the medical paradigm "box" to which they are ascribed.

From the Lead Editor. Each of the guest editors kindly responded to an invitation to submit an article for independent peer review. Dr. Margaret Sears presents an article discussing practical aspects of the environmental health field while a piece by Dr. Gerry Schwalfenberg highlights the profound clinical worth of vitamin D in healthcare. A manuscript by Dr. Robin Bernhoft gives an overview of the challenges and clinical management of mercury exposure. A couple of interesting papers including a piece by Dr. Janette Hope et al. draw attention to the serious and widespread clinical problem of mold and mycotoxins among exposed individuals and groups.

The publishers at the Journal of Environmental and Public Health deserve much credit for recognizing the importance of this expanding field and for inviting a special issue exploring the discipline of clinical environmental medicine. It is by initiatives such as this that the translation of new knowledge occurs and that innovative clinical trends are established. Challenging the status quo with the adoption of new ideas and skills has always been and remains the path to scientific and clinical progress.

As lead editor of this issue, I must admit there was reservation when we first embarked on this expedition. While there are many scientific researchers in the burgeoning field of environmental science and an ever-expanding number of scientific journals that focus on this subject, there is a paucity of clinicians worldwide who have both the knowledge and skills to assimilate information from environmental health research and translate it into clinical practice, and even fewer with the ability and commitment to prepare scientific articles. With that realization, I was concerned we might receive minimal response to the call for papers. I was wrong: the overwhelming response and submission of articles for this issue has been heart warming and exemplifies the mounting interest in this field. The time and work involved has been well worth it and I am most grateful to my fellow guest editors, who have been extraordinarily helpful in the process.

In the end, however, I remain disenchanted by the mounting divisions within the health care field. I am not fond of labels and disunity; I prefer medicine without descriptors. Scientific clinical medicine should be based on credible untainted research and reporting, reproducible observation, as well as competent and compassionate health care in order to provide favorable outcomes for patients and populations. I contend that it is time to incorporate credible research science and emerging evidence, whatever the source, into the practice of mainstream clinical medicine. It is to this end that this special issue has come forward. Thank you for your interest in this publication we have prepared.

Conflict of Interests

There are no conflicts of interest. No funding has been received for any part of this work.

Stephen J. Genuis
Margaret Sears
Gerry Schwalfenberg
Janette Hope
Robin Bernhoft

References

[1] J. M. Perrin, S. R. Bloom, and S. L. Gortmaker, "The increase of childhood chronic conditions in the United States," *Journal of the American Medical Association*, vol. 297, no. 24, pp. 2755–2759, 2007.

[2] R. Horton, "The neglected epidemic of chronic disease," *Lancet*, vol. 366, no. 9496, p. 1514, 2005.

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Research Article

Evaluation of the Quick Environmental Exposure and Sensitivity Inventory in a Danish Population

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Objectives. To evaluate a Danish translation of the Quick Environmental Exposure and Sensitivity Inventory (QEESI). *Methods*. The study included two groups: one comprised a random sample of 2000 individuals drawn from the Danish Civil Registration System; the other comprised 315 patients with chemical intolerance. *Results*. The evaluation suggested good reliability for the four QEESI scales in terms of internal consistency and coefficients between test and retest scores. The discriminatory validity was the largest for the Chemical (inhalant) Intolerance and Life Impact Scales. Using combined cut-off scores for these two scales provided a sensitivity of 92.1 and a specificity of 91.8 and yielded a prevalence of 8.2% in the population group. *Conclusions*. The Danish translation of the QEESI showed overall good reliability and validity. We recommend the use of the combined Chemical (inhalant) Intolerance and Life Impact Scales in future studies.

1. Introduction

Chemical intolerance, also referred to as multiple chemical sensitivities (MCS), is a disorder characterized by reports of nonspecific symptoms from various organ systems attributed by the individual to exposure to common airborne chemicals [1]. In general the reported symptoms are attributed to previous chemical exposures and recur on subsequent exposures to the same or structurally unrelated chemicals at levels normally considered to be nontoxic [1].

Symptoms of chemical intolerance are prevalent with estimates ranging from 9 to 33% in population-based studies; however, such studies are few [2–7]. Physician-diagnosed MCS or reports of disabling consequences in the form of social and occupational disruptions attributed to exposure to common airborne chemicals range from 0.5 to 6.3% [2–4, 7]. The reported symptoms typically vary between individuals with women being more sensitive and reporting more symptoms than do men [2, 5–7]. A typical symptom pattern is thus difficult to establish. Nonspecific central nervous system (CNS) complaints are frequently reported, including fatigue, headache, and difficulty concentrating [2, 8, 9]. Other symptoms include pain and respiratory complaints [2, 5, 6]. An association between asthma and chemical intolerance has been reported in several studies [2, 10, 11]. In a population-based twin study on the heritability of perfume-related respiratory symptoms, Elberling and colleagues reported a heritability of 0.35 [12]. A mutual genetic correlation of 0.39 was reported for perfume-related respiratory symptoms and atopic dermatitis, suggesting some genetic pleiotropy for these two factors. No genetic pleiotropy was found between perfume-related respiratory symptoms, hand eczema, contact allergy, or asthma [12], suggesting that the association with asthma might be caused by mechanisms other than genetic susceptibility. Increasing evidence points to an association between MCS and symptoms of psychological distress, that is, depressive symptoms, somatisation, negative affect, and anxiety [13–18], which are likely to add to the level of overall functional disability.

The label "MCS" was initially proposed by Cullen based on clinical observations [19]. Although more case definitions have been proposed since the introduction of Cullen's

criteria in 1987 [9, 20], none is currently widely accepted [20, 21]. The absence of widely accepted case criteria for establishing the presence and degree of chemical intolerance challenges epidemiological and clinical studies in this field. Several self-report questionnaires have been developed for research purposes [22–24]. The questionnaire that appears to have been most widely applied is the Quick Environmental Exposure and Sensitivity Inventory (QEESI) developed by Miller and Prihoda [25, 26]. QEESI is a reliable and valid self-administered questionnaire that was developed to gauge the multisystem symptoms and multiple intolerances often reported in chemical intolerance [25, 26]. QEESI consists of five scales measuring different domains related to chemical intolerance, that is, commonly reported symptoms, chemical (inhalant) intolerances, other intolerances (e.g., allergies, foods, alcohol), life impact attributed to chemical intolerances, and on-going exposures from routinely used products (Masking Index). Four of the QEESI scales consist of ten items where responses are rated on an eleven point scale ranging from "*not at all a problem*" (0) to "*disabling symptoms*" [10], resulting in a score range from 0 to 100. The fifth, the Masking Index, also consists of ten items, but the response format is dichotomous (0 or 1), resulting in a score range from 0 to 10. QEESI has been translated into a number of different languages, that is, Swedish [27], Japanese [28, 29], and Spanish [30], of which the Swedish and Japanese versions have also been evaluated in terms of validity and reliability. The Swedish study included a mildly ($n = 67$) and a moderately/severely chemically intolerant group ($n = 126$) and a control group ($n = 90$). The study concluded that the Swedish version of QEESI is reliable and valid for investigating chemical intolerance [27]. The Japanese study included a general population group ($n = 498$) and an outpatient group with self-reported MCS ($n = 131$) [28]. Based on principal components analyses, this study concluded that three of the QEESI subscales, that is, Symptom Severity, Chemical (inhalant) Intolerances, and Life Impact, were valid. To the best of our knowledge, no other study has established normative data based on a large population-based sample, and an evaluation of a Danish version of QEESI will not only strengthen future studies on chemical intolerance but will also enable international comparisons of data.

The objectives of the present study were (1) to evaluate a Danish translation of the QEESI in relation to validity and reliability, (2) to describe sensitivity and specificity, (3) to test whether asthma and high scores (based on Danish population norms) on SCL-92 subscales of depression and somatisation were associated with scores on QEESI, and (4) to establish normative data.

2. Methods

2.1. Participants. Two groups were invited to participate in the study: (1) individuals from the general population and (2) individuals who had contacted the Danish Research Centre because of symptoms attributed to common airborne chemicals, and patients with physician-diagnosed chemical intolerance.

2.1.1. General Population. A random sample of 18–69-year-old ($n = 2000$) from the general population was drawn from the Danish Civil Registration system in January 2010.

2.1.2. Patients. The patient sample ($n = 315$) comprised individuals who had contacted the Danish Research Centre for Chemical Sensitivities between January 1, 2006 and January 1, 2010 ($n = 183$) because of reactions consistent with chemical intolerance, and individuals who had received a diagnosis of chemical intolerance either at the Copenhagen University Hospital, Rigshospitalet, or at Hamlet, Private Hospital, Denmark, between January 1, 1990 and January 1, 2009 ($n = 132$) by the same ear-nose-and throat specialist.

2.2. Measurements

2.2.1. A Danish Translation of QEESI. The original version of QEESI [25, 26] was translated into Danish by a professional translation agency. The Danish translation was subsequently tailored to Danish usage and then translated back to the original language by a different professional translator. The back translation was then compared with the original version of QEESI to identify potential sense-altering discrepancies. Finally, the Danish translation was pilot tested among individuals with chemical intolerance for comprehension and ease of completion.

2.2.2. Symptom Checklist 92. Symptom Checklist 92 (SCL-92) subscales for depression and somatisation were included. These subscales comprise 25 items where responses are rated on a 5-point Likert scale ranging from *not at all* to *very much*. The SCL-92 has been validated in a general Danish population and normative data have been established [31, 32].

2.2.3. Asthma. Questions on asthma were adopted from the Stage 1 questionnaire of the European Community Respiratory Health Study (ECRHS) [33]. Asthma was defined according to criteria employed by the ECRHS as an affirmative answer to at least one of the following questions: (1) *Have you been woken by an attack of shortness of breath at any time in the last 12 months?* (2) *Have you had an attack of asthma in the last 12 months?* (3) *Are you currently taking any medicine (including inhalers, aerosols, or tablets) for asthma?* [34].

2.2.4. Procedure. A questionnaire was sent to the participants on two occasions. The first test occasion included (1) the QEESI, (2) questions on socioeconomic position, categorized in accordance with the British Registrar General's Classification I–V [35], (3) the SCL-92 somatisation and depression subscales, and (4) the ECRHS asthma questions. Demographic data, for example, age and sex, were available. Two months after responding to the first questionnaire, a random sample of the respondents from the general

population ($n = 200$) and 140 patients who had responded to the first questionnaire received a second questionnaire, which consisted of the QEESI only. The overall response rate to the first questionnaire was 64.5%. The response rates in the population sample were 65.3% ($n = 1305/2000$) on the first test occasion and 61.0% ($n = 122/200$) on the second test occasion. The response rates in the patient sample were 60.0% ($n = 189/315$) on the first test occasion and 80.0% ($n = 112/140$) on the second test occasion.

3. Statistical Analysis

3.1. Reliability and Validity. The internal consistency of the four QEESI scales (Chemical (inhalant) Intolerances, Symptom Severity, Other Intolerances, and Life Impact) was evaluated using Cronbach's alpha [36]. Coefficients were calculated for the patient sample and for age stratified samples of the population. Test-retest reliability was evaluated using Pearson correlations.

The discriminatory validity of the QEESI was evaluated using bivariate logistic regression, and multivariate analyses were also used for the Chemical (inhalant) Intolerance and Life Impact Scales. Criterion validity was addressed using the variables asthma, somatisation, and depression, for which associations with chemical intolerance have been reported. These variables were dichotomized using the ECRHS asthma criteria and the gender-based cut-off scores for the SCL-92 somatisation and depression subscales described by Olsen and colleagues [31, 32]. Further, cross-validation was performed by randomly dividing the data set in two and comparing results with those obtained for the entire data set.

3.2. Differential Item Functioning. Differential item functioning (DIF) is the phenomenon that performance of items differs across subpopulations or that items measure different things for members of one subpopulation as opposed to members of another. Instruments containing such items may have reduced validity for between-group comparisons because scores may be indicative of attributes other than those the instrument is intended to measure [37]. We tested DIF by testing conditional independence given the total score. We used the partial gamma coefficient [38], suggested by Kreiner when items are polytomous [39]. DIF with respect to asthma was tested using the ECRHS criteria, and depressive and somatising individuals were identified using the SCL-92 cut-off scores [31, 32].

Data were analysed using SPSS, version 15.0 for Windows and SAS version 9.2.

4. Approval

The study was approved by the Danish Data Protection Agency. According to Danish legislation questionnaire studies do not need approval from an ethics committee.

5. Results

5.1. Sample Characteristics. Characteristics of the patient- and population samples are shown in Table 1. Due to skewed distributions, the medians for the five QEESI scales (the Symptom Severity, Chemical (inhalant) Intolerances, Other Intolerances, Life Impact Scales, and the Masking Index) are presented. Table 1 also includes sex and age distributions for the two samples, mean and median scores on the two SCL-92 subscales, as well as occupational social class (SES). Table 1 shows that scores on the QEESI and SCL-92 differed significantly in the expected direction between the two samples. In analyses stratified by gender QEESI scores also differed significantly ($P < 0.0001$) between the two groups (data not shown). In terms of QEESI, scores also differed significantly ($P < 0.0001$) between women in the population and patient samples as well as between men (data not shown). In regards to age, the patient sample was significantly older than the population and differences were also seen in relation to SES classifications, which may be a consequence of the differences seen in age.

5.2. Reliability. Cronbach alpha coefficients and median scores on the four QEESI scales (Chemical (inhalant) Intolerances, Symptom Severity, Other Intolerances, and Life Impact) are shown in Table 2. The Cronbach alpha coefficients were overall high in both groups (range 0.64–0.94) for all four scales suggesting good internal consistency (Table 2).

Pearson correlation analyses of test-retest reliability showed statistically significant coefficients for the five scales: the Chemical (inhalant) Intolerances Scale (0.94, $n = 230$), the Symptom Severity Scale (0.89, $n = 234$), the Other Intolerances Scale (0.89, $n = 233$), the Life Impact Scale (0.96, $n = 232$), and the Masking Index (0.84, $n = 234$).

5.3. Validity. The discriminatory validity of the five QEESI scales is shown in Table 3. In the simple logistic regression analyses, the discriminatory power was the largest for the Chemical (inhalant) Intolerance and the Life Impact Scale, and these two scales were therefore selected for subsequent multivariate analyses. Calculating other pairwise comparisons resulted in lower values than the one specified for the Chemical (inhalant) Intolerance and the Life Impact Scales (data not shown). Including more scales in the analysis did not substantially change the result since the maximum value obtained for all five scales was 0.98 (data not shown).

To test whether other variables found to be associated with chemical intolerance, that is, asthma, somatisation, and depression, would influence the discriminatory validity of the Chemical (inhalant) Intolerance and the Life Impact Scales, other subsequent statistical analyses were performed. Area under the ROC curve was calculated using the ECRHS asthma criteria and the gender-based cut-off scores for the SCL-92 somatisation and depression subscales, as described by Olsen and colleagues [31, 32], in the analyses. The following results were obtained: for the Chemical (inhalant) Intolerance Scale the area under the ROC curve for asthma

Table 1: Characteristics of the patient and the population samples.

	Population sample			Patient sample			P-value[1]
	Men	Women	Total	Men	Women	Total	
	600	705	1305	25	163	188	
Age, mean (sd)	47.4 (14.1)	46.8 (14.3)	47.1 (14.2)	52.4 (14.7)	56.0 (10.8)	55.5 (11.5)	<0.0001
	QEESI (median)						P-value[2]
Symptoms	9.0	14.0	11.0	35.0	48.0	47.0	<0.0001
Chemical Int.	11.0	15.0	13.0	81.6	82.2	82.1	<0.0001
Other Int.	6.0	12.0	10.0	27.3	35.5	35.0	<0.0001
Life Impact	0.0	3.0	2.0	70.0	64.0	65.0	<0.0001
Masking Index	4.0	4.0	4.0	2.0	2.0	2.0	<0.0001
	SCL-92 (median)						
Depression	0.15	0.31	0.23	0.62	0.69	0.69	<0.0001
Somatisation	0.25	0.33	0.33	0.83	1.0	1.0	<0.0001
	SCL-92 (mean)						
Depression	0.39	0.53	0.47	1.0	0.86	0.88	—
Somatisation	0.38	0.51	0.45	1.0	1.13	1.12	—
	Occupational social class (n (%))						P-value[3]
							<0.0001
I + II:			224 (17.2)			17 (9.0)	
III + IV:			426 (32.6)			35 (18.5)	
V + VI + VII:			566 (43.4)			124 (65.6)	
Missing:			89 (6.8)			13 (6.9)	

Occupational social class: I + II: professionals and executives and medium-level white-collar employees; III + IV: low-level white-collar employees and skilled workers; V + VI + VII: unskilled and semiskilled workers, individuals receiving pension or disability benefits, and students.
[1]Independent samples t-test for equality of means (total) between population and patient samples.
[2]Mann-Whitney test (total) comparing population and patient samples.
[3]Chi-squared test comparing population and patient samples.

Table 2: Median scores and scale reliability coefficients (Cronbach's alpha).

Scale group	N	Symptom scale		Chemical intolerance scale		Other intolerance scale		Life Impact scale	
		Median (IQR)**	Alpha***	Median (IQR)	Alpha	Median (IQR)	Alpha	Median (IQR)	Alpha
Patient sample	189	47 (30–64)	0.84	80 (62–91)	0.91	34 (20–53)	0.83	64 (45–80)	0.89
Population	1309	11 (5–23)	0.86	13 (4–30)	0.92	10 (3–19)	0.77	2 (0–8)	0.86
Population*									
−30	201	12 (5–23)	0.83	11 (4–24)	0.87	11 (3–19)	0.64	4 (0–10)	0.74
30–40	218	11 (4–21)	0.85	15 (5–31)	0.93	11 (5–22)	0.79	2 (0–8)	0.86
40–50	288	11 (6–22)	0.89	14 (5–30)	0.92	11 (5–18)	0.79	2 (0–8)	0.90
50–60	312	12 (5–23)	0.86	14 (4–32)	0.94	8 (3–17)	0.78	1 (0–9)	0.87
60−	290	10 (4–23)	0.86	12 (1–29)	0.93	5 (0–17)	0.75	0 (0–5)	0.81

*Population sample grouped by age.
**Interquartile range.
***Cronbach's alpha.

was 0.93 (95% CI 0.90–0.95), for somatisation 0.89 (95% CI 0.86–0.94) (women) and 0.91 (95% CI 0.88–0.94) (men), and for depression 0.94 (95% CI 0.91–0.97) (women) and 0.94 (95% CI 0.91–0.96) (men); for the Life Impact Scale the area under the ROC curve for asthma was 0.94 (95% CI 0.91–0.96), for somatisation 0.88 (95% CI 0.84–0.93) (women) and 0.91 (95% CI 0.88–0.94) (men), and for depression 0.92 (95% CI 0.88–0.95) (women) and 0.93 (95% CI 0.89–0.95) (men) (data not shown). These results suggest that the area under the ROC curve is slightly lowered with coexisting asthma, depression, or somatisation; nevertheless the discriminatory validity of QEESI is still good. Randomly dividing the data set in two yielded results that corresponded with the results obtained for the entire data set: Chemical

Table 3: Discriminatory power of the five QEESI scales either when used alone (univariate analyses) or when combined in a multivariate logistic regression model.

Scale univariate	*P*-value	Odds ratio (95% CI) one-point increase	Area under ROC curve
Symptom severity	<0.0001	1.07 (1.06–1.08)	0.88 (0.85–0.90)
Chemical intolerances	<0.0001	1.11 (1.09–1.12)	0.97 (0.95–0.98)
Other Intolerances	<0.0001	1.07 (1.06–1.08)	0.84 (0.81–0.87)
Life Impact	<0.0001	1.10 (1.09–1.12)	0.97 (0.96–0.98)
Masking Index (rev)*	<0.0001	2.48 (2.16–2.06)	0.81 (0.78–0.84)
Multivariate			
Chemical intolerances	<0.0001	1.06 (1.05–1.08)	0.98
Life Impact	<0.0001	1.06 (1.05–1.07)	

* Scores on the Masking Index were reversed in the statistical analyses.

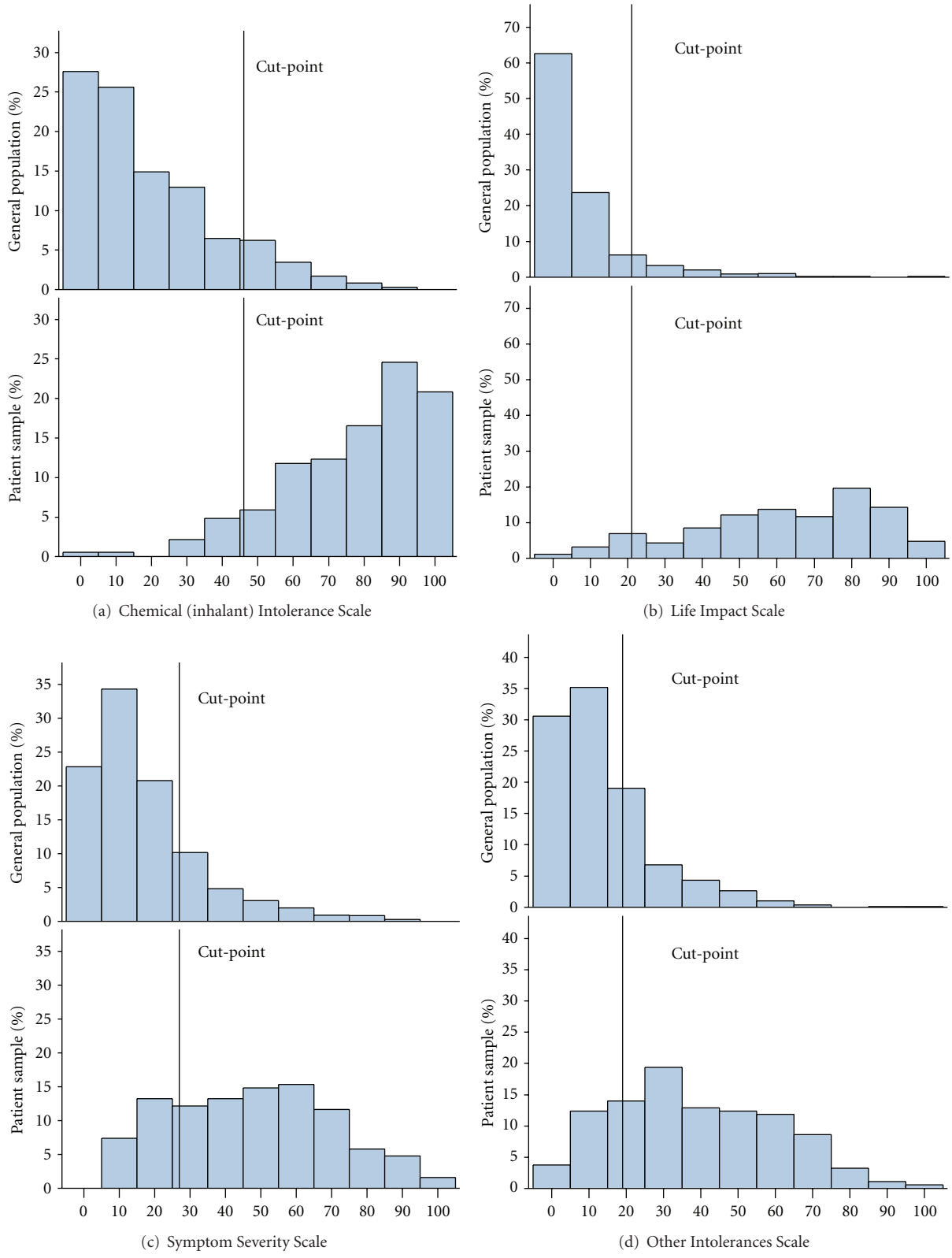
Table 4: Differential item functioning (DIF). Only significant results are shown.

Scale	Item	Asthmatics	Depressives	Somatisers
			partial γ coefficient	
Chemical int.	item 2 (tobacco smoke)	0.17 (se = 0.06)	−0.16 (se = 0.06)	—
	item 4 (gasoline)	−0.23 (se = 0.06)	—	—
	item 8 (tar)	—	0.16 (se = 0.04)	−0.17 (se = 0.06)
Life Impact	item 2 (work ability)	—	0.14 (se = 0.06)	—
	item 4 (choice of clothing)	—	−0.31 (se = 0.12)	—
	item 6 (choice of products)	−0.16 (se = 0.08)	—	—
	item 8 (choice of hobbies)	0.20 (se = 0.09)	—	—
	item 9 (relation with spouse)	—	—	−0.19 (se = 0.10)
Symptom severity	item 1 (muscle and joint pain)	0.23 (se = 0.06)	−0.19 (se = 0.06)	—
	item 2 (mucosal or respiratory)	0.47 (se = 0.04)	−0.30 (se = 0.07)	—
	item 4 (stomach and digestive)	—	−0.17 (se = 0.07)	—
	item 5 (concentration/memory)	−0.16 (se = 0.06)	0.36 (se = 0.06)	—
	item 6 (tension and nervousness)	−0.15 (se = 0.07)	0.79 (se = 0.04)	—
	item 7 (balance or coordination)	—	0.21 (se = 0.07)	0.24 (se = 0.08)
	item 10 (genital and urinary)	−0.20 (se = 0.06)	—	−0.27 (se = 0.08)
Other Int.	item 3 (unusual cravings)	−0.33 (se = 0.06)	—	—
	item 4 (feeling ill after meals)	—	0.42 (se = 0.08)	0.39 (se = 0.07)
	item 6 (feeling ill)	−0.21 (se = 0.08)	0.24 (se = 0.09)	—
	item 7 (alcoholic drinks)	—	0.27 (se = 0.09)	—
	item 10 (allergic reactions)	0.36 (se = 0.06)	−0.21 (se = 0.10)	—
Masking Index	item 2 (alcoholic intake)	−0.17 (se = 0.08)	−0.54 (se = 0.09)	—
	item 4 (fragranced products)	−0.51 (se = 0.06)	−0.35 (se = 0.11)	—
	item 10 (routine use of medicine)	0.62 (se = 0.05).	0.72 (se = 0.06)	—

(inhalant) Intolerance Scale (OR 1.07, 95% CI 1.05–1.09) and the Life Impact Scale (OR 1.05, 95% CI 1.03–1.07) (Area under the ROC curve 0.98).

Construct validity was tested by analysing differential item functioning (DIF), which investigates if item scores are affected by external variables. DIF was tested in asthmatics and in depressives and somatisers using the SCL-92 cut-off scores for caseness [31, 32]. Only statistically significant results are presented in Table 4.

5.4. Sensitivity and Specificity. Figure 1 shows the distribution of scores in the two groups and cut-off values for all four scales. Using all scales provided a sensitivity of 92.1% and a specificity of 93.1%. The ROC curves for the Chemical (inhalant) Intolerance and Life Impact Scales are shown in Figure 2. The 95% sensitivity and specificity and optimal cut-off scores for the Chemical (inhalant) Intolerance and the Life Impact Scales when used separately or when combined are shown in Table 5. When used separately, the cut-off



(a) Chemical (inhalant) Intolerance Scale

(b) Life Impact Scale

(c) Symptom Severity Scale

(d) Other Intolerances Scale

Figure 1: Distribution of the two study samples responses to the four QEESI scales.

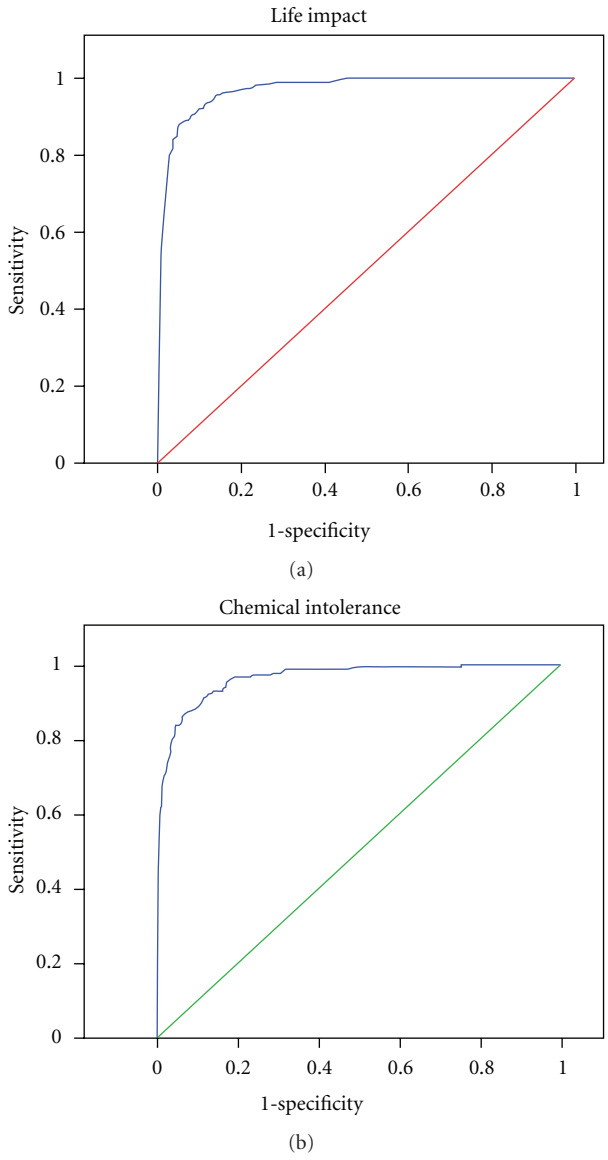


Figure 2: ROC curves for the Chemical (inhalant) Intolerance and Life Impact Scales.

values that provided the highest sensitivity and specificity for the two scales were 47 and 21 respectively (Table 5). Combining the two scale scores by using cut-offs of 35 (Chemical Intolerance scale) and 14 (Life Impact Scale) provided highest sensitivity and a specificity (Table 5). Miller and Prihoda [25, 26] used logistic regression to estimate a weighted sum of QEESI scales and an interaction term (the product of two scales) that could be used to provide an optimal cut-point. We used logistic regression finding no significant interactions. In our data this weighted approach yields a sensitivity of 94% and specificity of 91% by first computing $R = -3.9665 + 0.0619^*$ chemicalintolerance $- 0.0342^*$ otherintolerance $+ 0.6104^*$ maskingindex $+ 0.0767^*$ lifeimpactscale $- 0.00242^*$ symptoms and then computing the predicted probability prpr $= \exp(R)/(1 + \exp(R))$ and classifying a subject as "chemically sensitive" if prpr > 0.09. These analyses suggest that the difference between our approach and Miller and Prihoda's is minimal.

Table 5: Sensitivity, specificity, and optimal cut-off values for the chemical intolerance scale and the Life Impact Scale.

Chemical intolerance cut-off scores	Sensitivity (%)	Specificity (%)
37	95.2	82.8
47	**89.3**	**89.4**
58	83.9	95.2
Life Impact Scale cut-off scores	Sensitivity	Specificity
14	95.8	86.2
21	**91.0**	**90.9**
31	86.8	95.2
Combined scale scores	Sensitivity	Specificity
Chemical intolerance cut-off **35**/Life Impact Scale cut-off **14**	**92.1**	**91.8**

6. Discussion

The evaluation of the Danish version of QEESI suggested good reliability for the four scales, that is, Chemical (inhalant) Intolerances, Life Impact, Symptom Severity, and Other Intolerances, in terms of internal consistency and coefficients between test and retest scores.

The overall response rate to the first questionnaire was 64.5%. For the sample characteristics, the patients were significantly older than the general population sample and differences were also found in relation to SES, which may be a consequence of the age differences. In accordance with results reported in other studies, the patient group also scored significantly higher on the SCL-92 subscales [18, 40]. Scores on the QEESI differed between the samples in the expected direction as the patients scored significantly higher on all four scales, that is, the Chemical (Inhalant) Intolerances, Life Impact, Symptom Severity and Other Intolerances Scales, whereas the population scored significantly higher on the Masking Index.

Our results on the Cronbach alpha coefficients for all four scales and test-retest reliability showed good internal consistency and reliability and correspond to the results obtained in other studies evaluating the QEESI. The Cronbach alphas obtained in this study ranged from 0.64 to 0.94 in the population sample with a tendency to lower scores in the youngest age group, whereas the range in the patient sample was 0.83 to 0.91. Miller and Prihoda reported a corresponding range of 0.89–0.97 for the original American version of the questionnaire [25, 26]. Evaluating a Swedish version of the QEESI, Nordin and Andersson reported a range of 0.74 to 0.95 [27], and in the Japanese version the range was 0.87 to 0.94 for the Chemical (inhalant) Intolerances, the Life Impact, and the Symptom Severity scales [28].

The discriminatory validity was the largest for the Chemical (inhalant) Intolerance and Life Impact Scales. Testing the influence of other variables, that is, asthma, depression, and somatisation, by calculating area under the ROC curve did not substantially change the results. Using combined cut-off scores of 35 for the Chemical (inhalant) Intolerance Scale and 14 for the Life Impact Scale provided the best simultaneous sensitivity and specificity, that is, 92.1 and 91.8, respectively. The corresponding sensitivity and specificity for all five QEESI scales were 92.1% and 93.1%. Miller and Prihoda found that the discriminatory power for the Symptom Severity and the Chemical (inhalant) Intolerance Scales was largest [26]. They reported a sensitivity of 83.2% and a specificity of 84.2% using a cut-off score of ≥40 for the Chemical (inhalant) Intolerance Scale [26]. Using the cut-off scores collectively for the Chemical (inhalant) Intolerance Scale (≥40), the Symptom Severity Scale (≥40) and the Other Intolerance Scale (≥25) provided a sensitivity of 67.2% and a specificity of 90.9% [26]. In the Japanese evaluation of three of the QEESI scales, Hojo et al. reported the highest discriminatory ability for the Symptom Severity Scale with a cut-off score of ≥20, which provided a sensitivity of 84.8% and a specificity of 84.0% [29]. Sensitivity and specificity for the Life Impact Scale were 84.8% and 85.7%, respectively, with a cut-off score of ≥10. Contrary to our findings and the findings by Miller and Prihoda, the Japanese version of the Chemical (inhalant) Intolerance Scale had a low sensitivity (73.4%) and specificity (69.6) using a cut-off score of ≥40 [29]. The cut-off scores applied in the Japanese study were defined uniquely for the Japanese translation. Nordin and Andersson reported good discriminatory power for the Symptom Severity, Chemical (inhalant) Intolerance, and Life Impact Scales [27]. The different findings may reflect cross-cultural differences in the responses to QEESI or, perhaps more likely, reflect differences in study populations in relation to the selection and definition of cases. Nevertheless when applying the QEESI in epidemiological studies or in clinical research, our results suggest that using the combined cut-offs scores for the Chemical (inhalant) Intolerance and the Life Impact Scales provides a shorter and equally strong alternative.

Construct validity was tested by analysing differential item function (DIF). Item function is supposed to be invariant of other, and in this regard, irrelevant constructs [41]. Our analyses suggested that scores on several items on all five scales were influenced if the respondent had asthma according to the ECRHS criteria or had scores above the cut-off values for caseness on the SCL-92 subscales, which may have a negative impact on the construct validity of the Danish translation of QEESI. The use of the ECRHS definition on asthma has been validated with bronchial hyperresponsiveness to methacholine (BHR) [33] but not validated among individuals with chemical intolerance and might therefore overestimate a correlation. However, positive correlations between asthma and chemical intolerance have been described in studies using other self-reported asthma definitions [7, 42] as well as objective measurements of BHR [43]. Using the standards for interpretation of DIF applied by Bjorner et al. [41], the magnitude of DIF for the three items on the Chemical (inhalant) Intolerance Scale, that showed indication of DIF, was none or negligible. The same applied for the Life Impact Scale except for one item (choice of clothing) in relation to depression, which was in the slight to moderate range. However, taken together the magnitude of DIF appears to be of little importance for the construct validity of these two scales. For the remaining three scales the sizes of the gamma coefficients suggested that DIF may be a problem. However, this study is the first to test DIF in relation to the QEESI. Therefore, we cannot compare our results with others and thereby determine whether DIF occurs in other translations of the questionnaire than the Danish version. Accordingly, we recommend that future studies on QEESI address this issue. Altogether our results provide additional evidence of the reliability and validity of QEESI as a clinical survey tool for MCS. The size of the study and the response rate to the questionnaire on both the first and the second test occasion support the validity of our results. However, like most questionnaire-based studies, the information gathered relies upon self-reported and retrospective data, which must be kept in mind when interpreting the results. While reliability and validity of the different translations of QEESI have proven to be good and thereby support the use of the questionnaire in future studies, differences in the case definitions applied in the studies still point to difficulties in the comparisons of results across countries. As stated by Miller and Prihoda in their study published in 1999, the lack of a uniform approach for identifying individuals with chemical intolerance is a barrier for progress in this area [26]. Thus more research in this area is needed to establish internationally agreed diagnostic criteria. Meanwhile, the QESSI provides a good research tool with a response format that allows for continuous scores that may also be used in the evaluation of the effectiveness of treatments.

In conclusion, the Danish translation of the QEESI showed overall good reliability and validity, which is in accordance with the results reported in other studies. Our analyses of construct validity suggested that there may be problems with DIF in three of the QEESI scales. As our study is the first to conduct these analyses, we cannot conclude whether this applies only to the Danish translation. We therefore recommend that future studies on QEESI address this issue. For research purposes, we recommend use of the combined Chemical (inhalant) Intolerance and the Life Impact Scales scores, which provided a sensitivity of 92.1 and a specificity of 91.8 in this study.

Abbreviation List

MCS: Multiple chemical sensitivity
QEESI: Quick Environmental Exposure and Sensitivity Inventory
SCL-92: Symptom Checklist 92.

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References

[1] R. A. Graveling, A. Pilkington, J. P. K. George, M. P. Butler, and S. N. Tannahill, "A review of multiple chemical sensitivity," *Occupational and Environmental Medicine*, vol. 56, no. 2, pp. 73–85, 1999.

[2] N. D. Berg, A. Linneberg, A. Dirksen, and J. Elberling, "Prevalence of self-reported symptoms and consequences related to inhalation of airborne chemicals in a Danish general population," *International Archives of Occupational and Environmental Health*, vol. 81, no. 7, pp. 881–887, 2008.

[3] S. M. Caress and A. C. Steinemann, "A national population study of the prevalence of multiple chemical sensitivity," *Archives of Environmental Health*, vol. 59, no. 6, pp. 300–305, 2004.

[4] S. M. Caress and A. C. Steinemann, "Prevalence of multiple chemical sensitivities: a population-based study in the Southeastern United States," *American Journal of Public Health*, vol. 94, no. 5, pp. 746–747, 2004.

[5] C. Hausteiner, S. Bornschein, J. Hansen, T. Zilker, and H. Förstl, "Self-reported chemical sensitivity in Germany: a population-based survey," *International Journal of Hygiene and Environmental Health*, vol. 208, no. 4, pp. 271–278, 2005.

[6] A. Johansson, A. Brämerson, E. Millqvist, S. Nordin, and M. Bende, "Prevalence and risk factors for self-reported odour intolerance: the Skövde population-based study," *International Archives of Occupational and Environmental Health*, vol. 78, no. 7, pp. 559–564, 2005.

[7] R. Kreutzer, R. R. Neutra, and N. Lashuay, "Prevalence of people reporting sensitivities to chemicals in a population-based survey," *American Journal of Epidemiology*, vol. 150, no. 1, pp. 1–12, 1999.

[8] N. D. Berg, A. Linneberg, A. Dirksen, and J. Elberling, "Phenotypes of individuals affected by airborne chemicals in the general population," *International Archives of Occupational and Environmental Health*, vol. 82, no. 4, pp. 509–517, 2009.

[9] M. Lacour, T. Zunder, K. Schmidtke, P. Vaith, and C. Scheidt, "Multiple chemical sensitivity syndrome (MCS)—suggestions for an extension of the US MCS-case definition," *International Journal of Hygiene and Environmental Health*, vol. 208, no. 3, pp. 141–151, 2005.

[10] S. M. Caress and A. C. Steinemann, "Asthma and chemical hypersensitivity: prevalence, etiology, and age of onset," *Toxicology and Industrial Health*, vol. 25, no. 1, pp. 71–78, 2009.

[11] J. Elberling, A. Linneberg, H. Mosbech et al., "Airborne chemicals cause respiratory symptoms in individuals with contact allergy," *Contact Dermatitis*, vol. 52, no. 2, pp. 65–72, 2005.

[12] J. Elberling, A. Lerbaek, K. O. Kyvik, and J. Hjelmborg, "A twin study of perfume-related respiratory symptoms," *International Journal of Hygiene and Environmental Health*, vol. 212, no. 6, pp. 670–678, 2009.

[13] J. Bailer, M. Witthöft, and F. Rist, "Psychological predictors of short- and medium term outcome in individuals with idiopathic environmental intolerance (IEI) and individuals with somatoform disorders," *Journal of Toxicology and Environmental Health Part A*, vol. 71, no. 11-12, pp. 766–775, 2008.

[14] J. Bailer, M. Witthöft, C. Bayerl, and F. Rist, "Syndrome stability and psychological predictors of symptom severity in idiopathic environmental intolerance and somatoform disorders," *Psychological Medicine*, vol. 37, no. 2, pp. 271–281, 2007.

[15] S. Skovbjerg, R. Zachariae, A. Rasmussen, J. D. Johansen, and Jesper Elberling, "Attention to bodily sensations and symptom perception in individuals with idiopathic environmental intolerance," *Environmental Health and Preventive Medicine*, vol. 15, no. 3, pp. 141–150, 2010.

[16] E. C. V. Vliet, K. Kelly-McNeil, B. Natelson, H. Kipen, and N. Fiedler, "Anxiety sensitivity and depression in multiple chemical sensitivities and asthma," *Journal of Occupational and Environmental Medicine*, vol. 44, no. 10, pp. 890–901, 2002.

[17] K. Österberg, R. Persson, B. Karlson, F. Carlsson Eek, and P. Ørbæk, "Personality, mental distress, and subjective health complaints among persons with environmental annoyance," *Human and Experimental Toxicology*, vol. 26, no. 3, pp. 231–241, 2007.

[18] I. R. Bell, J. M. Peterson, and G. E. Schwartz, "Medical histories and psychological profiles of middle-aged women with and without self-reported illness from environmental chemicals," *Journal of Clinical Psychiatry*, vol. 56, no. 4, pp. 151–160, 1995.

[19] M. R. Cullen, "The worker with multiple chemical sensitivities: an overview," *Occupational Medicine*, vol. 2, no. 4, pp. 655–661, 1987.

[20] L. Bartha, W. Baumzweiger, D. S. Buscher et al., "Multiple chemical sensitivity: a 1999 consensus," *Archives of Environmental Health*, vol. 54, no. 3, pp. 147–149, 1999.

[21] R. Kreutzer, "Idiopathic environmental intolerance: case definition issues," *Occupational Medicine*, vol. 15, no. 3, pp. 511–517, 2000.

[22] S. Nordin, E. Millqvist, O. Löwhagen, and M. Bende, "A short chemical sensitivity scale assessment of airway sensory hyperreactivity," *International Archives of Occupational and Environmental Health*, vol. 77, no. 4, pp. 249–254, 2004.

[23] M. J. E. Andersson, L. Andersson, M. Bende, E. Millqvist, and S. Nordin, "The idiopathic environmental intolerance symptom inventory: development, evaluation, and application," *Journal of Occupational and Environmental Medicine*, vol. 51, no. 7, pp. 838–847, 2009.

[24] J. Bailer, M. Witthöft, and F. Rist, "The chemical odor sensitivity scale: reliability and validity of a screening instrument for idiopathic environmental intolerance," *Journal of Psychosomatic Research*, vol. 61, no. 1, pp. 71–79, 2006.

[25] C. S. Miller and T. J. Prihoda, "A controlled comparison of symptoms and chemical intolerances reported by Gulf War veterans, implant recipients and persons with multiple chemical sensitivity," *Toxicology and Industrial Health*, vol. 15, no. 3-4, pp. 386–397, 1999.

[26] C. S. Miller and T. J. Prihoda, "The environmental exposure and sensitivity inventory (EESI): a standardized approach for measuring chemical intolerances for research and clinical applications," *Toxicology and Industrial Health*, vol. 15, no. 3-4, pp. 370–385, 1999.

[27] S. Nordin and L. Andersson, "Evaluation of a Swedish version of the quick environmental exposure and sensitivity inventory," *International Archives of Occupational and Environmental Health*, vol. 83, no. 1, pp. 95–104, 2010.

[28] S. Hojo, H. Kumano, H. Yoshino, K. Kakuta, and S. Ishikawa, "Application of quick environment exposure sensitivity inventory (QEESI) for Japanese population: study of reliability and validity of the questionnaire," *Toxicology and Industrial Health*, vol. 19, no. 2-6, pp. 41–49, 2003.

[29] S. Hojo, K. Sakabe, S. Ishikawa, M. Miyata, and H. Kumano, "Evaluation of subjective symptoms of Japanese patients with

multiple chemical sensitivity using QEESI," *Environmental Health and Preventive Medicine*, vol. 14, no. 5, pp. 267–275, 2009.

[30] S. Nogué, J. Fernández-Solá, E. Rovira, E. Montori, J. M. Fernández-Huerta, and P. Munné, "Multiple chemical sensitivity: study of 52 cases," *Medicina Clinica*, vol. 129, no. 3, pp. 96–98, 2007.

[31] L. R. Olsen, E. L. Mortensen, and P. Bech, "The SCL-90 and SCL-90R versions validated by item response models in a Danish community sample," *Acta Psychiatrica Scandinavica*, vol. 110, no. 3, pp. 225–229, 2004.

[32] L. R. Olsen, E. L. Mortensen, and P. Bech, "Mental distress in the Danish general population," *Acta Psychiatrica Scandinavica*, vol. 113, no. 6, pp. 477–484, 2006.

[33] J. Pekkanen, J. Sunyer, J. M. Anto et al., "European community respiratory health study operational definitions of asthma in studies on its aetiology," *European Respiratory Journal*, vol. 26, no. 1, pp. 28–35, 2005.

[34] J. Sunyer, D. Jarvis, J. Pekkanen et al., "Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study," *Journal of Allergy and Clinical Immunology*, vol. 114, no. 5, pp. 1033–1039, 2004.

[35] U. Christensen, L. Schmidt, C. Ørsted Hougaard, M. Kriegbaum, and B. E. Holstein, "Socioeconomic position and variations in coping strategies in musculoskeletal pain: a cross-sectional study of 1287 40- and 50-year-old men and women," *Journal of Rehabilitation Medicine*, vol. 38, no. 5, pp. 316–321, 2006.

[36] L. J. Cronbach and W. G. Warrington, "Time-limit tests: estimating their reliability and degree of speeding," *Psychometrika*, vol. 16, no. 2, pp. 167–188, 1951.

[37] P. W. Holland and H. Wainer, *Differential Item Functioning*, Lawrance Erlbaum Associates, London, UK, 1993.

[38] J. A. Davis, "A partial coefficient for Goodman and Kruskal's gamma," *Journal of the American Statistical Association*, vol. 62, pp. 189–193, 1967.

[39] S. Kreiner, "Analysis of multidimensional contingencey tables by exact conditional tests," *Scandinavian Journal of Statistics*, vol. 14, pp. 97–112, 1987.

[40] I. R. Bell, C. S. Miller, G. E. Schwartz, J. M. Peterson, and D. Amend, "Neuropsychiatric and somatic characteristics of young adults with and without self-reported chemical odor intolerance and chemical sensitivity," *Archives of Environmental Health*, vol. 51, no. 1, pp. 9–21, 1996.

[41] J. B. Bjorner, S. Kreiner, J. E. Ware, M. T. Damsgaard, and P. Bech, "Differential item functioning in the Danish translation of the SF-36," *Journal of Clinical Epidemiology*, vol. 51, no. 11, pp. 1189–1202, 1998.

[42] S. M. Caress and A. C. Stainamann, "National prevalence of asthma and chemical hypersensitivity: an examination of potential overlap," *Journal of Occupational and Environmental Medicine*, vol. 47, no. 5, pp. 518–522, 2005.

[43] J. Elberling, A. Linneberg, A. Dirksen et al., "Mucosal symptoms elicited by fragrance products in a population-based sample in relation to atopy and bronchial hyper-reactivity," *Clinical and Experimental Allergy*, vol. 35, no. 1, pp. 75–81, 2005.

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Review Article

Solar Radiation and Vitamin D: Mitigating Environmental Factors in Autoimmune Disease

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This paper looks at the environmental role of vitamin D and solar radiation as risk reduction factors in autoimmune disease. Five diseases are considered: multiple sclerosis, type 1 diabetes, rheumatoid arthritis, autoimmune disease of the thyroid, and inflammatory bowel disease. Clinical relevant studies and factors that may indicate evidence that autoimmune disease is a vitamin D-sensitive disease are presented. Studies that have resulted in prevention or amelioration of some autoimmune disease are discussed. An example of the utility of supplementing vitamin D in an unusual autoimmune disease, idiopathic thrombocytic purpura, is presented.

1. Introduction

After cardiovascular disease and cancer, autoimmune diseases, taken as a group, are the third leading cause of morbidity and mortality in the industrialized world [1]. There are more than eighty defined autoimmune diseases [2] known in humans. A multifactorial interaction between genetic predisposition, immunologic, hormonal and environmental stimuli contributes to the development of autoimmune disease [3]. Agents that may trigger autoimmune disease include infections, vaccine immunogens, adjuvants used to increase immune response, smoking and stress, and so forth as outlined in the literature [4]. (See Table 1). The prevalence of some autoimmune diseases may be as high as 5% in the general population [5]. Little is known about mitigating factors until recently. Evidence that autoimmune disease may be a vitamin D-sensitive disease comes from many studies. Solar radiation (UVR) and vitamin D have been shown to inhibit the induction of a number of autoimmune diseases in animal models [6–8]. (See Table 2). Autoimmune disease should vary by season, temperature, level of ultraviolet irradiance, latitude, race or skin color, BMI, physical activity, and vitamin D supplementation, if it is a vitamin D-sensitive process.

This paper will discuss the interaction between the host, the agent, and the environment as depicted in Figure 1. The environmental factor being considered is UVB radiation induced vitamin D or supplemental vitamin D. Five autoimmune diseases will be discussed. Multiple sclerosis, type 1 diabetes, rheumatoid arthritis, autoimmune disease of the thyroid, and inflammatory bowel disease.

At the end of this paper, an example of the utility of vitamin D in idiopathic thrombocytic purpura, another autoimmune disease, is discussed.

2. A Brief Overview of Vitamin D and Its Potential Role in Autoimmune Disease

Vitamin D deficiency is common in latitudes far from the equator [10, 11], and solar abstinence has been in vogue for the past few decades for fear of inducing skin cancer. Sun exposure (a minimal erythemal dose with full body exposure) can rapidly produce 10,000 or more units of vitamin D [12], and toxicity has not been ascribed to this method of achieving normal vitamin D status. Vitamin D used orally at 10,000 IU a day for several months does not cause toxicity [13]. Vitamin D is a secosteroid hormone

Table 1: Agents that trigger autoimmune disease.

Infections	Epstein-Barr's virus, cytomegalovirus, parvovirus, enteropathogenic bacteria
Vaccine immunogens	Multiple sclerosis, Guillain-Barre's syndrome, autism, rheumatoid arthritis, reactive arthritis, systemic lupus erythematosus, diabetes, vasculitis, dermatomyositis, polyarteritis nodosa
Adjuvants used to enhance immune response	Lupus erythematosus, brain directed autoantibodies, arthritis, nephritis
Birth control, pregnancy	Autoimmune thyroid disease
Smoking	Rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, Graves' hyperthyroidism, Crohn's disease
Stress	Type 1 diabetes, Grave's disease

Adapted from [3, 4].

Table 2: Autoimmune diseases that are inhibited by 1,25(OH)2D in animal studies [6].

Autoimmune encephalomyelitis
Collagen-induced arthritis
Inflammatory bowel disease
Type 1 diabetes
Systemic erythematosus
Thyroiditis
Lyme arthritis
Rheumatoid arthritis
Multiple sclerosis

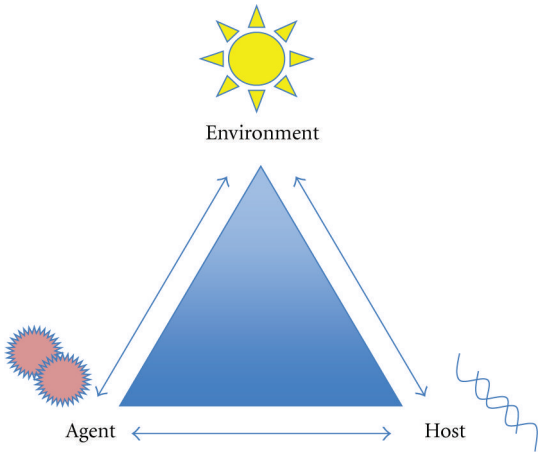

Figure 1: Autoimmune disease causation triangle. Adapted from [9]. Used with permission.

available in some foods and supplements or produced in the skin from 7-dehydrocholesterol after exposure to ultraviolet B light. The resulting previtamin D is then hydroxylated in the liver to hydroxyvitamin D (25(OH)D) and further hydroxylated in the kidney to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) which is the active hormone involved in calcium absorption in the gut. Circulating 25(OH)D (which is considered the measure of vitamin D adequacy) may also be used as substrate in many cells to locally produce ($1,25(OH)_2D$), the active hormone, via the CYP27B1 (1α-hydroxylase) enzyme and is inactivated by the CYP24A (24-hydroxylase) enzyme [14]. The classical role of vitamin D is to regulate calcium homeostasis [15]. Short latency diseases, such as rickets and osteomalacia, can be cured with 25(OH)D levels > 25 nmol/L. In a long-latency disease, such as osteoporosis, levels of 25(OH)D > 50 nml/L have been shown to reduce fractures.

In the last twenty years, the importance of vitamin D in the role of a hormone has been shown to influence numerous other diseases including cancer by increasing apoptosis in cancer cells and protecting DNA in normal cells [16]. Its effect on the immune system and infections is only beginning to be understood, and much higher doses of vitamin D may be needed to be effective in combating viruses, bacteria, and fungi [17]. Vitamin D is now recognized to be crucial in beta-defensin production in Crohn's disease [18].

The role in regulating the immune system in regards to self-tolerance and autoimmunity begins with an understanding of the impact of vitamin D on our genes. Research is showing that there are 2776 "binding sites" on the human genome to which vitamin D attaches, with at least 229 genes associated with Crohn's disease and type 1 diabetes [19]. Many of the sites are concentrated around genes linked to autoimmune conditions as described in this paper. Beyond this, vitamin D suppresses autoimmune disease pathology by regulating differentiation and activity of CD4+ T cells resulting in a more balanced T1/T2 response favoring less development of self-reactive T cells and autoimmunity [2].

3. Multiple Sclerosis

In regards to the host, it is known that only 30% of monozygotic twin pairs eventually get multiple sclerosis (MS) leading us to believe that exposure to one or more environmental risk factors is necessary for the development of MS [20]. Recently, two large genomic studies have confirmed the unambiguous associations with the DRB1 and DQB alleles of the human leukocyte antigen class II region and susceptibility to MS [21].

Multiple environmental factors may play a role in multiple sclerosis. An example is the Epstein-Barr virus infections [22]. The risk of developing multiple sclerosis following infectious mono is increased significantly for more than 30 years following infection [23]. Even after 10 years following infection, the risk has been defined as at least four-fold [24]. Sunlight and vitamin D may be protective, and MS demonstrates vitamin D sensitivity [25].

Evidence shows that MS correlates positively with higher latitude, with latitudes >37.5 degrees from the equator having significantly higher rates of MS [26–29]. The time of the first exacerbation after disease onset shows seasonal variation with 76% of exacerbations occurring in winter [30]. However, in another study, the likelihood and intensity of MS disease activity correlated positively with spring and summer (March to August) along with increased temperature and UVR [31]. Relapses of MS have a biphasic pattern with peaks in early spring when vitamin D levels are low and late fall when levels are declining [32]. MS also correlates positively with season of birth, with a significantly higher incidence of MS in those born in May, corresponding to low vitamin D levels in the winter months prior to giving birth [33]. As well, MS correlates inversely with altitude, with higher elevation (>2000 Meters) receiving more intense solar radiation having lower rates of MS [34]. Adiposity has been associated with lower vitamin D levels [35, 36], and a higher body mass index (BMI) has been associated with higher incidence of MS in adolescent women but not in adult women [37]. Multiple sclerosis correlates positively with skin color where sun avoidance is more likely (fair skin phenotype) [38]. In Norway, the intake of vitamin D in costal communities is estimated to be three times the average intake of those living inland and this is inversely associated with MS [39].

There are only a limited number of trials currently available in humans. An American study of more than 187,000 women followed for 10–20 years showed promising results with females taking at least 400 IU of supplemental vitamin D daily. The risk of developing MS was decreased by 40% [40]. Vitamin D_3 has been used safely in MS patients at high doses from 28 to 280, 000 IU per week. Mean levels of 25(OH)D rose to a mean of 385 nmol/L without causing hypercalcemia after being given the highest dose. Disease progression and activity were not affected in this study, but the number of gadolinium-enhancing lesions per patient assessed by nuclear magnetic brain scan was significantly reduced [41]. The highest dose in this study was only used for the last 6 weeks of the study, and longer-term use at this dose may risk significant toxicity in some patients. A trial using high-dose vitamin D_2 to achieve 25(OH)D levels of 130–195 nmol/L did not reduce MRI lesions in relapsing remitting multiple sclerosis [42]. In a recently reported trial using escalating doses up to 40,000 IU of vitamin D_3 for 28 weeks followed by 10,000 IU daily for 12 weeks, there were no significant adverse events and there appeared to be significantly less progression of disability in the treatment group [43].

4. Type 1 Diabetes

As with multiple sclerosis only about 34% of identical twins will develop type 1 diabetes [44]. Thus, exposure to other environmental stimuli must play an important role.

In the past rubella infection has been implicated as an inducer of type I diabetes. Several studies now indicate infection with enteroviruses seem to be linked to the induction of islet cell destruction and development of autoantibodies [45, 46]. Stress in the mother during pregnancy has been associated with elevated islet autoantibodies in cord blood [47].

The following is a summary of the evidence that type 1 diabetes is a vitamin D-sensitive disease. Type I diabetes varies by latitude with higher incidence in latitudes further from the equator (both north or south of the equator) except where local weather may influence this [48]. A yearly cyclical pattern (consistent over 20 years) of type 1 diabetes in Newfoundland reveals a peak incidence in winter [49]. Greater exposure to erythemal UVB radiation is negatively correlated with the incidence of type 1 diabetes [50]. The birth months of March to June correlate with an increased incidence of diabetes in Britain with prenatal exposure to low vitamin D levels during the winter months [51]. The average yearly temperature also correlates inversely with the incidence of type 1 diabetes [52]. Higher BMI as well as lower plasma levels of 25(OH)D correlate directly with development of type 1 diabetes in young adults [53, 54]. Maternal vitamin D levels have been shown to correlate inversely to the presence of islet autoantibodies in the offspring [55]. Supplementation of vitamin D in early childhood appears to reduce the chances of developing type 1 diabetes [56].

There is a large body of evidence showing that lack of vitamin D early in life is linked to the development of type 1 diabetes. Vitamin D used at a dose of 2000 IU in infants has been shown to reduced the subsequent development of type 1 diabetes over the next thirty years by 78 percent [57]. The use of cod liver oil in pregnancy and during the first year of life has been shown to reduce the risk of childhood onset type 1 diabetes. However, it was not possible to determine if vitamin D, omega-3 fatty acids, or the combination contributed to this result [58, 59]. The use of 400 IU of vitamin D has not been shown to reduce diabetes incidence [60]. In Finland the use of 2000 IU of vitamin D in children was recommended from 1964 to 1975 when it was lowered to 1000 IU of vitamin D supplementation with a small increase in type 1 diabetes incidence following this recommendation. A further reduction to the use of 400 IU of vitamin D in 1992 correlates with a significant rise in the incidence of type 1 diabetes since then [61]. A meta-analysis and systematic review on the use of vitamin D supplementation in early childhood in type 1 diabetes showed that the risk of developing type 1 diabetes was significantly reduced in infants with supplementation [56]. The pooled odds ratio was 0.71 (a CI of 0.60 to 0.84), and there was evidence of a dose-response effect showing a lower risk of developing type 1 diabetes with the use of higher amounts of vitamin D. In regards to sun exposure,

the incidence of type 1 diabetes approaches zero in areas with high levels of UVB irradiance [48].

5. Rheumatoid Arthritis (RA)

The maximum genetic contribution indicated by studies on monozygotic twins is about 15% when it comes to rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [62]. The HLA-DRB1 gene represents the major determinant of genetic predisposition to RA [63].

The association of infections and RA is still being debated in the literature. There is some evidence that RA-like diseases may result from the immunologic interaction between the host and bacterial peptidoglycans. Several agents relative to RA have been entertained such as heat shock proteins, bacterial IgG FC-binding proteins and rheumatoid factors [64]. Retroviruses and enteropathogenic bacteria continue to be intensively discussed candidates [65]. IgM antibodies to parvovirus B19 indicating recent infection correlate with juvenile idiopathic arthritis [66]. Certainly smoking has been cited as being a major risk factor both in RA and SLE as well as a risk factor for RF-positive and anti-citrulline antibody titers [67]. The risk only diminishes slowly after several years of cessation of smoking.

Increasing latitude in the northern hemisphere has been shown to correlate with increased risk of RA providing some support for a beneficial role of UVR [68]. Patients with undifferentiated connective tissue diseases have a seasonal variance in 25(OH)D levels, being significantly lower than controls in corresponding seasons [69]. Seasonal variations showed lower disease activity with higher 25(OH)D levels [70]. It appears that rheumatoid arthritis does not have association with month of birth [71]. Rheumatoid arthritis activity is inversely related to 25(OH)D levels [72]. Rheumatoid arthritis severity is associated positively with BMI with a high BMI having a greater risk of low 25(OH)D levels [73]. The incidence of arthritis in children is highest in Caucasian versus East Indian, First Nations as well as African American [74, 75]. The Iowa Women's Health Study showed an inverse association of vitamin D intake and rheumatoid arthritis [76] There was a 34% reduction in the development of rheumatoid arthritis with greater vitamin D intake. Women using a multivitamin with 400 IU of vitamin D reduced their risk of developing RA by 40% [76]. An open-label study using a high-dose vitamin D3 analogue resulted in improvement of symptoms in RA in 89% of patients with 45% of patients entertaining a complete remission [77].

6. Autoimmune Disease of the Thyroid

Autoimmune disease of the thyroid (AITD) is very prevalent with 5% of the population being affected. This includes both Hashimoto's (HT) and Graves' disease (GD) [5]. Several susceptibility genes for AITD have been identified, and it has been estimated that up to 80% may be attributable to genes [78]. However, the concordance rates in monozygotic twins is about 30–60%, while dizygotic twins is only 3–9%, and up to 50% of siblings of patients with GD having thyroid autoantibodies [79]. Thus, environmental factors contribute significantly to the expression of the disease.

Environmental factors are numerous and include iodine excess and deficiency, selenium deficiency, oral contraceptive use, parity, low birth weight, seasonal variation, allergy, smoking, radiation, viral and bacterial infections, and so forth [78]. Overall, data suggests that cumulative cigarette consumption significantly increases the risk of developing AITD [80]. Hashimoto's thyroiditis is associated with vitamin D insufficiency with AITD patients having significantly lower levels than controls [81, 82]. In AITD 72% of patients had 25(OH)D levels less than 25 nmol/L compared to 30.6% in healthy individuals. In patients with Hashimoto's thyroiditis, 79% had levels less than 25 nmol/L compared to 52% in normal controls.

The lowest incidence of AIDT disease was found in July to October (when vitamin D levels are high), and the highest incidence was in January to March when vitamin D levels tend to be low in the northern hemisphere [83]. The incidence of diagnosis of thyrotoxicosis is highest in May just after the winter when vitamin D levels are beginning to rise; however, this may also be a reflection of feeling hot with increasing ambient temperatures [84]. There is no information on altitude or temperature. Vitamin D levels are lower in AITD patients than in normal controls and supplementation is recommended [82]. There are presently no studies using vitamin D as an intervention in AITD; however, supplementation has been suggested.

7. Inflammatory Bowel Disease

In regards to genetic predisposition, a national German study has shown that concordance rates in monozygotic twins are about 35% for Crohn's disease (CD) and 16% for ulcerative colitis (UC), showing that environmental factors play a significant role in inflammatory bowel disease (IBD) [85].

An environmental trigger shown to increase the risk of development of CD is smoking. Many other triggers have been proposed such as infectious gastroenteritis, oral contraceptives, invasive *E. coli*, and antibiotics [86]. Vitamin D insufficiency is associated with inflammatory bowel disease [87, 88]. In another study 25(OH)D levels were lower in those with severe disease activity and less sun exposure [89].

A study in France has shown that there is an increasing incidence of UC with northern latitude [90]. Smaller studies did not show any seasonal pattern for IBD, but a larger study showed an increase incidence in those born in the first half of the year [91] while others show an increased incidence of exacerbations in summer. There is no information on altitude or temperature. Obesity is more common in Crohn's disease at the time of diagnosis [92]. Vitamin D deficiency is very prevalent in IBD [93] and the effects of low-dose supplementation are poor [94]. A clinical trial using 1200 IU of vitamin D_3 for 12 months resulted in a reduced risk of relapse from 29% to 13%, although this did not quite reach statistical significance [95]. Other trials are currently under way.

A striking example in which vitamin D made a significant change in morbidity in another autoimmune disease follows.

8. A Case History and Discussion of the Benefit of Vitamin D in Idiopathic Thrombocytopenic Purpura in an Adult

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease in which most patients have antibodies to specific membrane glycoprotein's on platelets. The incidence in adults is about 33/1,000,000 [96]of which about 10/1,000,000 become refractory. Spontaneous remission is uncommon in adults. The 5-year mortality rate is significantly elevated in adults over the age of 60 (47.8%) versus those below 40 years of age (2.2%), respectively. The most serious complication is hemorrhage of which intracranial hemorrhage is the most significant.

Vitamin D has been shown to improve outcomes and prevent some autoimmune diseases if taken early in life as discussed previously in the paper.

Treatment of ITP usually includes medications such as corticosteroids, splenectomy danazol, and various immune suppressant therapies [97]. The use of vitamin D_3 in ITP has not been described in the literature.

This example describes a patient who had refractory ITP who has been treated in the past with a splenectomy, danazol, and prednisone rescue during intercurrent illness. A review of the history of this case revealed a 48-year-old female who was found to have a very low platelet count in 1998, which remained persistent over time. After consultation with a hematologist, the diagnosis of ITP was made. Her platelets continued to drop so she had a splenectomy, which improved her platelet count, but it never achieved normal. Despite the use of danazol, her platelet count never normalized. She had frequent episodes of low platelets as low as 8×10^9 with intercurrent illnesses such as colds or flus. In 2006 she was found to have an inadequate level of 25(OH)D of 65 nmol/L. Her platelet count at that time was 8×10^9 after a viral illness. She was treated successfully with a tapering dose of Prednisone. She was started on vitamin D_3 2000 IU daily after this episode and during the next two years while she was on this dose she did not have any flus or colds and her platelet count never fell below $44 \times 10^9$and was usually from 70 to 80×10^9. This was quite out of the normal for her since she had at least one low episode a year. After being on this dose for two years, a neighbor in her building where she resided suggested that she was going to become toxic on this dose and she stopped her vitamin D. About three months later, she again had an upper respiratory infection (URI) and her platelets dropped only to 50×10^9 and she was started on prednisone with recovery of her platelets to 140×10^9. She was seen after this, and it was recommended that she resume her vitamin D_3 at a higher dose of 4000 IU daily. She was feeling quite well, and her platelets remained above 70 and continued to rise so she discontinued her danazol. Again, she had no further episodes of flu or colds for the next two years and her platelets did not drop below 70. Her vitamin D level on this dose was 88 nmol/L after 4 months. She phoned one day that she was sick with a URI, and it was suggested that she take 10,000 IU of vitamin D for a 3 days and have her platelets checked. She had her platelets checked after being on this dose for two days, and they were in the normal range much higher than they had been for years. The platelet count was 248×10^9. Her vitamin D level was 99 nmol/L at this time. She continues on the 4000 IU vitamin D, and she continues off the danazol and remains well. Her latest platelet count was 318×10^9.

This case presents a significant response to vitamin D_3 in ITP. As well, it demonstrates a recurrent failure with lack of vitamin D and restoration of a normal platelet count on a higher dosing of vitamin D_3, which did not result in toxicity. The vitamin D_3 rescue with 10,000 IU of vitamin D_3 appeared to result in a similar response as that of prednisone used in past treatments over the years. ITP has been shown to have spontaneous remission in some people; however, it is uncommon in older patients. Is it possible that restoration of vitamin D levels results in some of these cases of spontaneous resolution? At this time this is not known. Restoring vitamin D to a level that is safe appears to be sensible supportive therapy.

Restoration of adequate platelets has never been demonstrated with repletion of inadequate vitamin D levels in the literature. Certainly in this example, vitamin D_3 restoring reasonable platelet levels and reducing the number of infections is most fascinating. The reduction in platelet levels with removal of vitamin D_3 with restoration of normal levels with an increased dose of vitamin D_3, as well as rescue with higher levels of vitamin D_3, is furthermore more intriguing. More studies would be warranted to demonstrate the benefit of adequate 25(OH)D levels in ITP.

9. Discussion and Conclusion

This paper outlines a number of autoimmune diseases that show vitamin D sensitivity as provided by supplementation, UVR, or other factors. These factors have been summarized in Table 3. At this time the research on the role of vitamin D in autoimmune disease is not conclusive, and much of the data comes from epidemiologic or case control studies. However, the evidence is increasingly pointing towards a significant role of vitamin D in reducing the incidence and burden of autoimmune diseases.

The link between various autoimmune diseases and vitamin D in various ecological, population, and case control studies is summarized in Table 4. Interventional studies when available have been cited throughout this paper. Although not all linkages are positive in each disease, the ones that have been studied the most, such as MS and type 1 diabetes, are quite striking. Studies done in type 1 diabetes were done in infants, and supplementation during the prenatal and first year of life may result in a significant reduction of morbidity later in life. Vitamin D may very well be a significant factor in preventing the loss of tolerance to self and resultant autoimmune disease.

Population studies have shown consistently in many "northern" countries (i.e., Canada) that the majority of the

Table 3: Autoimmunity and factors that relate to vitamin D-sensitive diseases.

Parameters relating to vitamin D	Multiple Sclerosis	Type 1 diabetes	Rheumatoid arthritis	Autoimmune disease of thyroid	Inflammatory bowel disease
Incidence seasonality	+	+	+	+	−
Seasonality of birth	+	+	−	+	+
Latitude	+	+	+	N/A	+
Altitude	+	N/A	N/A	N/A	N/A
Temperature	+	+	N/A	+	N/A
BMI	+	+	+	N/A	+
Race (skin tone)	+	+	−	N/A	N/A
UV radiance	+	+	+	+	+
Vitamin D intake	+	+	+	+	+

Evidence from studies listed in the paper for positive correlation of vitamin D-sensitive parameters in each disease. +: positive correlation, −: negative correlation, N/A: information lacking.

Table 4: Human studies in autoimmune disease.

Autoimmune disease	Study design (N)	Results
Multiple sclerosis	Prospective cohort studies NHS, NHS II supplementation of vitamin D ($N = 187,365$) [40]	40% reduction in developing MS with supplementation of 400 IU vitamin D
	Open label progressive supplementation of vitamin D ($N = 12$) [41]	The number of gadolinium-enhancing lesions was reduced
	Randomized control using 1000 IU versus 6000 IU daily of vitamin D_2 for 6 months ($N = 23$) [42]	Vitamin D_2 was not effective in reducing MRI lesions in RRMS
	Open-label randomized controlled trial ($N = 49$) [43]	8% in the treatment group had worsening disability versus 38% of patients in the control group
Diabetes	Birth cohort study ($N = 12058$) [57]	Use of 2000 IU had a reduced risk of developing diabetes by 78%
	Newly diagnosed diabetic children from 1980–2005 ($N = 10737$) [61]	Significant increase in incidence noted after reduction in vitamin D intake recommendation (decreased daily recommendation from 1000 IU to 400 IU)
	Meta-analysis of supplementation of vitamin D in infants [56]	29% reduction in risk of developing type 1 diabetes
Rheumatoid arthritis	Prospective cohort study dietary and supplement vitamin D intake ($N = 29,368$) [76]	34% reduction in developing RA in the supplement group > 400 IU vitamin D
	Open-label trial using high-dose oral alphacalcidiol therapy, ($N = 19$) [77]	Result in a positive effect on disease activity in 89% of patients
Autoimmune thyroid disease	None available to date	
Crohn's disease	Randomized double-blind placebo-controlled study ($N = 94$) [95]	1200 IU of vitamin D_3 reduced the number of relapses in the treatment group by more than 50% during a 1 yr study

population is vitamin D deficient. Knowing that a large proportion of the population do not have adequate levels of vitamin D, it may be prudent to restore levels to >100 nmol/L for optimum function of the immune system. 2000 IU of vitamin D_3 has been shown to improve vitamin D status and achieve these levels in about 80% of patients [98]. This may decrease autoimmune disease incidence. Increasing vitamin D intake would benefit the general population with better bone health and prevention of cancer and infectious diseases. An estimate in the savings in healthcare expenditures with restoration of adequate vitamin D is in the billions of dollars [99, 100] These estimates include only a few of the many autoimmune diseases that vitamin D may mitigate. Further studies are warranted in addressing autoimmune disease and vitamin D. These studies should use vitamin D_3 since, as in the above outlined studies, vitamin D_2 was not effective and the latest evidence suggests that only vitamin D_3, not vitamin D_2, improves mortality [101].

References

[1] M. Harel and Y. Shoenfeld, "Predicting and preventing autoimmunity, myth or reality?" *Annals of the New York Academy of Sciences*, vol. 1069, pp. 322–345, 2006.

[2] M. T. Cantorna and B. D. Mahon, "Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence," *Experimental Biology and Medicine*, vol. 229, no. 11, pp. 1136–1142, 2004.

[3] V. Molina and Y. Shoenfeld, "Infection, vaccines and other environmental triggers of autoimmunity," *Autoimmunity*, vol. 38, no. 3, pp. 235–245, 2005.

[4] J. F. de Carvalho, R. M. Pereira, and Y. Shoenfeld, "The mosaic of autoimmunity: the role of environmental factors," *Frontiers in Bioscience*, vol. 1, pp. 501–509, 2009.

[5] E. M. Jacobson and Y. Tomer, "The genetic basis of thyroid autoimmunity," *Thyroid*, vol. 17, no. 10, pp. 949–961, 2007.

[6] E. Ginanjar, Sumariyono, S. Setiati, and B. Setiyohadi, "Vitamin D and autoimmune disease," *Acta Medica Indonesiana*, vol. 39, no. 3, pp. 133–141, 2007.

[7] M. Cutolo, K. Otsa, M. Uprus, S. Paolino, and B. Seriolo, "Vitamin D in rheumatoid arthritis," *Autoimmunity Reviews*, vol. 7, no. 1, pp. 59–64, 2007.

[8] A. L. Ponsonby, R. M. Lucas, and I. A. F. Van Der Mei, "UVR, vitamin D and three autoimmune diseases—multiple sclerosis, type 1 diabetes, rheumatoid arthritis," *Photochemistry and Photobiology*, vol. 81, no. 6, pp. 1267–1275, 2005.

[9] F. Garland, *Powerpoint presentation: Can Diabetes Be Prevented with Vitamin D?* Department of Family and Preventative Medicine UCSD School of Medicine, 2008.

[10] G. Schwalfenberg, "Not enough vitamin D: health consequences for Canadians," *Canadian Family Physician*, vol. 53, no. 5, pp. 841–854, 2007.

[11] G. K. Schwalfenberg, S. J. Genuis, and M. N. Hiltz, "Addressing vitamin D deficiency in Canada: a public health innovation whose time has come," *Public Health*, vol. 124, no. 6, pp. 350–359, 2010.

[12] M. F. Holick, "Environmental factors that influence the cutaneous production of vitamin D," *American Journal of Clinical Nutrition*, vol. 61, supplement 3, pp. 638S–645S, 1995.

[13] J. N. Hathcock, A. Shao, R. Vieth, and R. Heaney, "Risk assessment for vitamin D," *American Journal of Clinical Nutrition*, vol. 85, no. 1, pp. 6–18, 2007.

[14] A. W. Norman, "From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health," *American Journal of Clinical Nutrition*, vol. 88, no. 2, pp. 491S–499S, 2008.

[15] P. Lips, "Vitamin D physiology," *Progress in Biophysics and Molecular Biology*, vol. 92, no. 1, pp. 4–8, 2006.

[16] D. P. Hayes, "The protection afforded by vitamin D against low radiation damage," *International Journal of Low Radiation*, vol. 5, no. 4, pp. 368–394, 2008.

[17] G. K. Schwalfenberg, "A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency," *Molecular Nutrition and Food Research*, vol. 55, no. 1, pp. 96–108, 2011.

[18] T. T. Wang, B. Dabbas, D. Laperriere et al., "Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin β2 innate immune pathway defective in crohn disease," *Journal of Biological Chemistry*, vol. 285, no. 4, pp. 2227–2231, 2010.

[19] S. V. Ramagopalan, A. Heger, A. J. Berlanga et al., "A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution," *Genome Research*, vol. 20, no. 10, pp. 1352–1360, 2010.

[20] J. Hillert, "The genetics of multiple sclerosis," *Results and Problems in Cell Differentiation*, vol. 51, pp. 1–19, 2010.

[21] G. Giovannoni and G. Ebers, "Multiple sclerosis: the environment and causation," *Current Opinion in Neurology*, vol. 20, no. 3, pp. 261–268, 2007.

[22] E. L. Thacker, F. Mirzaei, and A. Ascherio, "Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis," *Annals of Neurology*, vol. 59, no. 3, pp. 499–503, 2006.

[23] T. R. Nielsen, K. Rostgaard, N. M. Nielsen et al., "Multiple sclerosis after infectious mononucleosis," *Archives of Neurology*, vol. 64, no. 1, pp. 72–75, 2007.

[24] S. V. Ramagopalan, W. Valdar, D. A. Dyment et al., "Association of infectious mononucleosis with multiple sclerosis," *Neuroepidemiology*, vol. 32, no. 4, pp. 257–262, 2009.

[25] M. P. Pender, "The essential role of Epstein-Barr virus in the pathogenesis of multiple sclerosis," *Neuroscientist*, vol. 17, no. 4, pp. 351–367, 2011.

[26] A. Ascherio and K. Munger, "Epidemiology of multiple sclerosis: from risk factors to prevention," *Seminars in Neurology*, vol. 28, no. 1, pp. 17–28, 2008.

[27] A. E. Handel, L. Jarvis, R. Mclaughlin, A. Fries, G. C. Ebers, and S. V. Ramagopalan, "The epidemiology of multiple sclerosis in Scotland: inferences from hospital admissions," *PLoS ONE*, vol. 6, no. 1, article e14606, 2011.

[28] J. S. Sloka, W. E. M. Pryse-Phillips, and M. Stefanelli, "The relation of ultraviolet radiation and multiple sclerosis in Newfoundland," *Canadian Journal of Neurological Sciences*, vol. 35, no. 1, pp. 69–74, 2008.

[29] J. G. McLeod, S. R. Hammond, and J. F. Hallpike, "Epidemiology of multiple sclerosis in Australia: with NSW and SA survey results," *Medical Journal of Australia*, vol. 160, no. 3, pp. 117–121, 1994.

[30] C. Bisgård, "Seasonal variation in disseminated sclerosis," *Ugeskrift for Laeger*, vol. 152, no. 16, pp. 1160–1161, 1990.

[31] D. S. Meier, K. E. Balashov, B. Healy, H. L. Weiner, and C. R. G. Guttmann, "Seasonal prevalence of MS disease activity," *Neurology*, vol. 75, no. 9, pp. 799–806, 2010.

[32] F. Salvi, I. Bartolomei, M. H. Smolensky et al., "A seasonal periodicity in relapses of multiple sclerosis? A single-center, population-based, preliminary study conducted in Bologna, Italy," *BMC Neurology*, vol. 10, article 105, 2010.

[33] C. J. Willer, D. A. Dyment, A. D. Sadovnick, P. M. Rothwell, T. J. Murray, and G. C. Ebers, "Timing of birth and risk of multiple sclerosis: population based study," *British Medical Journal*, vol. 330, no. 7483, pp. 120–123, 2005.

[34] J. F. Kurtzke, "On the fine structure of the distribution of multiple sclerosis," *Acta Neurologica Scandinavica*, vol. 43, no. 3, pp. 257–282, 1967.

[35] Y. Dong, N. Pollock, I. S. Stallmann-Jorgensen et al., "Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness," *Pediatrics*, vol. 125, no. 6, pp. 1104–1111, 2010.

[36] M. G. Bischof, G. Heinze, and H. Vierhapper, "Vitamin D status and its relation to age and body mass index," *Hormone Research*, vol. 66, no. 5, pp. 211–215, 2006.

[37] K. L. Munger, T. Chitnis, and A. Ascherio, "Body size and risk of MS in two cohorts of US women," *Neurology*, vol. 73, no. 19, pp. 1543–1550, 2009.

[38] T. Dwyer, I. Van Der Mei, A. L. Ponsonby et al., "Melanocortin 1 receptor genotype, past environmental sun exposure, and risk of multiple sclerosis," *Neurology*, vol. 71, no. 8, pp. 583–589, 2008.

[39] K. Westlund, "Distribution and mortality time trend of multiple sclerosis and some other diseases in Norway," *Acta Neurologica Scandinavica*, vol. 46, no. 4, pp. 455–483, 1970.

[40] K. L. Munger, S. M. Zhang, E. O'Reilly et al., "Vitamin D intake and incidence of multiple sclerosis," *Neurology*, vol. 62, no. 1, pp. 60–65, 2004.

[41] S. M. Kimball, M. R. Ursell, P. O'Connor, and R. Vieth, "Safety of vitamin D3 in adults with multiple sclerosis," *American Journal of Clinical Nutrition*, vol. 86, no. 3, pp. 645–651, 2007.

[42] M. S. Stein, Y. Liu, O. M. Gray et al., "A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis," *Neurology*, vol. 77, no. 17, pp. 1611–1618, 2011.

[43] J. M. Burton, S. Kimball, R. Vieth et al., "A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis," *Neurology*, vol. 74, no. 23, pp. 1852–1859, 2010.

[44] P. Olmos, R. A'Hern, D. A. Heaton et al., "The significance of the concordance rate for Type 1 (insulin-dependent) diabetes in identical twins," *Diabetologia*, vol. 31, no. 10, pp. 747–750, 1988.

[45] H. Hyöty and K. Taylor, "The role of viruses in human diabetes," *Diabetologia*, vol. 45, no. 10, pp. 1353–1361, 2002.

[46] C. H. Williams, S. Oikarinen, S. Tauriainen, K. Salminen, H. Hyoty, and G. Stanway, "Molecular analysis of an echovirus 3 strain isolated from an individual concurrently with appearance of islet cell and IA-2 autoantibodies," *Journal of Clinical Microbiology*, vol. 44, no. 2, pp. 441–448, 2006.

[47] B. Lernmark, K. Lynch, and A. Lernmark, "Cord blood islet autoantibodies are related to stress in the mother during pregnancy," *Annals of the New York Academy of Sciences*, vol. 1079, pp. 345–349, 2006.

[48] S. B. Mohr, C. F. Garland, E. D. Gorham, and F. C. Garland, "The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide," *Diabetologia*, vol. 51, no. 8, pp. 1391–1398, 2008.

[49] S. Sloka, M. Grant, and L. A. Newhook, "Time series analysis of ultraviolet B radiation and type 1 diabetes in Newfoundland," *Pediatric Diabetes*, vol. 9, no. 2, pp. 81–86, 2008.

[50] S. Sloka, M. Grant, and L. A. Newhook, "The geospatial relation between UV solar radiation and type 1 diabetes in Newfoundland," *Acta Diabetologica*, vol. 47, no. 1, pp. 73–78, 2010.

[51] P. M. Rothwell, A. Staines, P. Smail, E. Wadsworth, and P. McKinney, "Seasonality of birth of patients with childhood diabetes in Britain," *British Medical Journal*, vol. 312, no. 7044, pp. 1456–1457, 1996.

[52] H. K. Akerblom, D. J. Ballard, B. Bauman et al., "Geographic patterns of childhood insulin-dependent diabetes mellitus," *Diabetes*, vol. 37, no. 8, pp. 1113–1119, 1988.

[53] B. Littorin, L. Nyström, B. Gullberg et al., "Increasing body mass index at diagnosis of diabetes in young adult people during 1983–1999 in the Diabetes Incidence Study in Sweden (DISS)," *Journal of Internal Medicine*, vol. 254, no. 3, pp. 251–256, 2003.

[54] B. Littorin, P. Blom, A. Schölin et al., "Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS)," *Diabetologia*, vol. 49, no. 12, pp. 2847–2852, 2006.

[55] L. Marjamäki, S. Niinistö, M. G. Kenward et al., "Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring," *Diabetologia*, vol. 53, no. 8, pp. 1599–1607, 2010.

[56] C. S. Zipitis and A. K. Akobeng, "Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis," *Archives of Disease in Childhood*, vol. 93, no. 6, pp. 512–517, 2008.

[57] E. Hyppönen, E. Läärä, A. Reunanen, M.-R. Järvelin, and S. M. Virtanen, "Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study," *The Lancet*, vol. 358, no. 9292, pp. 1500–1503, 2001.

[58] L. C. Stene and G. Joner, "Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study," *American Journal of Clinical Nutrition*, vol. 78, no. 6, pp. 1128–1134, 2003.

[59] L. C. Stene, J. Ulriksen, P. Magnus, and G. Joner, "Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring," *Diabetologia*, vol. 43, no. 9, pp. 1093–1098, 2000.

[60] S. S. Harris, "Vitamin D in type 1 diabetes prevention," *Journal of Nutrition*, vol. 135, no. 2, pp. 323–325, 2005.

[61] V. Harjutsalo, L. Sjöberg, and J. Tuomilehto, "Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study," *The Lancet*, vol. 371, no. 9626, pp. 1777–1782, 2008.

[62] P. Jarvinen and K. Aho, "Twin studies in rheumatic disease," *Seminars in Arthritis and Rheumatism*, vol. 24, no. 1, pp. 19–28, 1994.

[63] Y. Kochi, A. Suzuki, R. Yamada, and K. Yamamoto, "Genetics of rheumatoid arthritis: underlying evidence of ethnic differences," *Journal of Autoimmunity*, vol. 32, no. 3-4, pp. 158–162, 2009.

[64] R. L. Wilder and L. J. Crofford, "Do infectious agents cause rheumatoid arthritis?" *Clinical Orthopaedics and Related Research*, no. 265, pp. 36–41, 1991.

[65] A. Krause, T. Kamradt, and G. R. Burmester, "Potential infectious agents in the induction of arthritides," *Current Opinion in Rheumatology*, vol. 8, no. 3, pp. 203–209, 1996.

[66] B. Gonzalez, C. Larrañaga, O. León et al., "Parvovirus B19 may have a role in the pathogenesis of juvenile idiopathic arthritis," *Journal of Rheumatology*, vol. 34, no. 6, pp. 1336–1340, 2007.

[67] L. Klareskog, L. Padyukov, and L. Alfredsson, "Smoking as a trigger for inflammatory rheumatic diseases," *Current Opinion in Rheumatology*, vol. 19, no. 1, pp. 49–54, 2007.

[68] M. Cutolo, "Solar light effects on onset/relapses and circannual/circadian symptomatology in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 21, no. 2, pp. 148–150, 2003.

[69] E. Zold, P. Szodoray, J. Gaal et al., "Vitamin D deficiency in undifferentiated connective tissue disease," *Arthritis Research and Therapy*, vol. 10, no. 5, article R123, 2008.

[70] M. Cutolo, K. Otsa, K. Laas et al., "Circannual vitamin d serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe," *Clinical and Experimental Rheumatology*, vol. 24, no. 6, pp. 702–704, 2006.

[71] W. W. Buchanan, L. G. Gregoire, and H. M. Buchanan, "Month of birth and rheumatoid arthritis," *The Lancet*, vol. 2, no. 8557, p. 517, 1987.

[72] M. Rossini, S. Maddali Bongi, G. La Montagna et al., "Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability," *Arthritis Research and Therapy*, vol. 12, no. 6, article R216, 2010.

[73] G. Westhoff, R. Rau, and A. Zink, "Radiographic joint damage in early rheumatoid arthritis is highly dependent on body

mass index," *Arthritis and Rheumatism*, vol. 56, no. 11, pp. 3575–3582, 2007.

[74] K. G. Oen and M. Cheang, "Epidemiology of chronic arthritis in childhood," *Seminars in Arthritis and Rheumatism*, vol. 26, no. 3, pp. 575–591, 1996.

[75] M. M. Schwartz, P. Simpson, K. L. Kerr, and J. N. Jarvis, "Juvenile rheumatoid arthritis in African Americans," *Journal of Rheumatology*, vol. 24, no. 9, pp. 1826–1829, 1997.

[76] L. A. Merlino, J. Curtis, T. R. Mikuls, J. R. Cerhan, L. A. Criswell, and K. G. Saag, "Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study," *Arthritis and Rheumatism*, vol. 50, no. 1, pp. 72–77, 2004.

[77] Z. Andjelkovic, J. Vojinovic, N. Pejnovic et al., "Disease modifying and immunomodulatory effects of high dose 1α(OH) D3 in rheumatoid arthritis patients," *Clinical and Experimental Rheumatology*, vol. 17, no. 4, pp. 453–456, 1999.

[78] M. F. Prummel, T. Strieder, and W. M. Wiersinga, "The environment and autoimmune thyroid diseases," *European Journal of Endocrinology*, vol. 150, no. 5, pp. 605–618, 2004.

[79] Y. Tomer and T. F. Davies, "The genetic susceptibility to Graves' disease," *Bailliere's Clinical Endocrinology and Metabolism*, vol. 11, no. 3, pp. 431–450, 1997.

[80] T. H. Brix, P. S. Hansen, K. O. Kyvik, and L. Hegedüs, "Cigarette smoking and risk of clinically overt thyroid disease: a population-based twin case-control study," *Archives of Internal Medicine*, vol. 160, no. 5, pp. 661–666, 2000.

[81] G. Tamer, S. Arik, I. Tamer, and D. Coksert, "Relative vitamin D insufficiency in Hashimoto's thyroiditis," *Thyroid*, vol. 21, no. 8, pp. 891–896, 2011.

[82] S. Kivity, N. Agmon-Levin, M. Zisappl et al., "Vitamin D and autoimmune thyroid diseases," *Cellular and Molecular Immunology*, vol. 8, no. 3, pp. 243–247, 2011.

[83] G. E. Krassas, K. Tziomalos, N. Pontikides, H. Lewy, and Z. Laron, "Seasonality of month of birth of patients with Graves' and Hashimoto's diseases differ from that in the general population," *European Journal of Endocrinology*, vol. 156, no. 6, pp. 631–636, 2007.

[84] D. I. W. Phillips, D. J. P. Barker, and J. A. Morris, "Seasonality of thyrotoxicosis," *Journal of Epidemiology and Community Health*, vol. 39, no. 1, pp. 72–74, 1985.

[85] M. E. Spehlmann, A. Z. Begun, J. Burghardt, P. Lepage, A. Raedler, and S. Schreiber, "Epidemiology of inflammatory bowel disease in a German twin cohort: results of a nationwide study," *Inflammatory Bowel Diseases*, vol. 14, no. 7, pp. 968–976, 2008.

[86] F. Carbonnel, P. Jantchou, E. Monnet, and J. Cosnes, "Environmental risk factors in Crohn's disease and ulcerative colitis: an update," *Gastroentérologie Clinique et Biologique*, vol. 33, supplement 3, pp. S145–S157, 2009.

[87] J. H. Ooi, J. Chen, and M. T. Cantorna, "Vitamin D regulation of immune function in the gut: why do T cells have vitamin D receptors?" *Molecular Aspects of Medicine*, vol. 33, no. 1, pp. 77–82, 2012.

[88] J. Gilman, F. Shanahan, and K. D. Cashman, "Determinants of vitamin D status in adult Crohn's disease patients, with particular emphasis on supplemental vitamin D use," *European Journal of Clinical Nutrition*, vol. 60, no. 7, pp. 889–896, 2006.

[89] A. J. Joseph, B. George, A. B. Pulimood, M. S. Seshadri, and A. Chacko, "25 (OH) vitamin D level in Crohn's disease: association with sun exposure & disease activity," *Indian Journal of Medical Research*, vol. 130, no. 2, pp. 133–137, 2009.

[90] V. Nerich, E. Monnet, A. Weill et al., "Fine-scale geographic variations of inflammatory bowel disease in france: correlation with socioeconomic and house equipment variables," *Inflammatory Bowel Diseases*, vol. 16, no. 5, pp. 813–821, 2010.

[91] A. Ekbom, C. Helmick, M. Zack, and H. O. Adami, "The epidemiology of inflammatory bowel disease: a large, population-based study in Sweden," *Gastroenterology*, vol. 100, no. 2, pp. 350–358, 1991.

[92] M. A. Mendall, A. Viran Gunasekera, B. Joseph John, and D. Kumar, "Is obesity a risk factor for Crohn's disease?" *Digestive Diseases and Sciences*, vol. 56, no. 3, pp. 837–844, 2011.

[93] H. M. Pappa, C. M. Gordon, T. M. Saslowsky et al., "Vitamin D status in children and young adults with inflammatory bowel disease," *Pediatrics*, vol. 118, no. 5, pp. 1950–1961, 2006.

[94] P. H. A. Bours, J. P. M. Wielders, J. R. Vermeijden, and A. van de Wiel, "Seasonal variation of serum 25-hydroxyvitamin D levels in adult patients with inflammatory bowel disease," *Osteoporosis International*, vol. 22, no. 11, pp. 2857–2867, 2010.

[95] S. P. Jørgensen, J. Agnholt, H. Glerup et al., "Clinical trial: vitamin D3 treatment in Crohn's disease—a randomized double-blind placebo-controlled study," *Alimentary Pharmacology and Therapeutics*, vol. 32, no. 3, pp. 377–383, 2010.

[96] D. R. Terrell, L. A. Beebe, S. K. Vesely, B. R. Neas, J. B. Segal, and J. N. George, "The incidence of immune thrombocytopenic purpura in children and adults: a critical review of published reports," *American Journal of Hematology*, vol. 85, no. 3, pp. 174–180, 2010.

[97] D. Provan, R. Stasi, A. C. Newland et al., "International consensus report on the investigation and management of primary immune thrombocytopenia," *Blood*, vol. 115, no. 2, pp. 168–186, 2010.

[98] G. K. Schwalfenberg and S. J. Genuis, "Vitamin D supplementation in a nursing home population," *Molecular Nutrition and Food Research*, vol. 54, no. 8, pp. 1072–1076, 2010.

[99] W. B. Grant, G. K. Schwalfenberg, S. J. Genuis, and S. J. Whiting, "An estimate of the economic burden and premature deaths due to vitamin D deficiency in Canada," *Molecular Nutrition and Food Research*, vol. 54, no. 8, pp. 1172–1181, 2010.

[100] W. B. Grant, H. S. Cross, C. F. Garland et al., "Estimated benefit of increased vitamin D status in reducing the economic burden of disease in western Europe," *Progress in Biophysics and Molecular Biology*, vol. 99, no. 2-3, pp. 104–113, 2009.

[101] G. Bjelakovic, L. L. Gluud, D. Nikolova et al., "Vitamin D supplementation for prevention of mortality in adults," *Cochrane Database of Systematic Reviews*, no. 7, article CD007470, 2011.

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Review Article

Arsenic, Cadmium, Lead, and Mercury in Sweat: A Systematic Review

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Arsenic, cadmium, lead, and mercury exposures are ubiquitous. These toxic elements have no physiological benefits, engendering interest in minimizing body burden. The physiological process of sweating has long been regarded as "cleansing" and of low risk. Reports of toxicant levels in sweat were sought in Medline, Embase, Toxline, Biosis, and AMED as well as reference lists and grey literature, from inception to March 22, 2011. Of 122 records identified, 24 were included in evidence synthesis. Populations, and sweat collection methods and concentrations varied widely. In individuals with higher exposure or body burden, sweat generally exceeded plasma or urine concentrations, and dermal could match or surpass urinary daily excretion. Arsenic dermal excretion was severalfold higher in arsenic-exposed individuals than in unexposed controls. Cadmium was more concentrated in sweat than in blood plasma. Sweat lead was associated with high-molecular-weight molecules, and in an interventional study, levels were higher with endurance compared with intensive exercise. Mercury levels normalized with repeated saunas in a case report. Sweating deserves consideration for toxic element detoxification. Research including appropriately sized trials is needed to establish safe, effective therapeutic protocols.

1. Introduction

No person is without some level of toxic metals in their bodies, circulating and accumulating with acute and chronic lifetime exposures. An individual may take numerous measures to minimize exposures and to optimize metabolism and excretion of toxic elements in the stool and urine with diet, supplements, and chelation therapy [1, 2]; however, an often overlooked route of excretion of toxicants is via the process of sweating [3].

Sweating with heat and/or exercise has been viewed throughout the ages, by groups worldwide, as "cleansing." As part of a scoping review regarding arsenic, cadmium, lead, and mercury, we reviewed the scientific literature pertaining to toxicant excretion in sweat.

1.1. Arsenic, Cadmium, Lead, and Mercury: Background. While many chemical elements are essential for life, arsenic, cadmium, lead, and mercury have no known beneficial effect in humans. On the contrary, all four elements are confirmed or probable carcinogens, and they exhibit wide-ranging toxic effects on many bodily systems, including the nervous, endocrine, renal, musculoskeletal, immunological, and cardiovascular systems [4–7].

Children and the fetus are most at risk of harm, with early exposures potentially predisposing the youngster over his/her lifetime to multisystem ailments, as well as lower IQ and dysfunctional behavior. In older populations there is increased likelihood of early cognitive decline, as well as a range of conditions including kidney and cardiovascular disease, diabetes, and osteoporosis [4–7].

Some populations are exposed to elevated levels of toxic elements by virtue of geochemistry, resulting in groundwater or foods with elevated levels of toxic elements (e.g., elevated arsenic in groundwater, most famously in parts of Asia such as Bangladesh but also elsewhere; cadmium that accumulates

in foods grown in particular locations with high levels in soils or from fertilizers, including shellfish [8], grains [9], and brassicas [10]; and mercury in fish and seafoods). Tobacco avidly accumulates cadmium and lead from soil, making smoking a major source of exposure. In addition, valuable and unique properties of arsenic, cadmium, lead, and mercury have made them integral in many products, including electronics, batteries, and alloys. Modern environmental exposures arise from mining, refining, and industrial processes (e.g., arsenic from precious metal mining and refining, mercury from chloralkali production, or lead and cadmium from mining, refining, and recycling these and other metals such as zinc); the vestiges of older products (e.g., pesticides, leaded gasoline, paint and plumbing, mercury-containing switches and thermometers, and arsenical wood preservatives); ongoing uses (e.g., arsenical veterinary drugs, and mercury-containing dental amalgams, preservatives, and lamps); as well as emissions from burning coal and other incineration (including cremation).

With toxic elements ubiquitous in our air, water, food, and the physical environment, as well as in many consumer products, prudent avoidance is not always possible. Although signs and symptoms of chronic disease are consistent with effects of arsenic, cadmium, lead, and/or mercury, physicians commonly have a low index of clinical suspicion, and therefore levels of toxic elements are seldom investigated. Diagnosis may be challenging because multiple chemicals may contribute to subtle effects in chronic illnesses of an individual, and the effects may be synergistic. A recent review called for mercury assessment in all patients presenting with hypertension or any vascular disease [11], but other toxic elements such as lead [12] may also be implicated at levels commonly observed in the population. "Interaction Profiles" [13] compiled by the US Agency for Toxic Substances and Disease Registry report that renal toxicities of mixtures of lead plus mercury are greater than would be predicted knowing the toxicity dose response of the individual elements. Similarly, neurological toxicities of mixtures of lead plus arsenic, lead plus methylmercury, and lead plus cadmium are supra-additive [13].

1.2. Sweating: Background. Increasing the thermal load on the body activates heat loss mechanisms including increased circulation throughout the skin and sweating [14], with blood flow to the skin increasing from a baseline of 5–10%, to 60–70% of the cardiac output [15]. Maximal sweating occurs within 15 minutes and the fluid loss may be as high as 2 L/h in an "acclimatized" person who regularly sweats [16].

Eccrine sweat is produced in tubular coil glands under the skin surface in response to heat and, or work stress. Capillaries as well as adjacent adipose tissue may contribute to secretions from sebaceous and apocrine glands, as has been seen in research using sweat patches to detect drugs of abuse [17]. Sweat arises from the blood supply to the sweat gland, but is not simply an ultrafiltrate of blood plasma; sodium and chloride are lower in sweat than in serum, as salt loss is restricted by reabsorption in the gland [18]. Both the concentration and total loss of salt (sodium chloride) in sweat vary widely among individuals [19], as well as with acclimatization to exercise and heat [20]. In an early study, Robinson et al. demonstrated that with serum salt depletion the kidneys responded within hours by restricting excretion into the urine, while the sweat glands responded only after days with decreased concentrations in the sweat [21]. Potassium, urea, ammonia, and lactic acid concentrations are higher in sweat than in plasma, although these levels are also regulated to some extent by reabsorption in the ductal tubule of the sweat gland [22]. In one study of successive exercise sessions with cool-down breaks, over the short-term sodium, potassium, calcium, and magnesium excretion in sweat remained constant, while zinc excretion fell [23]. It is unclear whether reabsorption or depletion of plasma supply resulted in diminishing zinc losses.

Children, with greater surface area in comparison to body mass, have been observed in research studies to sweat less than adults, with sweating increasing through puberty [24]. Although some research has indicated that children's thermoregulation and heat tolerance may be less robust than adults, these findings may be at least in part an artifact of study designs and models for interpretation [25]. In research involving exercise and heat, it may be a challenge to maintain ongoing, consistent motivation among children.

2. Methods

2.1. Search Strategy. Medline, Embase, Toxline, Biosis, and AMED were searched, with no restriction on date or language, to March 22, 2011. These records were supplemented with searches for other research by key authors, searches of citations and reference lists of key reports, and "related articles."

Neither sweating nor toxic elements are exclusively modern topics of research, so in order to search older literature for all chemical forms, the online version of the Chemical Rubber Company Handbook was searched for all arsenic, cadmium, lead, and mercury compounds, and lists of keywords were extracted from these lists. Searches using these keywords yielded records that were not identified in searches using the four chemical abstracts service (CAS) numbers or the medical subject headings (MeSHs) for arsenic, cadmium, lead, and mercury. CAS numbers and MeSHs are intended for specific individual chemicals or records referring to unspecified compounds—the tool cannot simultaneously be both specific and general. Toxic element records were searched for terms related to sweating, perspiration, sauna, steam baths, exercise, depuration, and secretion or excretion from skin. Bibliographic records were imported, duplicates were removed, and reports were screened using Zotero 2.03 (http://www.zotero.org/).

2.2. Report Screening and Inclusion. Titles and abstracts were screened by one investigator (MS), for primary reports with data on one or more of the toxic elements in sweat, with at least a substantial abstract in English. Reviews were included at this level, to search reference lists. Two investigators (MS and KK) independently screened studies

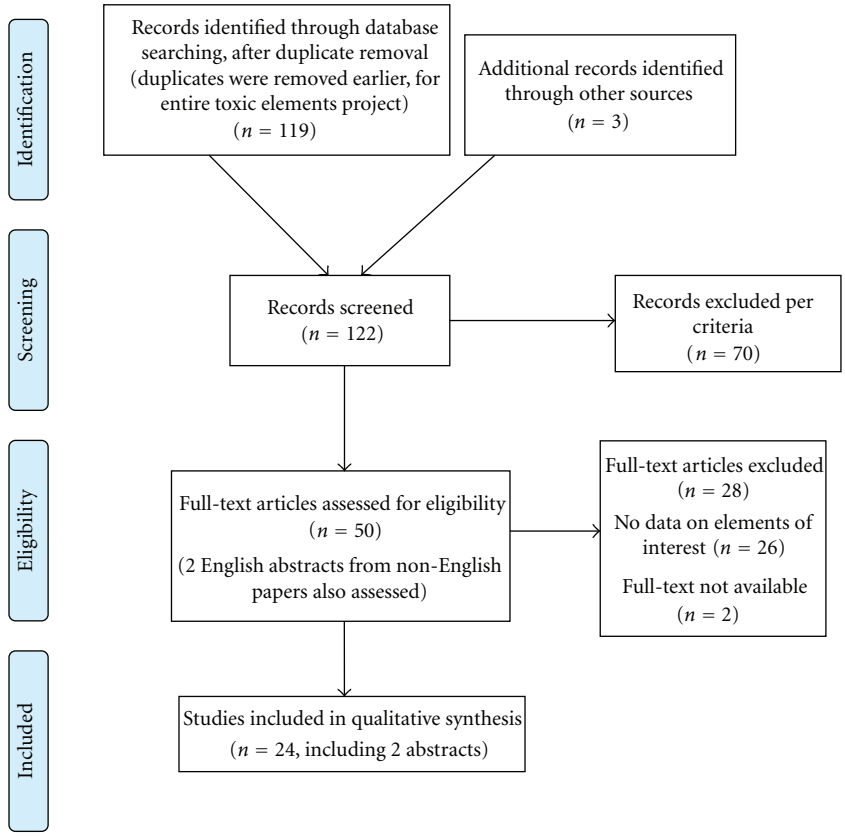

Figure 1: PRISMA flow diagram of evidence searches and inclusion.

for inclusion, and extracted and verified data. All studies presenting quantitative human data on levels of arsenic, cadmium, lead, and/or mercury were included, regardless of experimental design, or methods of sweat collection or chemical analysis.

3. Results

Of 122 bibliographic records identified, 70 did not meet inclusion criteria at first screening, 52 full-text articles were sought for full-text screening, and 50 were obtained and screened. Data from the extended abstract of a report in German [26] and the conclusion from the abstract of one report in Russian [27] that were not obtained in full text were noted. Twenty-four reports of 22 or 23 trials or studies (it is unclear if two studies from one institution reported results twice for a subset of participants [22, 28]) were included in evidence synthesis. Searching, screening, and study inclusion are summarized in the modified PRISMA flow diagram, Figure 1.

3.1. Excretion of Toxic Elements in Sweat. Along with essential minerals, sweat is an acknowledged excretory route for toxic metals. For instance, it is recommended to sample hair close to the scalp because content of toxic elements may be elevated along the shaft, from either environmental contamination or excreted toxins in sweat and sebum [32, 42]. The minerals generally arise from blood serum [28], with contribution from dermally absorbed occupational exposures, which might not be reflected in blood or urine [35, 37]. Sweating was induced by sauna, exercise, or pilocarpine iontophoresis to measure the concentration of the heavy metals in the sweat, while sauna and exercise were used for therapy. Study participants included workers with occupational exposures and individuals with no occupational exposures who were well or experiencing chronic ill health, and in two studies participants were intentionally dosed with lead [34, 37]. Studies that have examined the presence of toxic metals in sweat are summarized in Tables 1, 2, 3, and 4, for arsenic, cadmium, lead, and mercury, respectively.

Arsenic accumulates highly in the skin, and causes characteristic skin lesions, but little information is available on levels in sweat. Yousuf et al. recently found that excretion of arsenic was greatest from the skin of patients with skin lesions, slightly but not statistically significantly lower from arsenic-exposed controls, and severalfold lower from nonexposed controls [29]. Genuis et al. measured numerous toxic elements in blood plasma, urine, and sweat of 20 study subjects (10 healthy and 10 with chronic health problems) [3]. The maximum sweat arsenic concentration was 22 μg/L. On average, arsenic was 1.5-fold (in males) to 3-fold (in females) higher in sweat than in blood plasma; however,

Table 1: Studies of excretion of arsenic in sweat.

Study	Country, participants	Study design and intervention	Key findings (concentrations of μg/L unless otherwise indicated)
Yousuf et al. 2011 [29]	Bangladesh 20 arsenicosis patients with melanosis and leucomelanosis 20 controls with As in drinking water 20 unexposed controls	Secretions from chest, back, and abdomen collected for 24 h, on gauze pads (8-fold; 2 $\times$ 3 inches) attached to fitted T-shirt	As secretion severalfold greater for As-exposed groups No significant difference between patients and As-exposed controls 2 zinc atoms excreted per As atom Vitamin E excreted with As
Genuis et al. 2010 [3]	Canada 10 with chronic conditions 10 healthy	Simultaneous measurement of As in blood plasma, urine, and sweat Sweating induced by exercise or sauna, collected directly into bottle	17 participants with As detected in all samples Blood plasma mean: 2.5 (range 0.9–13) ($n = 17$) Urine mean: 37 (range 4.8–200) ($n = 20$) Sweat mean: 3.1 (range 3.7–22) ($n = 20$)

Table 2: Studies of cadmium excretion in sweat.

Study	Country, participants	Study design and intervention	Key findings (concentrations μg/L unless otherwise indicated)
Genuis et al., 2010 [3]	Canada 10 with chronic conditions 10 healthy	Simultaneous measurement of toxic trace elements in blood plasma, urine, and sweat Exercise or sauna Sweat collected directly into bottle	3 participants with cadmium detected in all samples Blood plasma mean: 0.03 (range 0.02–0.07) ($n = 11$) Urine mean: 0.28 (0.18–0.39) ($n = 3$) Sweat mean: 5.7 (0.36–36) ($n = 18$)
Omokhodion and Howard, 1994 [30]	UK 15 healthy participants	Sweat collected using modified arm bag (hand excluded) Participants exercised at room temperature	Cadmium detected in 13 sweat samples Mean 1.9 Range 1.1–3.1
Stauber and Florence, 1988 [28]	Australia 24 males 13 females taking oral contraceptives 26 females not taking oral contraceptives	Forearm sweat induced by pilocarpine iontophoresis and collected on a membrane filter	Males mean sweat cadmium 1.4 (range <0.5–10) Females not taking contraceptives 2.6 (<0.5–18) Females taking contraceptives 2.4 (<0.5–5.5)
Stauber and Florence, 1987 [22]	Australia 9 males 7 females taking oral contraceptives 6 not taking oral contraceptives (unclear overlap with 1988 participants)	Forearm sweat induced by pilocarpine iontophoresis and collected on a membrane filter	Cadmium not detected in sweat (0.5 detection limit) Mean blood cadmium 0.8
Robinson and Weiss, 1980 [31]	USA 28 males (university faculty members)	Exercise and shower preceded sauna for sweat collection. Sweat collected as drips from forehead or nose	Sweat cadmium (range 11–200) Urine cadmium (range ND–67) Sweat/urine ratio (range 1.0–16) No correlation between the concentrations in urine and sweat
Robinson and Weiss, 1980 [32] (companion to previous)	USA 2 males (university faculty members)	As previous, cadmium also measured in hair segments.	Daily excretion of cadmium estimated as follows: (i) 30 μg/day in urine (ii) 120 μg/day in sweat (iii) 0.2 μg/day in hair Cadmium concentrations in hair and sweat were lower in one participant than the other
Cohn and Emmett, 1978 [33]	USA 6 males 3 females	Total body washdown and arm bag techniques	Mean concentration of cadmium in sweat > urine Arm bags yielded lower levels than whole body measurements

Table 3: Studies of lead excretion in sweat.

Study	Country, participants	Study design and intervention	Key findings (concentrations μg/L unless otherwise indicated)
Genuis et al., 2010 [3]	Canada 10 with chronic health conditions 10 healthy	Analyses of blood plasma, urine, and sweat Sweating induced by exercise or sauna, collected directly into bottle	Sweat mean 31 (range 1.5–94) ($n = 20$) Blood plasma mean 0.12 (0.39–1.7) ($n = 20$) Urine mean 1.8 (0.91–7.5) ($n = 20$)
Omokhodion and Crockford, 1991 [34]	UK 2 participants	Blood, urine, and sweat lead measured before and following ingestion of lead chloride: 1 or 2 doses of lead chloride (20 mg $PbCl_2$ total, in 1 or 2 divided doses).	Blood lead peaked at 4 h Sweat concentrations did not increase significantly (range 0–11) Blood concentration range 6–51 Urine concentration range 10–97 Arm sweat collections varied by more than 2-fold between arms at the same time on the same person
Omokhodion and Howard, 1991 [35]	Unidentified "tropics" 19 workers in a lead battery factory 8 controls (medical students)	Measured lead in sweat, blood, and urine simultaneously Sweating induced by exercising at room temperature. Sweat collected in arm bags.	Workers: (i) blood lead 13–36 (ii) urine lead 28–290 μg/g creatinine (iii) sweat lead 72–260 Controls: (i) blood lead 90–120 (ii) urine lead 9–20 μg/g creatinine (iii) sweat lead 9–30
Omokhodion and Crockford, 1991 [36]	UK 24 normal, healthy subjects	Measured lead in sweat, urine, blood, and saliva Sweat collected in arm bags, sitting in a hot chamber	(i) Blood lead 86 (range 60–140) (ii) Urine lead 18 μg/g creatinine (range 7.7–44 μg/g creatinine) (iii) Mean sweat lead 5.2 (2.5–13) (iv) Saliva lead 4.8 (2.5–10)
Parpaleĭ et al., 1991 [27] (in Russian—English abstract only)	Russia NR in abstract	NR in abstract	"... sauna increased excretion with sweat fluid of toxic substances [lead] that penetrated the body during work. Sauna is recommended."
Lilley et al., 1988 [37]	Australia 9 lead workers volunteers had lead applied to skin	Lead dust 6 h/day for 4 days 20 mg Pb dust on L arm of volunteer $PbNO_3$ 24 h of 60 mg $PbNO_3$ on L arm of volunteer.	Sweat lead in workers: 71–18,000 Following exposure, sweat lead from R arm increased approximately by 10x, returning to baseline after approximately by 2–4 days. Saliva increased approximately 5-6x. Urine and blood levels were unchanged
Stauber and Florence, 1988 [28]	Australia 24 males 13 females taking oral contraceptives 26 not taking oral contraceptives	Sweating induced on the forearms by pilocarpine iontophoresis and collected on a membrane filter	Mean sweat lead: (i) males: 41 (range 6–87) (ii) females not taking contraceptives: 24 (<5–66) (difference with males $P < 0.01$) (iii) females taking contraceptives: 36 (<5–70)
Stauber and Florence, 1987 [22]	Australia 9 males 7 females taking oral contraceptives 6 not taking oral contraceptives (unclear overlap with 1988 participants)	Sweating induced in the forearms by pilocarpine iontophoresis and collected on a membrane filter	No significant differences among groups Mean blood lead 200 Mean blood plasma lead 10 Mean sweat lead 15

Table 3: Continued.

Study	Country, participants	Study design and intervention	Key findings (concentrations μg/L unless otherwise indicated)
Haber et al., 1985 [26] (in German-used extended abstract)	Germany 4 groups of 8 males 2 groups with occupational lead exposure 2 control groups	Comparison of precisely defined physical work (intensive cycling and extended rowing in a pool), examining lead excretion in persons with elevated blood levels compared with nonexposed controls	Aerobic endurance training (rowing) caused a significant drop in the blood lead level in the occupationally exposed group (mean 430 (range 320–580) decreased to 370 (240–450)) ($P < 0.05$) Endurance training was more effective than shorter, more intensive training (cycling) Urine lead levels were not significantly affected by training
Cohn and Emmett, 1978 [33]	USA 6 males 3 females	Total body washdown and arm bag techniques	The mean concentration of lead in sweat was similar to that in urine (1) Total body sweat lead mean: (i) males: 24 (SD 16) (ii) females: 53 (range 40–60) (2) Body minus 1 arm/arm bag sweat lead 60 (SD 16) (40–120)/83 (86) (20–250)
Hohnandel et al., 1973 [38]	33 healthy males 15 females	15 min of arm bag collection	Mean sweat lead: (i) males: 51 (range 8–180) (ii) females: 120 (SD 72) (49–280)

Table 4: Studies of mercury excretion in sweat.

Study	Country, participants	Study design and intervention	Key findings (concentrations μg/L unless otherwise indicated)
Genuis et al., 2010 [3]	Canada 10 with chronic conditions 10 healthy	Sweating induced by exercise or sauna, collected directly into bottle	16 participants had mercury detected in all samples Blood plasma mercury mean 0.61 (range 0.26–1.6) ($n = 16$) Urine mean 0.65 (range 0.32–1.3) ($n = 16$) Sweat mean 0.86 (range 0.48–1.5) ($n = 20$)
Robinson and Skelly, 1983 [39]	USA 21 males at university 7 sampled more than once	Mercury in sweat dripping from forehead or nose, compared with urine	Sweat mean 0.5 (range 0.1–1.4)
Sunderman 1978 [40]	USA 1 case with mercury intoxication	Case report of chelating agents to treat mercury intoxication, followed by a regimen of daily sweat and physiotherapy for a protracted period of several months	Appreciable quantities of mercury were excreted in sweat. With the sweating regimen mercury, levels in sweat decreased to within the normal range
Lovejoy et al., 1973 [41]	USA 3 mercury-exposed workers 3 nonexposed workers 1 control	Participants wore rubber chest waders from 7 : 30 to 9 : 00 am Sweat accumulated in the feet was collected, as well as a 16-hour urine sample	Exposed workers: 1.5 h sweat: 120–350 ng mercury 16 h urine: 160–190 ng mercury Unexposed workers: 1.5 h sweat: 5–8 ng mercury 16 h urine: 5–7 ng mercury Internal controls: 1.5 h sweat: 43–70 ng mercury 16 h urine: 30–46 ng mercury Mercury concentrations in sweat > urine for exposed workers; similar for controls

arsenic was excreted at lower concentrations in sweat than in urine [3].

Cadmium in sweat was examined in six studies [3, 22, 28, 30–33], with concentrations in sweat ranging from <0.5–10 μg/L [28] to 0.36–35.8 μg/L [3]. Stauber and Florence concluded that sweat may be an important route for excretion of cadmium when an individual is exposed to high levels [22, 28], a finding that was confirmed by observing that the total daily excretion of cadmium was greater in sweat than in urine [3, 32]. The maximum cadmium concentration observed in sweat was 35.8 μg/L [3].

Lead was examined in eleven studies [3, 22, 26–28, 33–38]. In 1973, Hohnadel et al. suggested that "sauna bathing might provide a therapeutic method to increase elimination of toxic trace metals" [38]. In two males, 36% and 50% of sweat lead was of molecular weight > 30,000, as measured by ultrafiltration, suggesting excretion of organic complexes rather than simple ions [22]. Lead excretion was lower in females taking birth control medications compared with females not taking medications, or males [28]. Haber et al. found that prolonged endurance workouts (rowing) ameliorated elevated blood lead levels in exposed workers but did not alter levels in control subjects and did not affect urine levels [26]. They suggested that the elimination route was not urine, but potentially sweat or/and bile. Omokhodion and Crockford carried out several studies of trace elements in sweat, including a study of lead ingestion by two human participants [34]. Sweat lead levels did not increase immediately with elevated blood lead, although the authors make reference to an older study with longer followup wherein lead in underarm pads doubled in the five days following ingestion. Omokhodion and Howard also reported higher lead in sweat of exposed workers compared with unexposed controls [35], and in another study that sweat and blood lead levels were the only two variables that correlated among blood, urine, sweat, and saliva [36]. The English abstract of a 1991 case report in Russian indicated that sauna increased excretion of toxic elements and resulted in clinical improvements [27]. Sweat lead levels up to 283 μg/L have been observed in nonoccupationally exposed subjects [38] and up to 17,700 μg/L in workers [37], where it is noted that lead in sweat may partially originate from material absorbed within the skin that was not removed by pretest cleaning protocols [35]. Indeed, although dermal application of lead via hair follicles, sweat ducts, and diffusion does not result in immediate increases in blood or urine lead concentrations, dermal absorption was demonstrated using the Pb-204 isotope [43], lead powder, and salt [37].

Mercury. In 1973, Lovejoy et al. noted that exposure to mercury does not always correlate with urine mercury levels and that elimination by other routes such as sweat may be an explanation [41]. They suggested, "sweating should be the initial and preferred treatment of patients with elevated mercury urine levels." In a 1978 case report, a severely poisoned worker was rescued with chelation therapy, followed by a regimen of daily sweat and physiotherapy over several months during which the sweat mercury level returned to normal and the patient recovered [40]. Robinson measured mercury in sweat repeatedly in two volunteers, observing sweat to urine concentration ratios ranging from less than 0.1 to greater than 5. Sweat mercury concentrations varied widely from day to day, and there was no correlation with urine levels. Sweat mercury levels of 1.5 μg/L were observed by Genuis et al. [3] and 1.4 μg/L by Robinson and Skelly [39].

4. Discussion

Arsenic, cadmium, lead, and mercury may be excreted in appreciable quantities through the skin, and rates of excretion were reported to match or even exceed urinary excretion in a 24-hour period. This is of particular interest should renal compromise limit urinary excretion of toxic elements.

Most of the research identified was over 20 years old, and collection methods varied widely. Although authors described thorough precleaning methods, sweat concentrations measured in research settings are not well validated and varied according to the location on the body, collection method, and from day to day according to other variables such as hydration. Sweat contains metals not only from the blood plasma, but also evidently originating from dermal layers (particularly with significant dermal exposures, as for workers in welding, smelting, or battery manufacturing). It would appear that large variabilities in measured concentrations, apart from collection methods as mentioned above, were likely the result of differences in excretion amongst widely varying individuals with ranges of body burdens, genetic polymorphisms affecting detoxification efficiency, and physiological states, coupled with necessarily crude if simple experimental techniques. These variations were very much greater than would be expected due to limitations of analytical methods. Although analytical methods have improved over the years, analysis of these metals was commonplace at the time of the studies. Authors generally reported analytical methods rigorously or provided references to thorough descriptions and included internal standards and some indication of sensitivity.

The observation that between a third and a half of lead in sweat may be associated with high-molecular-weight molecules [22] merits replication, including examination of additional toxic elements and characterization of the associated molecules previously observed. Excretion of these large molecules also suggests that sweating may be a means of excretion of metals complexed with natural or synthetic chelating agents.

Yousuf et al.'s recent study demonstrating a 2 : 1 molar ratio of zinc : arsenic and increased vitamin E in skin secretions suggests potential therapeutic supplementation to accommodate these biochemical requirements. Vitamin E, zinc, and other nutrients are required for methylation and detoxification of arsenic within the body, and vitamin E supplementation improves the skin manifestations in arsenicosis [29].

From an occupational health perspective, lead, and presumably other toxic elements, may be absorbed via the

skin, which supports showering at work and further suggests the possibility of purging workers' skin by washing with a chelating agent (e.g., EDTA rinses extracted lead from workers' skin in methods validation experimentation [38]). It is unknown if sweating during the workday may affect dermal absorption, or if forced sweating at the end of the workday would be beneficial. It is also unknown if increased blood flow to the skin could possibly enhance absorption into the bloodstream, or if worker health could be optimized by a combination of workplace skin cleaning and sweating interventions.

Sweating has long been perceived to promote health, not only accompanying exercise but also with heat. Worldwide traditions and customs include Roman baths, Aboriginal sweat lodges, Scandinavian saunas (dry heat; relative humidity from 40% to 60%), and Turkish baths (with steam). Infrared saunas heat exposed tissues with infrared radiation, while air temperatures remain cooler than in other saunas.

Sweating is a long-standing, if recently forgotten, aspect of mercury detoxification. Various strategies used to maintain the mercury mining workforce have been explored over the centuries. In Spain and colonies, long the western world's primary sources of mercury, sending ill workers to warmer climes away from the exposure to drink weak beer (the hydrogen peroxide catalase oxidation of elemental mercury to ionic mercury is competitively inhibited by alcohol, increasing mercury in exhaled breath [44]) and to work in the heat (presumably to sweat out the "vapors") was a common and effective strategy centuries ago; tremors, salivation, and mouth ulcers resolved generally within a few weeks [45].

With acclimatization and regular use, the sauna is generally well tolerated by all ages [46], though medical supervision may be recommended during initial sessions for children, the elderly, or those with compromised health. Varying qualities of evidence indicate potential short- and long-term improvements for cardiovascular, rheumatological and respiratory conditions; contraindications include unstable angina pectoris, recent myocardial infarction, severe aortic stenosis, and high-risk pregnancy [15, 46]. Sweating is not only observed to enhance excretion of the toxic elements of interest in this paper, but also may increase excretion of diverse toxicants, as observed in New York rescue workers [47], or in particular persistent flame retardants [48] and bisphenol-A [49].

Optimizing the potential of sweating as a therapeutic excretory mechanism merits further research. To date, the large body of research into homeostasis of the most common metals (sodium, potassium, and to a lesser extent, magnesium, calcium, and zinc) and conditioning or adaptation to regular sweating by athletes has not been matched with studies of excretion of trace elements. Limited research suggests indirectly that conditioning may not restrict excretion of nonessential elements. Combination therapies, such as administration of *n*-acetyl cysteine, vitamin C, a chelating agent, or low doses of ethanol (for mercury), to name a few possibilities, along with sauna and/or exercise therapy to induce sweating, may be fruitful avenues of investigation.

It has been noted that among people whose health is compromised by toxicants, heat regulatory mechanisms of the autonomic nervous system are often affected, resulting in a failure to sweat readily [3]. In these cases, along with diet and nutritional supplementation to remediate biochemical imbalances, interventions to consider include brushing the skin, niacin to assist with vasodilation, and exercise prior to sauna use [50]. Clinical experience is that with persistence and ample hydration patients do eventually start to sweat. This is often a sign that the autonomic nervous system function is beginning to improve. With enhanced ability to sweat, detoxification is facilitated, which can ultimately result in clinical improvement.

For biomonitoring and research purposes, modern validated methods are desirable to collect and measure elements in sweat, so this means of excretion may be considered in the context of other measures such as urine, blood, feces, and hair concentrations. Considerations for dry and wet collection methods were recently discussed in the context of essential solutes [51, 52].

Undoubtedly further research in this area would improve understanding, but the available evidence suggests that physicians could consider recommending sweating as tolerated via exercise (preferred) and/or use of a sauna as a low-risk, potentially beneficial treatment for individuals who may be experiencing effects of toxic elements, or for individuals with regular exposure to or accretion of toxicants.

5. Conclusions

Sweating offers potential and deserves consideration, to assist with removal of toxic elements from the body. As toxic elements are implicated in many serious chronic conditions, research is needed in patients with select conditions to evaluate the body burden and to test the efficacy of source removal, dietary choices and supplements, interventions that induce sweating, and treatments with drugs, all to enhance excretion of toxic elements with the goal of clinical improvement. There is a clear need for robust trials, appropriately sized to assess clinical outcomes, from which therapeutic protocols can be derived. Both biochemical and clinical outcomes should be examined in order to develop and monitor clinical interventions that are both safe and effective.

Conflicts of Interests

The authors declare that they have no conflicts of interests.

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References

[1] Institute for Functional Medicine, *Textbook of Functional Medicine*, Gig Harbor, Wash, USA, 3rd edition, 2010.

[2] M. E. Sears and S. J. Genuis, "Environmental determinants of chronic disease, and medical approaches: recognition, avoidance, supportive therapy and detoxification," *Journal of Environmental and Public Health*, 2012.

[3] S. J. Genuis, D. Birkholz, I. Rodushkin, and S. Beesoon, "Blood, urine, and sweat (BUS) study: monitoring and elimination of bioaccumulated toxic elements," *Archives of Environmental Contamination and Toxicology*, vol. 61, no. 2, pp. 344–357, 2011.

[4] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Arsenic, 2007, http://www.atsdr.cdc.gov/ToxProfiles/TP.asp?id=22&tid=3.

[5] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Cadmium, 2008, http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=48&tid=15.

[6] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Lead, 2007, http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=96&tid=22.

[7] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Mercury. US Department of Health and Human Services. Public Health Service, 1999, http://www.atsdr.cdc.gov/ToxProfiles/TP.asp?id=115&tid=24.

[8] R. Copes, N. A. Clark, K. Rideout, J. Palaty, and K. Teschke, "Uptake of cadmium from Pacific oysters (*Crassostrea gigas*) in British Columbia oyster growers," *Environmental Research*, vol. 107, no. 2, pp. 160–169, 2008.

[9] P. Perilli, L. G. Mitchell, C. A. Grant, and M. Pisantea, "Cadmium concentration in durum wheat grain (*Triticum turgidum*) as influenced by nitrogen rate, seeding date and soil type," *Journal of the Science of Food and Agriculture*, vol. 90, no. 5, pp. 813–822, 2010.

[10] W. Liu, Q. Zhou, J. An, Y. Sun, and R. Liu, "Variations in cadmium accumulation among Chinese cabbage cultivars and screening for Cd-safe cultivars," *Journal of Hazardous Materials*, vol. 173, no. 1–3, pp. 737–743, 2010.

[11] M. C. Houston, "Role of mercury toxicity in hypertension, cardiovascular disease, and stroke," *Journal of Clinical Hypertension*, vol. 13, no. 8, pp. 621–627, 2011.

[12] Health Canada. Government of Canada. Draft Human Health State of the Science Report on Lead—2011 Health Canada Consultation Document, 2011, http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/dhhssrl-rpecscepsh/index-eng.php.

[13] Agency for Toxic Substances and Disease Registry. Interaction Profiles Home Page, http://www.atsdr.cdc.gov/interactionprofiles/index.asp.

[14] J. Leppaluoto, "Human thermoregulation in sauna," *Annals of Clinical Research*, vol. 20, no. 4, pp. 240–243, 1988.

[15] M. L. Hannuksela and S. Ellahham, "Benefits and risks of sauna bathing," *American Journal of Medicine*, vol. 110, no. 2, pp. 118–126, 2001.

[16] A. Eisalo and O. J. Luurila, "The Finnish sauna and cardiovascular diseases," *Annals of Clinical Research*, vol. 20, no. 4, pp. 267–270, 1988.

[17] J. A. Levisky, D. L. Bowerman, W. W. Jenkins, and S. B. Karch, "Drug deposition in adipose tissue and skin: evidence for an alternative source of positive sweat patch tests," *Forensic Science International*, vol. 110, no. 1, pp. 35–46, 2000.

[18] G. W. Cage and R. L. Dobson, "Sodium secretion and reabsorption in the human eccrine sweat gland," *Journal of Clinical Investigation*, vol. 44, no. 7, pp. 1270–1276, 1965.

[19] D. B. Dill, F. G. Hall, and W. van Beaumont, "Sweat chloride concentration: sweat rate, metabolic rate, skin temperature, and age," *Journal of Applied Physiology*, vol. 21, no. 1, pp. 96–106, 1966.

[20] D. B. Dill, F. G. Hall, and H. T. Edwards, "Changes in composition of sweat during acclimatization to heat," *American Journal of Physiology*, vol. 123, no. 2, p. 412, 1938.

[21] S. Robinson, J. R. Nicholas, J. H. Smith, W. J. Daly, and M. Pearcy, "Time relation of renal and sweat gland adjustments to salt deficiency in men," *Journal of Applied Physiology*, vol. 8, no. 2, pp. 159–165, 1955.

[22] J. L. Stauber and T. M. Florence, "The determination of trace metals in sweat by anodic stripping voltammetry," *Science of the Total Environment*, vol. 60, pp. 263–271, 1987.

[23] S. J. Montain, S. N. Cheuvront, and H. C. Lukaski, "Sweat mineral-element responses during 7 h of exercise-heat stress," *International Journal of Sport Nutrition and Exercise Metabolism*, vol. 17, no. 6, pp. 574–582, 2007.

[24] B. Falk, O. Bar-Or, R. Calvert, and J. D. MacDougall, "Sweat gland response to exercise in the heat among pre-, mid-, and late- pubertal boys," *Medicine and Science in Sports and Exercise*, vol. 24, no. 3, pp. 313–319, 1992.

[25] B. Falk and R. Dotan, "Children's thermoregulation during exercise in the heat—a revisit," *Applied Physiology, Nutrition and Metabolism*, vol. 33, no. 2, pp. 420–427, 2008.

[26] P. Haber, F. Ring, O. Jahn, and V. Meisinger, "Influence of intensive and extensive aerobic circulatory stress on blood lead levels," *Zentralblatt für Arbeitsmedizin, Arbeitsschutz, Prophylaxe und Ergonomie*, vol. 35, no. 10, pp. 303–306, 1985 (German).

[27] I. A. Parpaleĭ, L. G. Prokof'eva, and V. G. Obertas, "The use of the sauna for disease prevention in the workers of enterprises with chemical and physical occupational hazards," *Vracebnoe Delo Kiev*, no. 5, pp. 93–95, 1991, English abstract.

[28] J. L. Stauber and T. M. Florence, "A comparative study of copper, lead, cadmium and zinc in human sweat and blood," *Science of the Total Environment*, vol. 74, pp. 235–247, 1988.

[29] A. K. M. Yousuf, M. Misbahuddin, and M. S. Rahman, "Secretion of arsenic, cholesterol, vitamin E, and zinc from the site of arsenical melanosis and leucomelanosis in skin," *Clinical Toxicology*, vol. 49, no. 5, pp. 374–378, 2011.

[30] F. O. Omokhodion and J. M. Howard, "Trace elements in the sweat of acclimatized persons," *Clinica Chimica Acta*, vol. 231, no. 1, pp. 23–28, 1994.

[31] J. W. Robinson and S. Weiss, "The direct determination of cadmium in urine and perspiration using a carbon bed atomizer for atomic absorption spectroscopy," *Journal of Environmental Science and Health, Part A*, vol. 15, no. 6, pp. 635–662, 1980.

[32] J. W. Robinson and S. Weiss, "The direct determination of cadmium in hair using carbon bed atomic absorption spectroscopy. Daily rate of loss of cadmium in hair, urine and sweat," *Journal of Environmental Science and Health, Part A*, vol. 15, no. 6, pp. 663–697, 1980.

[33] J. R. Cohn and E. A. Emmett, "The excretion of trace metals in human sweat," *Annals of Clinical and Laboratory Science*, vol. 8, no. 4, pp. 270–275, 1978, (only abstract available online).

[34] F. O. Omokhodion and G. W. Crockford, "Sweat lead levels in persons with high blood lead levels: experimental elevation of blood lead by ingestion of lead chloride," *Science of the Total Environment*, vol. 108, no. 3, pp. 235–242, 1991.

[35] F. O. Omokhodion and J. M. Howard, "Sweat lead levels in persons with high blood lead levels: lead in sweat of lead

workers in the tropics," *Science of the Total Environment*, vol. 103, no. 2-3, pp. 123–128, 1991.

[36] F. O. Omokhodion and G. W. Crockford, "Lead in sweat and its relationship to salivary and urinary levels in normal healthy subjects," *Science of the Total Environment*, vol. 103, no. 2-3, pp. 113–122, 1991.

[37] S. G. Lilley, T. M. Florence, and J. L. Stauber, "The use of sweat to monitor lead absorption through the skin," *Science of the Total Environment*, vol. 76, no. 2-3, pp. 267–278, 1988.

[38] D. C. Hohnadel, F. W. Sunderman, M. W. Nechay, and M. D. McNeely, "Atomic absorption spectrometry of nickel, copper, zinc, and lead in sweat collected from healthy subjects during sauna bathing," *Clinical Chemistry*, vol. 19, no. 11, pp. 1288–1292, 1973.

[39] J. W. Robinson and E. M. Skelly, "The direct determination of mercury in sweat," *Spectroscopy Letters*, vol. 16, no. 2, p. 133, 1983.

[40] F. W. Sunderman, "Clinical response to therapeutic agents in poisoning from mercury vapor," *Annals of Clinical and Laboratory Science*, vol. 8, no. 4, pp. 259–269, 1978.

[41] H. B. Lovejoy, Z. G. Bell, and T. R. Vizena, "Mercury exposure evaluations and their correlation with urine mercury excretions. 4. Elimination of mercury by sweating," *Journal of Occupational Medicine*, vol. 15, no. 7, pp. 590–591, 1973.

[42] D. Airey, "Mercury in human hair due to environment and diet: a review," *Environmental Health Perspectives*, vol. 52, pp. 303–316, 1983.

[43] J. L. Stauber, T. M. Florence, B. L. Gulson, and L. S. Dale, "Percutaneous absorption of inorganic lead compounds," *Science of the Total Environment*, vol. 145, no. 1-2, pp. 55–70, 1994.

[44] G. Sällsten, S. Kreku, and H. Unosson, "A small dose of ethanol increases the exhalation of mercury in low-level-exposed humans," *Journal of Toxicology and Environmental Health, Part A*, vol. 60, no. 2, pp. 89–100, 2000.

[45] K. W. Brown, "Workers' health and colonial mercury mining at Huancavelica, Peru," *The Americas*, vol. 57, no. 4, pp. 467–496, 2001.

[46] K. Kukkonen-Harjula and K. Kauppinen, "Health effects and risks of sauna bathing," *International Journal of Circumpolar Health*, vol. 65, no. 3, pp. 195–205, 2006.

[47] J. Dahlgren, M. Cecchini, H. Takhar, and O. Paepke, "Persistent organic pollutants in 9/11 world trade center rescue workers: reduction following detoxification," *Chemosphere*, vol. 69, no. 8, pp. 1320–1325, 2007.

[48] S. J. Genuis, D. Birkholz, M. Ralitsch, and N. Thibault, "Human detoxification of perfluorinated compounds," *Public Health*, vol. 124, no. 7, pp. 367–375, 2010.

[49] S. J. Genuis, S. Beesoon, D. Birkholz, and R. A. Lobo, "Human excretion of bisphenol-A: blood, urine and sweat (BUS) study," *Journal of Environmental and Public Health*, 2012.

[50] G. H. Ross and M. C. Sternquist, "Methamphetamine exposure and chronic illness in police officers: significant improvement with sauna-based detoxification therapy," *Toxicology and Industrial Health*. In press.

[51] L. B. Weschler, "Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes," *Journal of Applied Physiology*, vol. 105, no. 4, pp. 1376–1377, 2008.

[52] L. E. Armstrong, "Commentary on viewpoint: sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes," *Journal of Applied Physiology*, vol. 105, no. 4, p. 1378, 2008.

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Clinical Study

Efficacy of Sublingual Immunotherapy versus Subcutaneous Injection Immunotherapy in Allergic Patients

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While it is generally accepted that Subcutaneous Injection Immunotherapy (SCIT) and Sublingual Immunotherapy (SLIT) are both efficacious, there is not yet a significant amount of information regarding their comparative efficacy. In this paper, we performed a retrospective chart review and compared treatment results in two groups of patients (both with nasal allergies with or without asthma) that were treated either with SCIT or SLIT. Both treatment modalities were found to be of similar efficacy.

1. Introduction

Allergic disease is an increasingly prevalent problem affecting up to one-third of the general population in industrialized countries. Immunotherapy is a treatment modality that can modify the immunological response of the allergy sufferer so that the affected individual will stop reacting to involved allergens. Immunotherapy is indicated for the treatment of allergic rhinitis (AR) and asthma [1], and it may prevent development of asthma in patients with AR [1, 2].

Immunotherapy can be administered by different routes amongst which we find injectable and oral vaccines. Injectable vaccines refers to the classical subcutaneous injection immunotherapy (SCIT) usually known as "allergy shots." Oral vaccines refer to sublingual immunotherapy (SLIT) where the allergens are administered as drops to the sublingual area even though the term oral vaccines may also include allergy tablets [3].

The purpose of this study is to compare the efficacy of treatment results in patients with nasal allergies, with or without asthma, that were treated with either one or the other of these two treatment modalities: SCIT or SLIT.

There is a voluminous body of scientific evidence that proves that these two treatment modalities are efficacious for the management of allergic conditions but the issue of these two modalities having similar efficacy has not yet been fully addressed. A review of the literature reveals only a few articles that directly address this issue [4–10]. In five of these reports [5–9] SCIT and SLIT are found to be equally effective. In one report [4] SCIT is found to have better results, and one report [10] finds both equally effective for AR patients but SCIT more effective for asthmatic patients. In our own experience, SLIT and SCIT appear to be of similar efficacy [11] In this report the efficacy of one will be compared against the other.

SCIT is a well-established treatment modality that has been successfully used for many decades and is relatively well tolerated. Occasionally patients can develop severe reactions that very rarely can result in mortality [12].

SLIT is also a very old treatment modality (earliest description is from 1900) and yet, while commonly used in Europe, it is still not well established in the USA [13]. Over the last 20 years the European medical community produced a large amount of high-quality evidence suggesting that SLIT is safer than SCIT [14, 15]. While no single case of mortality has ever been reported with SLIT [12, 16] this is not the case with SCIT [17, 18]. SLIT is so safe and easy to administer that patients treat themselves at home [19].

2. Methods

This study constitutes a retrospective, consecutive chart review of allergy patients treated by the author at his private office. The charts of active patients were alphabetically reviewed to determine eligibility. Inclusion criteria were as follows: a patient of any age with nasal allergies with or without asthma that was treated with immunotherapy for at

least for 6 months and had at least 2 complete evaluations. A complete evaluation implies symptom scoring, evaluation of medication use, and determination of the peak flow meter (PFM) value. These evaluations are done every 3–6 months as treatment progresses. Because evaluations depend on patient's cooperation not all the patients had the same number of evaluations, but any patient that was considered a candidate had to have 2 evaluations as a minimum. We compared the first evaluation (pretreatment) and the last evaluation the patient had just at the time of inclusion for the study. These were considered pretreatment and post-treatment evaluations. The symptoms in the pretreatment evaluation and the amount of medications the patient was taking at that time reflect how the patient was doing without immunotherapy treatment.

Ethical Considerations. Subjects' privacy was respected by collecting and recording data in such a way that the subjects could not be identified, directly or indirectly, through identifiers linked to the subject. In other words, a patient's confidentiality would be protected by entering data in a simple spreadsheet with nonspecific identifiers as patient no. 1, patient no. 2, and so forth with subsequent refiling of the patient's chart, according to usual procedure. The content of the spread sheet became anonymous and ready for statistical analysis.

2.1. Decision to Use SCIT or SLIT. After discussing with patient about their allergies and advising about environmental modification maneuvers a discussion about treatment options including immunotherapy follows. In our office SCIT or SLIT is used to treat patients with inhalant allergies with or without bronchial involvement. The decision to use one or the other is sometimes made by the patient, sometimes advised by the treating physician. Economical considerations, living far from the office, busy schedule, or "needle phobia," are examples of when a patient may chose SLIT. Having severe asthma, being a very young patient or having medical problems that may render administration of SCIT risky are examples of why the treating physician will advise SLIT.

2.2. Testing and Treatment Administration. All patients were tested using a fivefold intradermal dilution skin test (IDT) as taught by the AAOA [20, 21]. The test includes several panels: dust, dander, epidermals, molds, and pollens for our geographic area (Table 1).

Standardized antigens were used for testing and treatment whenever these were available; otherwise weight/volume antigen extracts were used [22].

After identifying the minimally reactive antigen concentration (meaning first reactive wheal) for each of the patient's reactive allergens, SCIT vials or SLIT bottles were formulated including all of the positive results (reactive allergens in the intradermal test) in the treatment mixture. Patients on SCIT were treated according to AAOA guidelines [21, 23]. Patients on SLIT were treated according to a previously published protocol [11] where the dose is slowly advanced from 1 drop per day to 5 drops per day until attaining the most concentrated mixture in the SLIT bottle. The formulation was the same for both injectable and oral vaccines.

2.3. Amount of Antigen Delivered. While the concentration of antigens is exactly the same for both SCIT and SLIT but SLIT is administered daily [11], patients on SLIT will receive a larger amount of antigen each week than those treated with SCIT. The injectable vials are mixed with a volume of 5.0 mL. The SLIT bottles are mixed with 7.5 mL. If we consider a single allergen, for example, *Dermatophagoides pteronyssinus* (DP), standardized dust mite DP has a concentration of 10,000 AU/mL containing 68 mcg/mL of Der p 1 and 71 mcg/mL of Der p 2 antigens [22]. If the minimally reactive antigen concentration occurred at dilution no. 3 and dose was advanced until mixing a vial from manufacturer's concentrate, the cumulative dose this patient would receive weekly by SCIT would be 200 AU per week, while a patient treated by SLIT would receive 464 AU per week [11]. As stated before, the initial allergen concentration in both SCIT and SLIT is the same: 80 AU/mL as in both circumstances the extract (with 10.000 AU/mL) will be diluted 125 times. After one year of treatment the patient on SCIT would receive 9680 AU and the patient treated by SLIT would receive 21149 AU or 2.18 times more allergen [11].

2.4. Sample Comparison in reference to Allergen Reactivity. A chi-square test was applied for the following allergens: dust mite, cat, roach, mold, tree-pollens, grass-pollens, and weed-pollens for both groups, SCIT and SLIT.

2.5. Asthma Diagnosis. Asthma diagnosis was based on the presence of recurrent cough, chest tightness, SOB, or wheezing [24], having a spirometry consistent with airflow obstruction or having the symptoms respond to the administration of a short-acting broncho-agonist (SABA).

2.6. Scoring. Recorded symptoms included runny nose, sneezing, nasal obstruction, itchy eyes, itchy ears, cough, shortness, and wheezing. These were scored according to Fell's method [25] with a numerical analog from 0 through 3 as follows:

0 = symptom not present,

1 = symptom is mild,

2 = symptom is moderate,

3 = symptom is severe.

Medication use was also evaluated on a similar numerical scale as follows:

0 = medication is not being used,

1 = medication is being used once a week or less,

2 = medication is being used 2–3 times per week,

3 = medication is being used 4 or more times per week.

Table 1: Allergy test panels.

Dust, dander. and epidermals	Molds	Trees	Grasses	Weeds
Mite pteronyssinus	Alternaria	Ash	Bermuda	Cocklebur
Mite farinae	Aspergillus	Beech	Johnson	English Plantain
Dog	Cladosporium	Birch	Timothy	Goldenrod
Cat	Curvularia	Box Elder		Lambs Quarters
Roach americana	Epicoccum	Elm		Pigweed
Roach germanica	Fusarium	Hickory		Ragweed
	Helminthosporium	Oak		Sagebrush
	Mucor	Sycamore		Sheep Sorrel
	Penicillium			
	Pullularia			

Table 2: Symptom Results.

Symptom	No. of patients	Before (mean)	After (mean)	P value of t-test	Significance of SCIT/SLIT × before/after interaction
Runny nose SCIT	47	2.1	0.7	<0.001	Not significant
Runny nose SLIT	34	1.8	0.5	<0.001	
Sneezing SCIT	47	2.0	0.8	<0.001	Not significant
Sneezing SLIT	39	1.9	0.8	<0.001	
Nasal obstruction SCIT	48	2.4	0.8	<0.001	Not significant
Nasal obstruction SLIT	40	2.2	0.9	<0.001	
Itchy ears SCIT	38	1.5	0.5	<0.001	Not significant
Itchy ears SLIT	30	1.3	0.5	<0.001	
Itchy eyes SCIT	46	1.9	0.7	<0.001	Not significant
Itchy eyes SLIT	37	1.8	0.7	<0.001	
Cough SCIT	46	1.7	0.4	<0.001	Greater improvement for SCIT ($P = 0.037$)
Cough SLIT	30	1.2	0.4	<0.001	
SOB SCIT	6	1.4	0.5	0.041	Not significant
SOB SLIT	9	2.0	0.8	0.005	
Wheezing SCIT	4	1.3	0.5	0.042	Greater improvement for SLIT. ($P = 0.024$)
Wheezing SLIT	7	2.5	0.3	0.001	

Medications were generically grouped as allergy pills, intranasal steroids (INSs), and short-acting broncho-agonists (SABAs) in the case of asthmatic patients.

The value of the PFM determination was used as the parameter to be recorded at each patient's encounter.

3. Results

Ninety-three charts met the inclusion criteria, 50 on SCIT and 43 on SLIT. Among the 50 patient's on SCIT, 20 (40%) were male, 30 (60%) female ranging in age from 2.33 to 75 years (mean 45 ± 17.8 SD). This compared to 43 patients on SLIT of whom 21 (49%) were male, 22 (51%) female ranging in age from 1.66 to 75 years (mean 35 ± 20.8 SD). There are no statistical differences between the demographics of both groups. Analysis of covariance for the dependent variables for which a significant pre/posttreatment by treatment modality interaction effects was obtained did not reveal gender or age to account for significant dependent variable variance; in other words the results were not affected by age or gender so both groups can be considered homogeneous. Both groups were also compared in reference to test results. A chi-square test was applied for the following allergens: dust mite, cat, roach, mold, tree-pollens, grass-pollens, and weed-pollens. Results indicate that there are no statistical differences between both groups (at the $P < 0.05$ level); therefore in their reactivity to allergens both groups can also be considered homogeneous.

There were 3 children <12 years on SCIT (mean 7.8 years) versus 11 on SLIT (mean 6.9 years). Ten (20%) SCIT patients had asthma versus 12 (28%) on SLIT. Thus a greater percentage of asthmatics (12/22 or 55%) and more children under 12 years of age (11/14 or 79%) were on SLIT. Length of treatment for the SCIT group was 12 to 86 (mean 31 ± 18.7 SD) months and for the SLIT group was 10 to 32 (mean 19 ± 6.3 SD) months.

For all patients the pre- and posttreatment averages for each symptom, medication use, and PF value were statistically compared through the use of repeated measure analysis of variance (ANOVA). The results for the two treatment modalities (SCIT versus SLIT) were also compared using the between-subjects factor of the ANOVA (Table 2). The same analyses were completed for medication use (Table 3). For the PF evaluation the pre- and post-treatment values were compared (Table 4).

Table 3: Medication use.

Medication	no. of patients	Before (mean)	After (mean)	P-value of t-test	Significance of SCIT/SLIT × before/after interaction
Pills in SCIT	37	2.0	0.5	<0.001	Not significant
Pills in SLIT	25	1.5	0.4	<0.001	
INS in SCIT	28	1.5	0.3	<0.001	Not significant
INS in SLIT	26	1.2	0.2	<0.001	
SABA in SCIT	6	1.6	0.9	0.047	Not significant
SABA in SLIT	9	1.1	0.2	0.010	

Table 4: Peak Flow Meter determinations. (L/m = litters per minute).

PFM (L/m)	no. of patients	Before (mean)	After (mean)	P-value of t-test	Significance of SCIT/SLIT × before/after interaction
PFM in SCIT	44	368	467	<0.001	Not significant
PFM in SLIT	36	323	422	<0.001	

3.1. Symptom Results. In Table 2 the mean value for each symptom score before treatment and at the time of data collection is shown for both treatment modalities. The result of the test of significance is shown for each symptom within each treatment modality (paired t-test). Lastly, the result of the statistical analysis comparing symptom improvement with one or the other treatment modality is shown.

All symptoms had significant improvement with both treatment modalities. Shortness of breath and wheezing had significant improvements at $P < 0.05$ for both treatment modalities. The remaining symptoms had a significant improvement at $P < 0.001$ for both treatment modalities.

Wheezing and coughing were the only symptom scores which seemed to respond better to either SCIT (coughing slightly better, $P = 0.037$) or SLIT (wheezing slightly better, $P = 0.024$), though both symptoms significantly improved regardless of treatment modality. For the remaining symptoms there was no significant difference between both treatment modalities.

3.2. Results of Medication Use. Both SCIT and SLIT provided equally significant reduction in use of medication ($P < 0.001$) including allergy pills, INS, and, to a slightly lesser but still significant degree, SABA (Table 3) but without no significant difference between both treatment modalities.

3.3. Results of Changes in PFM Values. PF value before treatment and at the time of the last patient evaluation is shown in Table 4. Both treatment modalities were equally effective in achieving a significant increase in PF values ($P < 0.001$) but there was no significant difference between both treatment modalities.

4. Discussion

This paper is a retrospective chart review and as such lacks the rigor of a prospective randomized study with a placebo control group which is very difficult to do in a private office setting. While an analysis of covariance is useful, it is not a perfect solution. A future, larger-scale study should be planned to include the above design characteristics.

We observed that patients usually come to the office already using one or more allergy medications. This study, like others, demonstrates that immunotherapy, whether SCIT or SLIT, will lead to the reduction of medication use for AR and/or asthma. It was not the purpose of this paper to evaluate the effect of medications on allergy symptoms but rather to compare the effects of SCIT versus SLIT on medication use. Both treatment modalities resulted in the reduction of antihistamines, inhaled nasal steroids, and SABAs.

The slight imbalances in demographic characteristics between the groups on SCIT versus SLIT were not statistically significant and did not affect the statistical results. The reason why there are more young patients and more asthmatic patients in the SLIT group can be explained by the fact that SLIT is safer and easier to administer therefore it is suggested more frequently for these difficult-to-manage patients. Indeed we would have expected a much more pronounced difference; yet fewer than expected chose SLIT because it is not covered by insurance.

Patients on SCIT have been treated for a longer period of time because SLIT was added to our practice later than SCIT.

The improvement of the asthmatic symptoms wheezing and SOB and the decrease in SABA use were significant at $P < 0.05$ yet because of sample size this is not as strong as the improvement in other symptoms or medications that had an improvement at the level of $P < 0.001$.

The advantage for SCIT in treating coughing is real, but the effect size (eta-squared) is only 0.025, meaning that it only accounts for 2.5% of the variance in pre- versus posttreatment differences, which is not much. Therefore, it can be concluded that SCIT and SLIT exhibit similar efficacy. The advantage of SLIT in treating wheezing may have been influenced by our own bias of suggesting SLIT use to asthmatic patients as a safer treating modality. It is therefore more likely that patients with higher symptom scores were present in the SLIT group.

Our findings demonstrate that SLIT is not only effective in controlling symptoms in nasal allergy patients with or without asthma, in decreasing medication use in such patients, and in improving parameters of pulmonary function, but it also appears that SLIT is as effective as SCIT

These findings are in agreement with those published in the European literature [26, 27] but certainly this presentation lacks the scientific validity of other reports [9] that present a prospective, randomized, controlled study;

therefore this presentation we hope will serve as a stimulus for centers with the capability to undertake such a study to continue with this line of research. This would help the FDA to finally recognize SLIT as an effective and safe treatment modality. If SLIT became an FDA-approved treatment modality (and hopefully) reimbursed by insurance companies many more patients might be receptive to immunotherapy which is a treatment capable of altering the immunological mechanisms responsible for the development of allergic conditions [28].

PF values for asthma control should be taken as a guideline only because the predicted lung function has a high degree of variability with significant differences in PF values according to presence or not of lung disease, smoking, age, sex, and even patient's social environment [29–31].

Having the advantage of providing results quickly, and requiring little training (from the patient as well as from the technical staff), the PFM device is useful to monitor progress during immunotherapy [32]. It is most useful when the changes in PF values are compared to the initial value of each patient, recorded at the time of treatment initiation [32]. For the purpose of this study individual improvement with therapy is not reported, but rather an overall trend, thus the use of PFM provides a gross indicator of change.

Immunotherapy is administered over a long period of time. Some of our patients were children, and it is expected they grow during treatment. Certainly using a PFM as a tool to determine improvement in pulmonary function adds uncertainty as to whether the improvement in PF value is related to clinical improvement or to the growth of the patient during treatment. In this study the number of young patients was not large. On the other hand we have demonstrated that the PF value in patients treated by immunotherapy increases regardless of age or asthmatic condition [32].

In our experience, the use of SLIT with multiple antigens has enabled us to treat patients that otherwise would have not received immunotherapy, or would have not continued to receive immunotherapy, like asthmatic patients with poorly controlled asthma, patients that had severe arm reactions, very young patients to whom it is difficult to administer shots or patients whose schedules prevent them from being compliant.

5. Conclusions

These results suggest that SCIT and SLIT exhibit similar efficacy. SLIT objectively improves symptom scores for asthma and AR while decreasing medication usage of allergy medications and SABAs.

Given the increased risk and difficulty in treating asthmatic and young patients, these results would suggest that SLIT should be considered as the main treatment modality for these patients, considering SCIT only for treatment failures.

The results of this study are in agreement with the European literature and therefore would support the inclusion of SLIT in the routine management of the allergic disease.

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References

[1] D. S. Theodoropoulos and R. F. Lockey, "Allergen immunotherapy: guidelines, update, and recommendations of the World Health Organization," *Allergy and Asthma Proceedings*, vol. 21, no. 3, pp. 159–166, 2000.

[2] L. Jacobsen, B. Niggemann, S. Dreborg et al., "Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-Year follow-up on the PAT study," *Allergy*, vol. 62, no. 8, pp. 943–948, 2007.

[3] J. Kleine-Tebbe, M. Ribel, and D. A. Herold, "Safety of a SQ-standardised grass allergen tablet for sublingual immunotherapy: a randomized, placebo-controlled trial," *Allergy*, vol. 61, no. 2, pp. 181–184, 2006.

[4] B. Wedi, "Status and indications for SLIT in comparison to SCIT," *Hautarzt*, vol. 59, no. 7, pp. 537–543, 2008.

[5] M. S. Khinchi, L. K. Poulsen, F. Carat, C. André, A. B. Hansen, and H. J. Malling, "Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study," *Allergy*, vol. 59, no. 1, pp. 45–53, 2004.

[6] M. Mauroa, M. Russelloa, C. Incorvaiab, G.-B. Gazzolaa, G. Di Carac, and F. Frati, "Comparison of efficacy, safety and immunologic effects of subcutaneous and sublingual immunotherapy in birch pollinosis: a randomized study," *European Annals of Allergy and Clinical Immunology*, vol. 39, no. 4, pp. 119–122, 2007.

[7] J. Pokladnikova, I. Krcmova, and J. Vlcek, "Economic evaluation of sublingual vs subcutaneous allergen immunotherapy," *Annals of Allergy, Asthma and Immunology*, vol. 100, no. 5, pp. 482–489, 2008.

[8] C. Antúnez, C. Mayorga, J. L. Corzo, A. Jurado, and M. J. Torres, "Two year follow-up of immunological response in mite-allergic children treated with sublingual immunotherapy. Comparison with subcutaneous administration," *Pediatric Allergy and Immunology*, vol. 19, no. 3, pp. 210–218, 2008.

[9] A. O. Eifan, T. Akkoc, A. Yildiz et al., "Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial," *Clinical and Experimental Allergy*, vol. 40, no. 6, pp. 922–932, 2010.

[10] D. Mungan, Z. Misirligil, and L. Gürbüz, "Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma—a placebo controlled study," *Annals of Allergy, Asthma and Immunology*, vol. 82, no. 5, pp. 485–490, 1999.

[11] D. Saporta and A. B. McDaniel, "Efficacy comparison of multiple-antigen subcutaneous injection immunotherapy and multiple-antigen sublingual immunotherapy," *Ear, Nose and Throat Journal*, vol. 86, no. 8, pp. 493–497, 2007.

[12] H. H. Windom and R. F. Lockey, "An update on the safety of specific immunotherapy," *Current Opinion in Allergy and Clinical Immunology*, vol. 8, no. 6, pp. 571–576, 2008.

[13] D. Saporta, "Sublingual immunotherapy: a novel, albeit not so new, immunotherapy treatment modality," *American Journal of Rhinology*, vol. 22, no. 3, pp. 253–257, 2008.

[14] G. W. Canonica and G. Passalacqua, "Noninjection routes for immunotherapy," *Journal of Allergy and Clinical Immunology*, vol. 111, no. 3, pp. 437–448, 2003.

[15] C. André, C. Vatrinet, S. Galvain, F. Carat, and H. Sicard, "Safety of sublingual-swallow immunotherapy in children and adults," *International Archives of Allergy and Immunology*, vol. 121, no. 3, pp. 229–234, 2000.

[16] G. Ciprandi and G. L. Marseglia, "Safety of sublingual immunotherapy," *Journal of Biological Regulators and Homeostatic Agents*, vol. 25, no. 1, pp. 1–6, 2011.

[17] W. E. Davis, P. R. Cook, J. P. McKinsey, and J. W. Templer, "Anaphylaxis in immunotherapy," *Otolaryngology—Head and Neck Surgery*, vol. 107, no. 1, pp. 78–83, 1992.

[18] P. R. Cook, J. L. Bryant, W. E. Davis, T. T. Benke, and A. S. Rapoport, "Systemic reactions to immunotherapy: the American Academy of Otolaryngic Allergy morbidity and mortality survey," *Otolaryngology—Head and Neck Surgery*, vol. 110, no. 6, pp. 487–493, 1994.

[19] L. Cox, "Sublingual immunotherapy in pediatric allergic rhinitis and asthma: efficacy, safety, and practical considerations," *Current Allergy and Asthma Reports*, vol. 7, no. 6, pp. 410–420, 2007.

[20] R. L. Mabry, *Skin End Point Titration*, AAOA Monograph Series, Thieme Medical Publishers, New York, NY, USA, 1994.

[21] J. A. Fornadley, "Skin testing in the diagnosis of inhalant allergy," in *Allergy and Immunology, an Otolaryngic Approach*, J. H. Krouse, S. J. Chadwick, B. R. Gordon, and M. J. Derebery, Eds., pp. 114–123, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 2002.

[22] Alk-Abello, Inc., *1700 Royston Lane*, Round Rock, Tex, USA.

[23] R. C. Haydon and B. R. Gordon, "Aeroallergen immunotherapy," in *Allergy and Immunology, an Otolaryngic Approach*, J. H. Krouse, S. J. Chadwick, B. R. Gordon, and M. J. Derebery, Eds., pp. 151–182, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 2002.

[24] http://www.nhlbi.nih.gov/guidelines/asthma/03_sec2_def.pdf.

[25] W. R. Fell, R. L. Mabry, and C. S. Mabry, "Quality of life analysis of patients undergoing immunotherapy for allergic rhinitis," *Ear, Nose and Throat Journal*, vol. 76, no. 8, pp. 528–536, 1997.

[26] Z. Calamita, H. Saconato, A. B. Pelá, and A. N. Atallah, "Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method," *Allergy*, vol. 61, no. 10, pp. 1162–1172, 2006.

[27] V. Di Rienzo, F. Marcucci, P. Puccinelli et al., "Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study," *Clinical and Experimental Allergy*, vol. 33, no. 2, pp. 206–210, 2003.

[28] H. S. Nelson, "Allergen immunotherapy: where is it now?" *Journal of Allergy and Clinical Immunology*, vol. 119, no. 4, pp. 769–777, 2007.

[29] F. R. Radziavicius, L. C. Martins, C. C. Radziavicius et al., "Peak expiratory flow values are higher in older and taller healthy male children: an observational study," *Clinical and Investigative Medicine*, vol. 33, no. 2, pp. E92–E98, 2010.

[30] G. R. Pesola, P. O'Donnell, V. M. Chinchilli, and A. F. Saari, "Peak expiratory flow in normals: comparison of the mini wright versus spirometric predicted peak flows," *Journal of Asthma*, vol. 46, no. 8, pp. 845–848, 2009.

[31] R. Hauser, C. Daskalakis, and D. C. Christiani, "A regression approach to the analysis of serial peak flow among fuel oil ash exposed workers," *American Journal of Respiratory and Critical Care Medicine*, vol. 154, no. 4, part 1, pp. 974–980, 1996.

[32] D. Saporta, "MD changes in peak flow meter values during immunotherapy administration," *Journal of Environmental and Public Health*, vol. 2012, Article ID 212867, 9 pages, 2012.

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Research Article

Human Impairment from Living near Confined Animal (Hog) Feeding Operations

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Problem. To determine whether neighbors around manure lagoons and massive hog confinement buildings who complained of offensive odors and symptoms had impaired brain and lung functions. *Method.* We compared near hog manure neighbors of lagoons to people living beyond 3 kilometers in Ohio and to unexposed people controls in a nearby state for neurophysiological, cognitive, recall and memory functions, and pulmonary performance. *Results.* The 25 exposed subjects averaged 4.3 neurobehavioral abnormalities, significantly different from 2.5 for local controls and 2.3 for Tennessee controls. Exposed subjects mean forced vital capacity and expiratory volume in 1 sec were reduced significantly compared to local and regional controls. *Conclusions.* Near neighbors of hog enclosures and manure lagoon gases had impaired neurobehavioral functions and pulmonary functions and these effects extended to nearby people thought to be controls. Hydrogen sulfide must be abated because people living near lagoons cannot avoid rotten egg gas.

1. Introduction

Centralizing animal production has increased efficiency and reduced costs of meat and dairy products. The disadvantage is that objectionable odors emanate from huge quantities of manure that are generated daily. Confined animal feed operations (CAFOs) exposed their human neighbors to effluent gases including hydrogen sulfide, and other sulfur gases, ammonia, pork antigens, and aerosols.

Beyond being unpleasant, these gases adversely affect human lungs, brains, and other organs. More than 100 parts per million (ppm) of hydrogen sulfide is lethal [1–7]. Permanent central nervous system impairment was described 20 years ago after nonlethal "knockdown" by hydrogen sulfide. Downwind neighbors of oil refineries, desulfurization plants and a cattle hide operation showed neurobehavioral impairment for balance, color discrimination, reaction time, and verbal recall from exposures ranging from 0.1 to 25 ppm of hydrogen sulfide [8–10].

Many human brain functions can be measured to estimate brain performance and losses thereof. Standing balance is simple in concept, but requires integrating in the cerebellum inputs from the vestibular apparatus (the 8th cranial nerve), ascending proprioceptive impulses, motor cortical corrections, and visual monitoring of sway. Visual perception and recognition occupies over 40% of cortical function from retinal cones for color discrimination and rods for perception in dim light. Thresholds for perception are mapped for hearing, the other 8th cranial nerve function as sound thresholds, are perceived in the brain's temporal lobes. Fingertip number writing tests parietal lobe perception. Simple/complex acts that depend upon perception decision making and response and are represented by simple and choice visual reaction time are easily tested. Most tests are measured as time needed for an act. Included are reaction times, peg placement, and making trails (connection of 25 circles in numerical order or alternate numbers and letter in alphabetical order), digit symbol substitution, and problem solving as in Culture Fair or Raven's Matrices. Comparison of observed values for each test against population-based predicted values quantifies as nearly as possible, performance before and after exposure for each of the 26 tests. Expressing

performance as percentage of predicted, observed divided by predicted, improves sensitivity for individuals and when averaged, for groups.

After 4 patients living near an Ohio hog manure lagoon were diagnosed with neurobehavioral and pulmonary impairment [10], they organized their nearby neighbors and those living beyond 3 kilometers for testing. These groups were compared to each other and to unexposed people in a nearby state.

2. Methods

2.1. Exposure Measurements. Several homes were sampled and H_2S found using direct reading Jerome meter with a 3 ppb detect limit for the near exposed people. Subsequently on May 6, 2003, air in twelve homes were monitored indoors and out for hydrogen sulfide using NIOSH method 3013-k and for ammonia with NIOSH method 5347 ISE and a handheld Jerome meter by TMC Ltd., Macedonia, OH, USA. Well water samples were analyzed for hydrogen sulfide, ammonia, oil, nitrate/nitrite, and coliform bacteria. Coliform bacteria were elevated in 5 wells but E. coli were not found. H_2S was elevated in 2 samples to 0.11 and 1.91 mg/l whereas nitrate was under 0.5 mg/l. On February 23-24, 2005, 9 homes were monitored for H_2S using 2 Jerome 631-x meters and 10 minutes in each room and outdoors by Burgess and Niple of Columbus, OH, USA. Water was run in bathrooms or kitchens and sample reading after 10 minutes were compared to initial values, all in ppb.

Twenty-five people who lived in proximity to hog manure lagoons near Paulding, OH, were recruited by neighborhood canvas and scheduled for testing. Twenty-two matching unexposed people living at least 3 km from the lagoons were also tested. Hydrogen sulfide exposed and unexposed subjects were invited to volunteer without regard to complaints.

Most homes were closer than 900 meters to lagoons (range from 180 to 2,180 meters). Samples were obtained after a rain storm. Durations of residential exposure were considered, as surrogate for exposure although lagoons had existed for only 4 years. Findings are described in results.

The 22 unexposed and 25 exposed subjects were tested together on April 26, 27, 2003. Their exposure status was not identified to the testing staff to avoid bias in neurobehavioral testing as done previously [7, 11–13]. The local control group participants matched the exposed group for age and gender. They were paid $30. No subject was excluded for symptoms, annoyance or their opinions about hydrogen sulfide. No unexposed subject was medically disabled.

Comparisons were made of 22 unexposed and 25 exposed subjects to 58 Tennessee controls. Although in 2003 probably no one was truly "unexposed to chemicals," the Tennessee registered voters from two communities were without chemical contamination by historical review and onsite inquiry. Their 2.3 average brain functional abnormalities, were distributed asymmetrically (strongly skewed left) and were similar to the population studied to derive the prediction equations [12].

These 58 reference subjects (30 women and 28 men) included 28 from Spring Hill Columbia and 30 from Waverly, TN, USA. All were volunteers who were recruited from voter registration rolls, interviewed briefly to verify their freedom from workplace and home chemical exposures, and reimbursed for time and mileage. Exposed and control subjects were intermingled for testing and their exposure status was withheld from examiners.

Exposed and referent subjects questionnaires were completed after rectifying omissions recognized by computer-guided card reading [13, 14]. Frequencies of 35 common health complaints (and two questions as validity checks) were self-rated: as rare equal to 1 to daily rated 11 [14, 15]. Other inquiries included standard lupus erythematosus questions [16], a standard respiratory questionnaire [17], histories of occupational and other exposures to chemicals, pesticides and herbicides, tobacco, alcohol, and drug use (prescription and illicit), anesthetic agents, unconsciousness, head trauma, and neurological and medical histories [14].

The questionnaires and test battery were developed and standardized in previous studies of histology technicians [18], fire fighters exposed to thermolysis products of PCBs [15], a chlorinated solvent exposed population [14], people exposed to toluene rich chemical waste, those exposed to hydrogen sulfide, and groups of unexposed subjects [5, 7, 8, 12].

Alcohol and carbon monoxide (CO) in expired alveolar air were tested by expelling a big breath held for 20 seconds using specific fuel cell analyzers [8]. No alcohol levels were above 1 ppm. Most CO levels were 0 but varied to peak at 27 ppm in persons who had smoked cigarettes within 24 hours.

2.2. Neurophysiological Tests. Simple reaction time (SRT) and visual two-choice reaction time (CRT) were measured with a computerized instrument [19] and the fastest median of the last 7 of two groups of 20 trials was recorded for SRT and CRT. It tests the retina and optic cortex, integrative radiation to the motor cortex, and descending corticospinal tracts.

Body balance was measured with the subject standing erect with feet together. A sound generating stylus on a head band tracked by two microphones and processed in a computer-expressed balance as mean speed of sway in cm/sec [20]. The minimal sway speed of 3 consecutive 20-second trials was counted for sway each with eyes open and eyes closed. Balance depends on ascending proprioceptive tracts, the vestibular division of the 8th nerve, cerebellum, visual integrative, and motor tracts.

Blink reflex was measured with surface electromyographic electrodes from lateral orbicularis oculi muscles bilaterally [21, 22] after tapping the right and left supraorbital notches with a light hammer which also triggered a recording computer. Its circuit is the trigeminal nerve, pons-cross over, and motor innervation via the facial nerve. Ten firings of the first wave, R-1 were averaged for each side and failures were recorded [22].

Hearing was measured in left and right ears with standard audiometers (model ML-AM Microaudiometrics, So. Daytona, FL, USA) at stepped frequencies of 500 to 8,000 Hertz and summed for each ear. It tests the auditory division of the 8th cranial nerve.

A dynamometer measured grip for cortical motor nerve and muscle function.

Color discrimination errors were measured with the desaturated Lanthony 15 hue test under constant illumination [23] and scored with Bowman's method [24]. It tests the cones of the retina and the visual cortex.

Visual fields were tested with a computerized (Med Lab Technology, New Wales, PA, USA) automated perimeter recording to a computer which mapped the central 30° of the right and left eye fields individually by measuring perceptual thresholds to 80 light emitting diodes. Performance was the sum of scores for each eye. Visual score counted the abnormal quadrants (scotoma or other defects) for both eyes [25]. Thus, rod functions in the retina, the optic nerve, cortical radiation were evaluated.

2.3. Neuropsychological Tests. Immediate verbal recall was measured by stories from Wechsler's Memory Scale-revised [26] which tests the limbic system of the temporal lobes. Culture Fair (battery 2A) and vocabulary were done in groups of 8 to 12 subjects. Culture Fair tested nonverbal nonarithmetical intelligence with 4 sets of designs for similarity, difference, completion, and pattern recognition and transfer [27, 28]. It resembles Raven's progressive matrices [29]. The 46-word vocabulary test was from Jackson's [30] multidimensional aptitude battery. Digit symbol substitution from the Wechsler Adult Intelligence Scale-revised (WAIS-R) [31] tested attention and integrative capacity. Information, picture completion and similarities from the WAIS-R tested long term retention of cultural information; a frontal lobe function that is usually maintained until chemical brain damage becomes severe [32].

Time to place 25 pegs in the Lafayette slotted pegboard with the preferred hand was measured and trail making A and B measured dexterity (optic to motor cortex), coordination and decision making. Fingertip number writing assessed peripheral sensation and discrimination were from the Halstead-Reitan battery [33, 34].

Subjects' moods were appraised by responses to 65 terms describing emotional status for the past week using the Profile of Mood States (POMSs) [35]. It assays feeling states and the limbic system.

2.4. Respiratory Flows and Vital Capacities. were measured after subjects took a full inspiration and exhaled into a volume displacement (Ohio) spirometer while standing and using a nose clip and repeated until two forced expirations agreed within 5% following ATS [36] criteria. Records were traced with a digitizer, measured by a computer, compared to predicted values that adjusted for height, sex, age, and the volume and flow reducing effects of cigarette smoking, and expressed as percent of predicted [17, 37].

2.5. Statistical Analysis. Scores and computed data were transferred to a computer for analysis using Stata Statistical Software Version 8 (Stata Corporation, College Station, TX, USA). Without neurobehavioral testing before people were exposed to hydrogen sulfide the reasonable alternative was to calculate expected values. The combined "unexposed" population was from Tennessee [13]. The steps were the following.

(1) Expected values were calculated for each test for each person using regression equations [12].

(2) Expected values were based on testing unexposed general population groups with appropriate age distributions. (Test scores were mathematically transformed when this improved the symmetry of data distributions.)

(3) Coefficients were retained for age for most tests, sex for many and educational attainment for problem solving, recall, long-term memory, and perceptual motor tests. Distances of homes to the nearest manure lagoon and hydrogen sulfide levels indoors were tested for influence on abnormalities score and individual test scores. Family income, hours of general anesthesia, weight, and Mood States (POMSs) scores did not influence any test.

(4) Observed scores were divided by expected (predicted) scores and multiplied by 100 and expressed as percent predicted. This procedure compared each test or function to that person's calculated value that approximated baseline measurements.

(5) The differences in means as percent predicted for groups were tested for statistical significance by analysis of variance.

(6) Exposed groups' averaged total abnormalities were compared to averages for control groups by analysis of variance.

(7) P values were adjusted for simultaneous inference using Holm's modification of Bonferroni procedure [38].

Each participant's total abnormality score was the sum of tests outside the 95% confidence interval (variance 92% to 97%) which was 1.5 times each test's standard deviation of each test. Balance and vision were so important in detecting effects of chemical exposure in several thousand subjects [9, 22], that each sway measures was scored 2, and visual fields performance was scored 1 for each eye. Bilateral hearing, blink reflex latency, grip strength, and fingertip number errors were assigned 0.5 per side with 1 for other functions. Regression analyses examined the effects of mood states scores, symptom frequencies, specific exposures, and other factors such as distance of their home from the hog confinement buildings.

3. Results

3.1. Exposures. Indoor air of 12 homes had hydrogen sulfide levels of 0 to 2,100 ppb. Ranges indoor and outdoor varied 10-fold or more in one-day's spot check samples. Two outdoor samples were above 1,100 ppb. Water running increased the H_2S levels 2 to 10 times for a peak of 430 ppb.

Distances to homes from lagoons varied from 170 to 3,000 meters, the inverse of distance squared from hog confinement lagoons did not predict scores or number of

abnormalities. Exposure was less than 4 years in only two people.

3.2. Neurobehavioral Testing. Comparison of test means for the 25 near exposed people to those of the local for exposed group of 22 showed statistically significant differences (ssd) for balance with eyes open was 1.0 abnormality, digit symbol substitution 1.0 abnormality, and vocabulary 1.0 abnormality (Table 1). These abnormalities were not statistically significant after Holm's [38] adjustment for simultaneous inference. However, comparison to the Tennessee unexposed group showed 7 physiological test differences: balance measured with eyes open and with eyes closed for 2 abnormalities, simple and choice reaction time 2 abnormalities, color discrimination errors 1 abnormality, and visual field performance 2 abnormalities. For the psychological tests: digit symbol substitution, vocabulary, verbal recall (immediate and delayed), and picture completion were significantly different. Testing for simultaneous inference reduced the significant differences for both sets of comparisons, but choice reaction time, balance and color discriminating errors, visual field performance, digit symbol substitution, vocabulary, visual recall, immediate and delayed verbal recall, and picture completion remained different compared to TN unexposed, only simple reaction time was dropped. Ohio near exposed had 7 differences from TN unexposed that were for color discrimination errors, visual field performance, immediate and delayed verbal recall, and picture completion.

Profile of Mood States mean scores were elevated at 53.1 in the 25 near exposed people versus 5.6 in the 22 distant exposed, people versus 5.6 in the 22 distant exposed, and 22.1 in the Tennessee unexposed people ($P < 0.0001$) (Table 2). Total abnormalities were correlated with symptom frequencies but not Profile of Mood States scores by regression analysis ($P > .007$) with 27.4% of the variance (r^2) explained.

More of the near exposed than for exposed or unexposed groups had ever smoked cigarettes: 40% versus 28%, but similar proportions, 16% versus 13% continued to smoke. Unexposed smokers from Tennessee were not different from nonsmokers for total neurobehavioral impairments. Regression of total neurobehavioral abnormalities against age, duration of smoking in years, and educational attainment showed only age was significant in the near exposed and no factor was significant in the far exposed (data are not shown).

The difference in total abnormalities, mean 4.3 ± 3.0, for the 25 near exposed compared to a mean of 2.5 ± 2.3 in the 22 distant exposed was statistically significant (by ANOVA, $P < .011$). The comparison of the near exposed group mean abnormalities of 4.3 to the Tennessee control's mean of 2.3 ± 2.1 was also statistically significant ($P < .0001$), as was the comparison of Ohio far-exposed people to Tennessee unexposed people that showed 6 differences and an abnormality score of 2.5 which was not significant ($P < .879$).

The near-exposed group had increased frequencies for shortness of breath when climbing stairs, but not at rest, or while walking nor when was wheezing more frequent. Their expiratory flows and vital capacities were significantly decreased (comparisons were adjusted for years of smoking) compared to the far exposed and to the unexposed (Table 3).

Frequencies of 18 of 35 symptoms were statistically significantly elevated in near exposed compared to far exposed (Table 4), and mean frequencies were ssd, 3.2 ± 1.7 in near exposed versus 1.9 ± 0.9 in far ($P < .002$) exposed and 2.6 ± 1.1 in the Tennessee unexposed ($P < .038$). Nine symptom frequencies were elevated compared to the Tennessee unexposed. Three general symptoms namely extreme fatigue, headache, and decreased smell joined eye irritation, loss of balance, loss of concentration, and losses of recent and long term memory. The near to farther away neighbor comparison added 6 chest symptoms: chest tightness, palpitation, shortness of breath, dry cough, dry mouth, throat tightness. This comparison added dizziness and lightheadedness to the balance category and somnolence, irritability and unstable mood. There were no differences between the exposed and unexposed groups for rheumatic or for lupus erythematosus complaints or for neurological diseases and psychiatric illnesses. No subject had substance dependency. The unexposed and near exposed and far exposed groups' did not differ in their occasional exposures to 15 occupations and groups of chemicals.

The 58 Tennessee unexposed people's individual abnormality scores averaged 2.3 with distributions skewed to the left as plotted in Figure 1(a). The comparative abnormality scores for the 25 near exposed (mean 4.3) and 22 far exposed (mean 2.5) are plotted in Figures 1(b) and 1(c). The distribution of abnormalities of the Tennessee controls was skewed, increasing sharply from many with zero to one or two going to eight abnormalities. In contrast, the 25 near-exposed subjects had a symmetrical distribution around the mean of 4.3. The 22 Ohio distant-exposed subjects also had a symmetrical distribution of abnormalities around a mean of 2.5.

4. Discussion

The number of neurobehavioral impairments in people exposed around lagoons emitting hydrogen sulfide differed significantly from local more distant people and differed greatly from unexposed people in a nearby state. Significantly, lower expiratory flows indicated pulmonary impairment. Spot-check sampling in May 2003 and February 2005 shows H_2S odors were mainly from several hog lagoons. Average indoor air hydrogen sulfide concentrations ranged from 0 to 30 ppb. Outdoor samples peaked at 1,600 ppb and indoor at 2,100 ppb with tap water running. Nevertheless, neurobehavioral impairments in these people were consistent with those from other hydrogen sulfide exposures, where levels were 1 to 5 ppm with peaks up to 100-fold higher [5, 10, 11]. Although distances to homes of impaired subjects from hog confinement, as a surrogate for hydrogen sulfide dose and total neurobehavioral abnormalities did not correlate, neither peak concentrations nor cumulative exposures were characterized and prevailing wind and humidity were assayed only on the days of sampling not for period of each season that would be needed to characterize doses.

Table 1: 25 people near hog lagoons compared to 22 distant exposed and to 58 Tennessee unexposed (compared as means of percent predicted values, by analysis of variance, ANOVA).

Percent predicted	A: distant exposed 22 mean ±sd	B: near exposed 25 mean ± sd	A versus B *P* value	C: unexposed TN 58 mean ± sd	B versus C *P* value (Holm p)	A versus C *P* value (Holm p)
Age (years)	56.6 ± 16.0	50.4 ± 16.8	.284	56.8 ± 18.1	.0001	.977
Educational level (years)	12.1 ± 1.7	13.0 ± 2.2	.195	12.1 ± 1.2	.817	.236
Simple reaction time	100.9 ± 4.2	101.3 ± 3.4	.757	103.1 ± 5.3	.017* .24+	.228
Choice reaction time	101.2 ± 3.0	101.5 ± 2.9	.824	103.3 ± 4.0	.0005* .01+	.113
Balance sway speed						
Eyes open	104.6 ± 2.6	136.4 ± 29.5	.0014*	106.9 ± 34.4	.0002* .004+	.831
Eyes closed	109.7 ± 26.7	149.3 ± 43.5	.003	95.1 ± 24.5	.0016* .03+	.147
Blink reflex latency R-1						
Right	90.4 ± 7.3	101.2 ± 13.0	.010	96.6 ± 15.4	.745	.473
Left	91.8 ± 10.3	92.7 ± 12.5	.861	105.2 ± 16.7	.963	.019*
Color discrimination errors						
Right	63.9 ± 47.0	42.3 ± 39.2	.173	66.7 ± 52.5	.0002* .004+	.883+
Left	56.2 ± 35.5	48.8 ± 41.8	.583	44.3 ± 38.3	.0001* .0027+	.394
Visual field performance						
Right	121.7 ± 14.8	110.1 ± 14.5	.033	119.9 ± 8.0	.0001* .003+	.0001* .0028+
Left	122.9 ± 21.3	110.6 ± 14.2	.070	124.1 ± 10.6	.0001* .0032+	.0001* .003+
Grip strength						
Right	105.7 ± 17.8	99.0 ± 18.9	.264	93.7 ± 14.5	.733	.040*
Left	102.1 ± 17.2	95.4 ± 22.1	.336	90.9 ± 12.4	.784	.070
Cognition						
Culture fair	107.1 ± 27.9	101.4 ± 15.9	.493	97.6 ± 23.2	.842	.348
Digit symbol	101.3 ± 12.6	88.2 ± 20.5	.030* .60+	91.4 ± 18.0	.0001* .0024+	.089
Vocabulary	89.9 ± 34.9	66.5 ± 27.2	.046	72.5 ± 34.1	.002* .03+	.197
Verbal recall						
Immediate	84.8 ± 27.5	78.5 ± 23.3	.488	75.3 ± 17.4	.0008* .015+	.309
Delayed	57.9 ± 33.5	65.1 ± 35.1	.555	38.9 ± 29.5	.0001* .0023+	.131
Pegboard	117.7 ± 20.3	102.2 ± 23.2	.050	108.9 ± 15.3	.769	.221
Trails A	99.9 ± 7.0	101.4 ± 5.6	.512	106.5 ± 10.0	.149	.044
Trails B	100.5 ± 7.9	103.7 ± 7.6	.238	96.3 ± 16.8	.423	.372
FTNWE right	94.4 ± 8.5	98.6 ± 7.4	.151	97.1 ± 8.4	.249	.395
FTNWE left	96.5 ± 8.9	102.6 ± 8.1	.054	99.6 ± 12.3	.442	.428
Information	98.4 ± 39.5	85.4 ± 31.9	.318	94.3 ± 36.4	.403	.780

Table 1: Continued.

Percent predicted	A: distant exposed 22 mean ±sd	B: near exposed 25 mean ± sd	A versus B *P* value	C: unexposed TN 58 mean ± sd	B versus C *P* value (Holm p)	A versus C *P* value (Holm p)
Picture Completion	69.7 ± 36.1	69.7 ± 42.4	.998	58.8 ± 40.4	.0009* .016+	.450
Similarities	102.6 ± 30.5	86.5 ± 41.5	.209	95.3 ± 47.5	.650	.608
Total Abnormalities	2.5 ± 2.3	4.3 ± 3.0	.011	2.3 ± 2.1	.001	.879

*: $P < 0.05$.
+: $P < 0.05$ after Holm's correction for multiple inference [38].

Table 2: Profile of mood states (POMSs) for 25 near exposed compared to 22 distant exposed in Paulding, Ohio, and 58 unexposed Tennessee subjects (compared as means of percent predicted values, by analysis of variance, ANOVA).

POMS	A: 22 distant exposed mean ± sd	B: 25 near distant exposed mean ± sd	A versus B *P* values	C: 58 TN unexposed mean ± sd	A versus C *P* values
Score*	5.6 ± 18.6	53.1 ± 45.5	.0001	22.1 ± 25.0	.0001
Range	−28 to 46	−11 to 164	—	5 to 130	—
Tension	7.2 ± 4.0	14.9 ± 7.7	.0001	8.9 ± 4.6	.0001
Depression	4.1 ± 4.1	14.7 ± 12.1	.0003	7.9 ± 7.1	.002
Anger	4.8 ± 4.5	15.4 ± 11.9	.0003	7.7 ± 6.5	.0003
Vigor	19.8 ± 7.0	14.7 ± 7.2	.018	17.0 ± 6.2	.137
Fatigue	5.5 ± 3.4	12.2 ± 7.6	.0004	8.3 ± 5.6	.01
Confusion	3.8 ± 2.5	10.6 ± 5.8	.0001	6.4 ± 3.7	.0001

* Vigor subtracts from the sum of affective states, so can be minus 28.

Table 3: Pulmonary function tests in 25 near-exposed subjects compared to 22 distant exposed in Paulding, Ohio, and 58 unexposed Tennessee subjects (compared as means of percent predicted values, by analysis, of variance, ANOVA).

	A: 22 distant exposed Mean ± sd	B: 25 near exposed Mean ± sd	A versus B *P* values	C: 58 TN unexposed Mean ± sd	B versus C *P* values	A versus C *P* values
FVC	97.0 ± 12.8	87.6 ± 10.7	.014*	101.6 ± 15.2	.0001*	.180
FEV_1	94.5 ± 11.7	85.5 ± 15.4	.028*	93.6 ± 15.2	.025*	.070
FEF_{25-75}	98.4 ± 20.0	91.9 ± 30.8	.223	88.1 ± 35.0	.633	.070
FEF_{75-85}	90.8 ± 24.2	96.4 ± 54.1	.080	78.1 ± 52.7	.133	.191
FEV_1/FVC	77.9 ± 3.3	76.1 ± 7.3	.301	72.8 ± 9.5	.130	.014*

* Statistically significant values.

There were significantly more abnormal tests in Paulding people near exposed and distant exposed than in regional Tennessee [13] referents and other unexposed (control) groups [7, 8, 39, 40]. These abnormalities are attributed primarily to hydrogen sulfide and other effluents from hog manure lagoons making it reasonably probable that local controls were significantly more abnormal than were regional referents, which suggests that both near and distant local groups shared exposures, probably to H_2S. Although the inverse square of the distance from sources did not correlate with abnormalities. Among possible explanations are (1) inhaling a few breaths of a spike of H_2S of 200 or more ppm can greatly impair human brain function and (2) the dynamic nature of this heavier-than-air gas movement that spread concentrations irregularly dependant on wind, protection, and depressions in the ground or buildings. Also, consider that hydrogen sulfide is attached to particles and particles contain Gram-negative bacterial endotoxin to cause the measured impairments.

Other possibilities such as considering that Ohio far-exposed people were biased for abnormality are inconsistent because fewer subjects were abnormal for tests of: blink reflex latency, peg placement, trail making, information, and similarities. Sectional differences in unexposed peoples function or impairment in the United States has not been found [39]. No adverse demographic, geographic, or other factors were found. If hydrogen sulfide is not the factor affecting both groups, we hypothesize a parallel (chemical) exposure, a shared Ohio factor. This problem has been encountered before [40]. No exposures to chlorinated solvents were found in reports of Environmental Protection Agency's monitoring of community culinary water. Other possible Ohio factors include atrazine, a herbicide widely used on corn fields, phosphorothioic acid (Famphur),

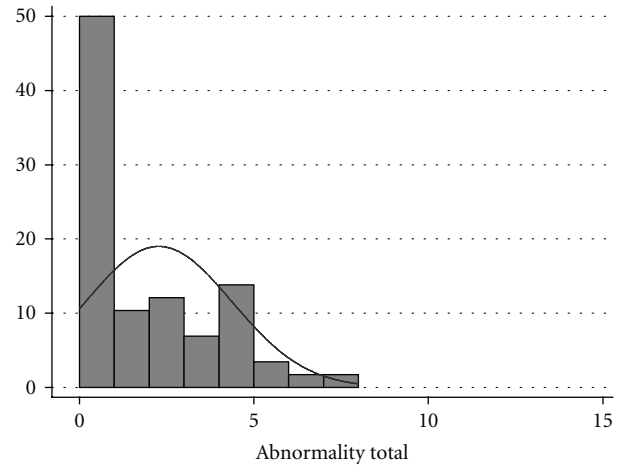
Table 4: Symptom frequencies (1 to 11 scale) for 25 near-exposed subjects compared to 22 distant-exposed and 58 unexposed subjects.

Symptom	A: 25 near exposed	B: 22 distant exposed	A versus B *P* values	C: 58 TN unexposed	A versus C *P* values
Skin irritation	4.1 ± 3.5	2.5 ± 2.1	.071	3.2 ± 2.5	.222
Deformed finger nails	1.4 ± 1.3	1.4 ± 1.2	.893	1.5 ± 1.3	.655
Chest tightness	2.6 ± 2.5	1.5 ± 0.8	.049*	2.0 ± 1.5	.234
Palpitations	2.0 ± 1.5	1.6 ± 1.7	.034*	2.3 ± 2.1	.580
Burning-tightness of chest	2.0 ± 1.9	1.4 ± 0.7	.170	1.9 ± 1.6	.977
Shortness of breath	2.7 ± 2.2	1.5 ± 1.1	.035*	3.1 ± 2.0	.418
Dry cough	3.0 ± 2.2	1.9 ± 1.0	.040*	2.5 ± 1.8	.362
Cough with mucus	2.9 ± 2.4	2.0 ± 1.3	.127	2.6 ± 1.9	.521
Cough with blood	1.4 ± 1.2	1.0 ± 0.0	.133	1.1 ± 0.6	.195
Dry mouth	4.1 ± 3.0	2.0 ± 0.9	.002*	3.1 ± 2.5	1.25
Throat tight	3.8 ± 2.9	2.3 ± 1.5	.035*	2.8 ± 1.9	.091
Eye irritation	3.8 ± 2.9	2.1 ± 1.6	.015*	2.4 ± 2.1	.013*
Decreased smell	3.4 ± 3.1	2.1 ± 2.2	.095	2.1 ± 2.0	.021*
Headache	5.7 ± 3.5	3.2 ± 2.4	.007*	4.1 ± 2.4	.017*
Nausea	2.2 ± 1.6	1.8 ± 1.5	.313	2.5 ± 1.7	.575
Dizziness	3.0 ± 2.7	1.5 ± 0.9	.017*	2.1 ± 1.6	.059
Lightheadedness	2.8 ± 1.9	1.7 ± 0.8	.019*	2.5 ± 1.7	.486
Exhilaration (unusual)	1.2 ± 1.6	1.1 ± 0.5	.704	1.8 ± 1.8	.062
Loss of balance	3.3 ± 2.6	1.5 ± 0.9	.003*	1.9 ± 1.2	.001*
Loss of consciousness	1.3 ± 1.1	1.0 ± 0.2	.243	1.2 ± 0.4	.356
Extreme fatigue	4.9 ± 3.4	1.8 ± 1.6	.0003*	3.1 ± 2.3	.005*
Somnolence	3.2 ± 2.9	1.3 ± 0.6	.004*	2.5 ± 2.2	.242
Insomnia	3.3 ± 3.1	2.3 ± 2.1	.204	2.6 ± 2.5	.291
Wake frequently	3.9 ± 3.1	2.5 ± 2.0	.089	2.7 ± 2.5	.014
Sleep few hours	3.7 ± 2.7	2.4 ± 2.4	.100	2.6 ± 2.5	.086
Irritability	4.5 ± 3.4	2.3 ± 1.7	.009*	3.7 ± 2.4	.236
Loss of concentration	4.6 ± 3.6	2.1 ± 1.7	.005*	3.2 ± 2.1	.034*
Loss of recent memory	5.8 ± 3.5	2.3 ± 1.6	.0001*	3.2 ± 2.6	.0002*
Long-term memory loss	4.1 ± 3.1	1.7 ± 1.3	.001*	2.4 ± 2.2	.006*
Unstable moods	3.6 ± 3.2	1.3 ± 0.5	.002*	2.4 ± 2.1	.064
Loss of libido	4.2 ± 3.2	2.7 ± 2.1	.060	3.8 ± 3.3	.537
Decreased alcohol tolerance	1.6 ± 1.2	1.6 ± 1.2	.992	2.2 ± 1.9	.165
Indigestion	3.2 ± 1.9	3.0 ± 2.2	.737	2.8 ± 2.2	.430
Loss of appetite	2.2 ± 1.7	1.5 ± 1.2	.121	2.4 ± 1.9	.570
Swollen stomach	3.0 ± 3.0	2.0 ± 2.0	.177	2.8 ± 2.5	.759
Tingling navel	1.0 ± 0.2	1.0 ± 0.2	.928	1.2 ± 0.8	.404
Itching gums	1.0 ± 0.0	1.0 ± 0.0	1.00	1.2 ± 0.5	.068
Symptom frequency mean	3.2 ± 1.70	1.9 ± .91	.002	2.6 ± 1.1	.038*
Symptom frequency range	1.22 to 6.57	1.11 to 5.11		1.18 to 5.68	

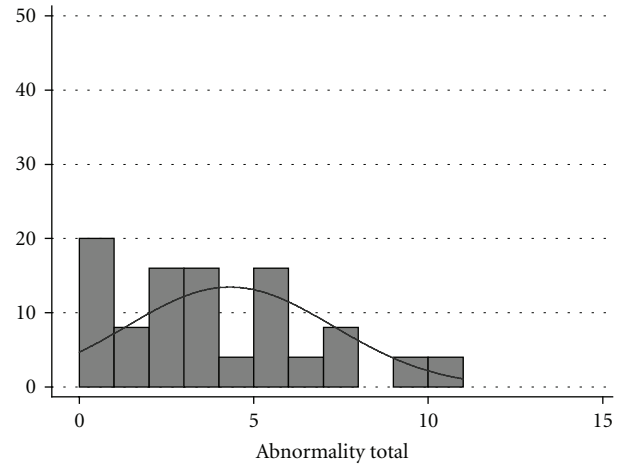
* Statistically significant values.

an organophosphate insecticide used on corn and animals, and Gram-negative bacterial endotoxin that has been measured in hog confinement workers [41].

A review of 2,786 workers in swine confinement buildings from 14 studies [42] showed elevated frequencies of chronic cough, phlegm, chest tightness, wheezing, and acute intermittent symptoms. Respiratory symptoms were accompanied by decrements in flow and further drops during work. Chronic fatigue, muscle and joint pains, and dizziness were also described [42]. A later study of 54 male workers



(a)



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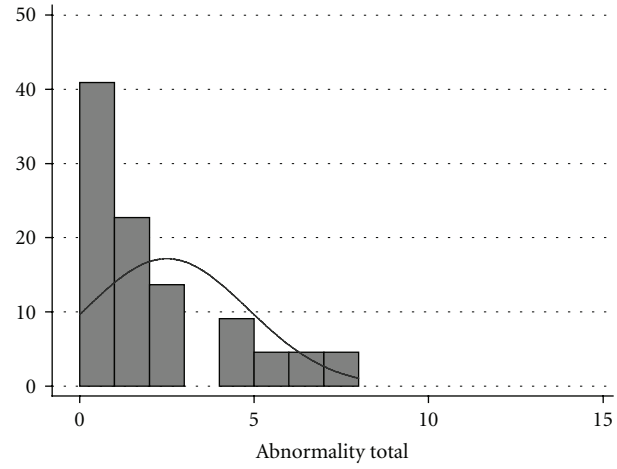

(c)

Figure 1: (a) Individual abnormality frequencies in regional Tennessee controls show a mean of 2.3 and a distribution skewed leftward. (b) The 25 hog-farm-exposed people had a symmetrical distribution of abnormalities with a mean frequency of 4.3. (c) The 22 Ohio hog-farm-distant-exposed people also had a skewed distribution of abnormalities with a mean frequency of 2.5.

correlated reduced forced vital capacities to increased endotoxin levels in dust (mean 11,443 endotoxin units) by regression analysis [41].

Objectionable odor has been associated with elevated scores on the 5 adverse moods of the Profile of Mood States (POMSs) thus increasing the total Profile of Mood States score in 44 people living nearby indoor hog operation compared to age- and sex-matched control people [43]. The stench of these operations has made national news repeatedly [44, 45].

Interviews of 55 people living near confined animal feeding operations in North Carolina found increased headaches, burning eyes, running nose, sore throat, excessive coughing, and diarrhea compared to 50 more distant neighbors [46]. These observations increase health concerns about exposure to confined animal feeding operations [47] particularly as to whether there are measurable effects on functions related to these exposures. Such concern is irrespective of whether our exposed and control people are considered as one group varying in proximity to hydrogen sulfide sources or sharing an additional and as yet unknown toxic exposure.

The demonstration of neurobehavioral impairment from proximity to confined animal feeding operations in Ohio neighbors of hog raising indoors, CAFOs, adds abnormal functions to excessive symptoms that were quantified earlier. We also confirmed in downwind neighbors of hog confinement the reductions in vital capacity and flows found in hog confinement workers [41].

4.1. Limitations and Alternate Explanations. The results agree with those from occupational groups [3, 4] and environmental exposures [7, 8, 10]. Some people who lived on farms nearby may have had occupational exposure to hydrogen sulfide but this is unlikely as most farms had no meat or dairy animals. Increased complaints from being in an exposed group [48] do not impair performance on our neurobehavioral tests [9, 40]. Conscious manipulation is impossible for blink reflex latency, a test that can be done on unconscious subjects. In contrast, a subject could deliberately slow peg placement and trail making, but test givers recognize and correct such slowing. Also, such manipulation is unlikely because local near-exposed and distant-exposed groups performed equally to Tennessee unexposed. For balance and reaction time, the best of several trials was the score. Finally, intentional poor performance is unlikely because we asked experienced testers' to fake impairment on these tests and they could not do so. It would be even less likely for people naïve to these tests to coordinate "a group effort to affect scores." The methods for visual field mapping were comparable for Ohio people without differences between exposed and controls. The systematic difference from Tennessee controls was due to a different method for the fields. Color testing was done on right and left eyes, the scores compared and consistent results were accepted. An explanation for variable results was sought in history and interview.

Information, picture completion, and similarity scores from the well-learned cultural domain were correlated with the highest grade attained in school as shown by others

[32]. Inebriation had no role as no alcohol levels in expired breath were elevated. Cigarette smoking that raised carbon monoxide levels in alveolar air from 5 to 30 ppm has no adverse neurobehavioral effects [13, 49]. The reverse is true, chronic smoking of cigarettes has improved the speed of choice reaction time and peg placement (personal observation) and nicotine improves mental and physical functions [50, 51]. Decreased respiratory flows after adjusting for effects of smoking appeared due to hydrogen sulfide, as observed previously [10, 11].

After sewer workers died from inhalation of gas in Paris and London in the mid 1800s, Christison [1] attributed the deaths to sulfurated hydrogen, now known as hydrogen sulfide. Poisonings of bystanders are still reported regularly from hydrogen sulfide escaping from geothermal sites, refineries, desulfurization plants, pipelines, hog husbandry buildings, waste lagoons, cattle feed lots, dairy buildings, wood pulping lagoons, and so forth [5, 6, 10]. Occupational exposures to hydrogen sulfide include shale oil [52], ocean fishing [53], oil refining [54, 55], and cleaning geothermal (hot) springs [56].

4.2. Mechanisms of Toxicity. Hydrogen sulfide poisons the brain and mitochondria by irreversibly combining with iron in respiratory enzymes, cytochrome oxidases, thus stopping oxidative phosphorylation. It stimulates the respiratory center, increasing hydrogen sulfide intake for a breath or two [57, 58]. Lower doses increase brain neuromediators by inhibiting monoamine oxidase [58] and there is evidence that hydrogen sulfide is the brain's third gaseous mediator [59].

Sensitive testing showed permanent brain dysfunction in workers thought to have recovered from hydrogen sulfide exposures [52, 54, 55]. They had cognitive and recall memory deficits reduced problem solving ability, impaired balance, slowed reaction time, and scotomata, losses in their visual fields [11, 25, 57]. The present observations from human exposure to hydrogen sulfide at the lower end of concentrations expected to produce adverse effects are tentative. They replicate earlier studies and invite validation by others. Unfortunately, it appears that studies of groups of people exposed environmentally in incidents associated with symptoms will have similar limitations in dose estimation as did this study.

Data are insufficient to propose a safe dose of hydrogen sulfide. This is because single brief exposures to sublethal doses may severely impair brain function and so does years of exposure to levels below 1 ppm. A dose-time relationship has not been found [11, 53]. As a safe level cannot be proposed, it would be prudent to separate people from all sources of hydrogen sulfide: feed lots, tanneries, oil refineries, and natural gas processing (desulphurization), and ponds and lagoons contain sulfur that becomes anaerobic as in geothermal sites such as hot springs. Information from many sources suggests that proximity is the important and geothermal factor in toxicity despite effects of other gases, wind velocity and direction, land contour, and temperature.

The precautionary principle recommends that odors, perceived at levels of H_2S above 30 ppb, are the cue to escape further exposure. Delay may let olfactory fatigue abolish the warning and invite damage.

Acknowledgment

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References

[1] R. Christison, *A Treatise on Poisons*, Adam and Charles Black, Edinburgh, Scotland, 4th edition, 1845.

[2] T. L. Guidotti, "Occupational exposure to H_2S in the sour gas industry: some unresolved issues," *International Archives of Occupational and Environmental Health*, vol. 66, no. 3, pp. 153–160, 1994.

[3] H. H. Wasch, W. J. Estrin, P. Yip, R. Bowler, and J. E. Cone, "Prolongation of the P-300 latency associated with hydrogen sulfide exposure," *Archives of Neurology*, vol. 46, no. 8, pp. 902–904, 1989.

[4] B. Tvedt, A. Edland, K. Skyberg, and O. Forberg, "Delayed neuropsychiatric sequelae after acute hydrogen sulfide poisoning: affection of motor function, memory, vision and hearing," *Acta Neurologica Scandinavica*, vol. 84, no. 4, pp. 348–351, 1991.

[5] B. Tvedt, K. Skyberg, O. Aaserud, A. Hobbesland, and T. Mathiesen, "Brain damage caused by hydrogen sulfide: a follow-up study of six patients," *American Journal of Industrial Medicine*, vol. 20, no. 1, pp. 91–101, 1991.

[6] J. W. Snyder, E. F. Safir, G. P. Summerville, and R. A. Middleberg, "Occupational fatality and persistent neurological sequelae after mass exposure to hydrogen sulfide," *American Journal of Emergency Medicine*, vol. 13, no. 2, pp. 199–203, 1995.

[7] K. H. Kilburn and R. H. Warshaw, "Hydrogen sulfide and reduced-sulfur gases adversely affect neurophysiological functions," *Toxicology and Industrial Health*, vol. 11, no. 2, pp. 185–197, 1995.

[8] K. H. Kilburn and R. H. Warshaw, "Neurotoxic effects from residential exposure to chemicals from an oil reprocessing facility and superfund site," *Neurotoxicology and Teratology*, vol. 17, no. 2, pp. 89–102, 1995.

[9] K. H. Kilburn, *Chemical Brain Injury*, John Wiley, New York, NY, USA, 1998.

[10] K. H. Kilburn, "Effects of hydrogen sulfide on neurobehavioral function," *Southern Medical Journal*, vol. 96, no. 7, pp. 639–646, 2003.

[11] K. H. Kilburn, "Evaluating health effects from exposure to hydrogen sulfide: central nervous system dysfunction," *Environmental Epidemiology and Toxicology*, vol. 1, no. 3-4, pp. 207–216, 1999.

[12] K. H. Kilburn, J. C. Thornton, and B. Hanscom, "A field method for blink reflex latency R-1 (BRL R-1) and prediction equations for adults and children," *Electromyography and Clinical Neurophysiology*, vol. 38, no. 1, pp. 25–31, 1998.

[13] K. H. Kilburn, "Visual and neurobehavioral impairment associated with polychlorinated biphenyls," *NeuroToxicology*, vol. 21, no. 4, pp. 489–499, 2000.

[14] K. H. Kilburn and R. H. Warshaw, "Effects on neurobehavioral performance of chronic exposure to chemically contaminated

well water," *Toxicology and Industrial Health*, vol. 9, no. 3, pp. 391–404, 1993.

[15] K. H. Kilburn, R. H. Warsaw, and M. G. Shields, "Neurobehavioral dysfunction in firemen exposed to polychlorinated biphenyls (PCBs): possible improvement after detoxification," *Archives of Environmental Health*, vol. 44, no. 6, pp. 345–350, 1989.

[16] R. E. Levin, A. Weinstein, and M. Peterson, "A comparison of the sensitivity of the 1971 and 1982 American Rheumatism Association criteria for the classification of systemic lupus erythematosis," *Arthritis and Rheumatism*, vol. 27, no. 5, pp. 530–538, 1984.

[17] B. G. Ferris, "Epidemiology standardization project," *American Review of Respiratory Disease*, vol. 118, no. 6, pp. 7–54, 1978.

[18] K. H. Kilburn and R. H. Warshaw, "Neurobehavioral effects of formaldehyde and solvents on histology technicians: repeated testing across time," *Environmental Research*, vol. 58, no. 2, pp. 134–146, 1992.

[19] J. A. Miller, G. S. Cohen, R. Warshaw, J. C. Thornton, and K. H. Kilburn, "Choice (CRT) and simple reaction time (SRT) compared in laboratory technicians: factors influencing reaction times and a predictive model," *American Journal of Industrial Medicine*, vol. 15, no. 6, pp. 687–697, 1989.

[20] K. H. Kilburn, R. H. Warshaw, and B. Hanscom, "Balance measured by head (and trunk) tracking and a force platform in chemically (PCB and TCE) exposed and referent subjects," *Occupational and Environmental Medicine*, vol. 51, no. 6, pp. 381–385, 1994.

[21] B. T. Shahani and R. R. Young, "Human orbicularis oculi reflexes," *Neurology*, vol. 22, no. 2, pp. 149–154, 1972.

[22] K. H. Kilburn, J. C. Thornton, and B. Hanscom, "Population-based prediction equations for neurobehavioral tests," *Archives of Environmental Health*, vol. 53, no. 4, pp. 257–263, 1998.

[23] P. Lanthony, "The desaturated panel D-15," *Documenta Ophthalmologica*, vol. 46, no. 1, pp. 185–189, 1978.

[24] K. J. Bowman, "A method for quantitative scoring of the Farnsworth Panel D-15," *Acta Ophthalmologica*, vol. 60, no. 6, pp. 907–916, 1982.

[25] D. R. Anderson, *Perimetry: With and Without Automation*, Mosby, St. Louis, Mo, USA, 2nd edition, 1987.

[26] D. Wechsler, "A standardized memory scale for clinical use," *Journal of Psychology*, vol. 9, pp. 87–95, 1945.

[27] R. B. Cattell, "Classical and standard score IQ standardization of the I.P.A.T: culture-free intelligence scale 2," *Journal of Consulting Psychology*, vol. 15, no. 2, pp. 154–159, 1951.

[28] R. B. Cattell, S. N. Feingold, and S. B. Sarason, "A culture-free intelligence test: II evaluation of cultural influence on test performance," *Journal of Educational Psychology*, vol. 32, no. 2, pp. 81–100, 1941.

[29] J. C. Raven, J. H. Court, and J. Raven, *Standard Progressive Matrices*, Oxford Psychologists, London, UK, 1988.

[30] D. N. Jackson, *Multidimensional Aptitude Battery*, Sigma Assessments Systems, Inc., Port Huron, Mich, USA, 1985.

[31] D. Wechsler, *Adult Intelligence Scale Manual—Revised*, The Psychological Corporation, New York, NY, USA, 1997.

[32] C. M. Ryan, L. A. Morrow, E. J. Bromet, and D. K. Parkinson, "Assessment of neuropsychological dysfunction in the workplace: normative data from the Pittsburgh occupational exposures test battery," *Journal of Clinical and Experimental Neuropsychology*, vol. 9, no. 6, pp. 665–679, 1987.

[33] R. M. Reitan, "A research program on the psychological effects of brain lesions in human beings," in *International Review of Research in Mental Retardation*, N. R. Ellis, Ed., pp. 153–216, Academic Press, New York, NY, USA, 1966.

[34] R. M. Reitan, "Validity of the trail-making test as an indicator of organic brain damage," *Percept Motor Skills*, vol. 8, pp. 271–276, 1958.

[35] "Educational and industrial testing service," *Profile of Mood States*, San Diego, Calif, (1971/1981).

[36] ATS Statement, "Standardization of spirometry—1987 update," *The American Review of Respiratory Disease*, vol. 136, pp. 1285–1298, 1987.

[37] A. Miller, J. C. Thornton, and R. Warshaw, "Mean and instantaneous expiratory flows, FVC and FEV1: prediction equations from a probability sample of Michigan, a large industrial state," *Clinical Respiratory Physiology*, vol. 22, no. 6, pp. 589–597, 1986.

[38] S. P. Wright, "Adjusted P-values for simultaneous inference," *Biometrics*, vol. 48, no. 4, pp. 1005–1013, 1992.

[39] K. H. Kilburn, "Do duration of exposure, proximity to electronic manufacturing plants, and involvement in a lawsuit affect chlorinated solvent toxicity?" *Archives of Environmental Health*, vol. 57, no. 2, pp. 121–126, 2002.

[40] K. H. Kilburn, "Is neurotoxicity associated with environmental trichloroethylene (TCE)?" *Archives of Environmental Health*, vol. 57, no. 2, pp. 113–120, 2002.

[41] J. E. Zejda, E. Barber, J. A. Dosman et al., "Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels," *Journal of Occupational Medicine*, vol. 36, no. 1, pp. 49–56, 1994.

[42] K. J. Donham, "Health effects from work in swine confinement buildings," *American Journal of Industrial Medicine*, vol. 17, no. 1, pp. 17–25, 1990.

[43] S. S. Schiffman, E. A. S. Miller, M. S. Suggs, and B. G. Graham, "The effect of environmental odors emanating from commercial swine operations on the mood of nearby residents," *Brain Research Bulletin*, vol. 37, no. 4, pp. 369–375, 1995.

[44] E. B. Smith, "Stench chokes Nebraska meatpacking towns," *USA Today*, February, 2000.

[45] J. Lee, "Neighbors of vast hog farms say foul air endangers their health," *The New York Times*, May, 2003.

[46] S. Wing and S. Wolf, "Intensive livestock operations, health, and quality of life among eastern North Carolina residents," *Environmental Health Perspectives*, vol. 108, no. 3, pp. 233–238, 2000.

[47] K. M. Thu, "Public health concerns for neighbors of large-scale swine production operations," *Journal of Agricultural Safety and Health*, vol. 8, no. 2, pp. 175–184, 2002.

[48] P. R. Lees-Haley and R. S. Brown, "Biases in perception and reporting following a perceived toxic exposure," *Perceptual and Motor Skills*, vol. 75, no. 2, pp. 531–544, 1992.

[49] R. D. Stewart, "The effect of carbon monoxide on humans," *Annual Review of Pharmacology*, vol. 15, pp. 409–423, 1975.

[50] N. L. Benowitz, "Nicotine addiction," *New England Journal of Medicine*, vol. 362, no. 24, pp. 2250–2303, 2010.

[51] W. -H. Piao, D. Campagnolo, C. Dayao, R. J. Lukas, J. Wu, and F. -D. Shi, "Nicotine and inflammatory neurological disorders," *Acta Pharmacologica Sinica*, vol. 30, no. 6, pp. 715–722, 2009.

[52] G. Ahlborg, "Hydrogen sulfide poisoning in shale oil industry," *Archives of Industrial Hygiene and Occupational Medicine*, vol. 3, no. 3, pp. 247–266, 1951.

[53] J. B. Dalgaard, F. Dencker, B. Fallentin et al., "Fatal poisoning and other health hazards connected with industrial fishing,"

British Journal of Industrial Medicine, vol. 29, no. 3, pp. 307–316, 1972.

[54] W. W. Burnett, E. G. King, M. Grace, and W. F. Hall, "Hydrogen sulfide poisoning: review of 5 years' experience," *Canadian Medical Association Journal*, vol. 117, no. 3, pp. 1277–1280, 1977.

[55] I. M. F. Arnold, R. M. Dufresne, B. C. Alleyne, and P. J. W. Stuart, "Health implication of occupational exposures to hydrogen sulfide," *Journal of Occupational Medicine*, vol. 27, no. 5, pp. 373–376, 1985.

[56] J. F. Deng and S. C. Chang, "Hydrogen sulfide poisonings in hot-spring reservoir cleaning: two case reports," *American Journal of Industrial Medicine*, vol. 11, no. 4, pp. 447–451, 1987.

[57] C. P. Chengelis and R. A. Neal, "Studies of carbonyl sulfide toxicity: metabolism by carbonic anhydrase," *Toxicology and Applied Pharmacology*, vol. 55, no. 1, pp. 198–202, 1980.

[58] M. W. Warenycia, K. A. Smith, C. S. Blashko, S. B. Kombian, and R. J. Reiffenstein, "Monoamine oxidase inhibition as a sequel of hydrogen sulfide intoxication: increases in brain catecholamine and 5-hydroxytryptamine levels," *Toxicology*, vol. 63, no. 2, pp. 131–136, 1989.

[59] R. Wang, "Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter?" *FASEB Journal*, vol. 16, no. 13, pp. 1792–1798, 2002.

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Clinical Study

Changes in Peak Flow Value during Immunotherapy Administration

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Nasal allergies are prevalent affecting a large percentage of the population. Not only the upper respiratory tract but the whole body is involved. Allergies produce morbidity (and even occasional mortality) as they can lead to asthma development, and increased number of accidents. Immunotherapy results can be evaluated by following symptom scores, medication use, and objective measurements. Using a Peak Flow Meter (PFM) to evaluate immunotherapy results, it became evident that patients with and without asthma exhibited an improvement in the Peak Flow (PF) value, suggesting that lower airway involvement in allergic patients could be more prevalent than assumed. A consecutive chart review was performed including patients of any age with nasal allergies (with or without asthma) treated with immunotherapy for at least 6 months that had at least 2 complete evaluations. When immunotherapy was successful, most patients exhibited an increase in the PF value regardless of asthma status. A very significant finding was that most allergy sufferers may have lower airway inflammation. The use of the PF value to assess immunotherapy results and the potential failure to diagnose asthma in allergy sufferers are discussed. A better diagnosis of lower airway inflammation could be substantial in the management of these patients.

1. Introduction

Nasal allergies are common and their prevalence in industrialized societies appears to be increasing. While nasal allergies were rarely diagnosed in the 19th century, their occurrence markedly increased during the 20th century. For example, studies show the incidence of nasal allergies in parts of the United States at 10% in 1974, 20% in 1986, and 42% in 1994 [1]. Similar figures of up to 40% have been reported in other parts of the world too [2]. In addition, similar increases have occurred in conditions not usually associated with allergies but with clear allergic etiology, such as eczema and asthma. These figures indicate an explosive increase. It is possible that an increasingly polluted environment is a causative factor [1].

Asthma is a common chronic disorder of the airways characterized by an underlying inflammation that leads to bronchial hyperresponsiveness and recurring airflow obstruction. In some cases patients develop persistent changes in airway structure, including fibrosis, smooth muscle hypertrophy, and angiogenesis. In the United States asthma affects more than 22 million people. It is one of the most common chronic diseases of childhood, affecting more than six million children in the USA [3].

The occurrence of asthma also has increased. Hospitalization rates are higher among young children. Collectively, individuals with asthma account for more than 497,000 hospitalizations annually. The onset of asthma for most patients begins early in life with the pattern of disease persistence. Recognizable risk factors include atopic disease. Current asthma treatment with anti-inflammatory therapy does not appear to prevent progression of the underlying disease severity [3].

The allergic disease affects the whole body. Fatigue is one of the most common complaints of the allergy sufferer, [4] and nasal congestion is recorded as the most bothersome symptom of allergic rhinitis [5]. Nasal obstruction is often the most severe symptom in patients with nasal allergies and it can lead to the onset or worsening of obstructive sleep apnea (OSA) [6].

Sleep-related symptoms are extremely common in patients with allergic rhinitis. Sleep impairment associated

with allergic rhinitis is likely a major contributor to the overall disease morbidity, health care costs, and loss of work productivity [7].

Individuals with OSA are at an increased risk for motor vehicle accidents [8] and lack of sleep has been implicated in job related accidents as well. [9] In addition, there are patients where the influence of nasal obstruction in sleep disordered breathing is critical [10]. Nasal obstruction also is a contributing factor to development of dentofacial abnormalities in the developing child [11].

So it is clear that having allergies means dealing with more than just nasal congestion, rhinorrhea, and itchy eyes. Not only can quality of life be significantly impaired for those with allergies, but also this condition can be potentially life threatening given that a patient with allergies is susceptible to fatigue, sleep deprivation, a higher incidence of accidents, and lower airway inflammation with bronchoconstriction.

While medical management of nasal allergies will control symptoms in the best of circumstances, immunotherapy is the only treatment modality available that can potentially cure the allergic condition [12, 13]. Immunotherapy works by modifying the immunological response of the allergic individual through stimulating the production of IgG (usually known as a "blocking antibody") and eliciting more complex changes in the activity of the T-cells [14], where T-cell tolerance is attained mainly by generation of allergen-specific Treg cells leading to suppressed T-cell proliferation and Th1 and Th2 cytokine responses against the allergen. This is accompanied by a significant increase in allergen-specific IgG_4, IgG_1, and IgA and a decrease in IgE in the late stage of the disease [13].

In the clinical setting immunotherapy treatment is usually evaluated by following changes in symptoms scores. Specifically, the patient rates his/her symptoms usually using a numerical scale, and the change of this value over time is followed to assess improvement or lack thereof. Even though this is a subjective tool, different symptoms scores have been scientifically validated as useful [15–17]. Some of the objective measurements of symptomatic improvement include use of acoustic rhinomanometry [18].

In our office we use a symptom questionnaire (see Figure 1) based on the scoring method followed by Fell [17]. We added objective measurements including nasal resistance determined by acoustic rhinomanometry and determination of PF value. While acoustic rhinomanometry requires significant training of the technician, it is time consuming, and yields results that are difficult to interpret, using a PFM device requires minimal training and yields easy-to-interpret results in seconds. It became clear that determining the PF value was a simpler procedure that could be easily incorporated in a private practice clinical setting. Soon after the use of the PFM was incorporated in our practice, it was observed that patients with allergies exhibited an improvement in PF value if immunotherapy was successful (as measured by a decrease in symptom scores and medication use) and that such improvement occurred not only in the patients with asthma but also in the allergy sufferer without any asthma symptoms.

Table 1: Symptoms considered for scoring.

Sneezing
Runny nose
Nasal obstruction
Post nasal drip
Watery eyes
Itchy eyes
Itchy ears
Itchy nose
Itchy throat
Itchy skin
Clogged ears
Facial pain/pressure
Headaches
Cough
Sensation of tight chest
Wheezing
Shortness of breath
Exercise-induced SOB
Exercise-induced cough
Exercise-induced wheezing
Waking up with symptoms

To verify this hypothesis, we collected the data presented herein, with the understanding that this is only an observational study that lacks the rigor of a prospective randomized study with a control group.

2. Methods

A consecutive chart review was performed. Inclusion criteria for the study were patients of any age with nasal allergies (with or without asthma) treated with immunotherapy for at least six months that had at least two complete evaluations. A complete evaluation included symptom scoring, evaluation of medication use, and determination of PF value.

Ethical Considerations. Subjects' privacy was assured by the way data was collected and recorded so that subjects could not be directly or indirectly identified. In other words, a patient's privacy was protected by entering data for statistical analysis in a simple spreadsheet with nonspecific identifiers, such as patient number 1 and patient number 2, with subsequent refiling of the patient's chart according to usual procedure.

In our practice we follow Fell's method [17] for symptom scoring, which classifies each symptom with a numerical analog from 0 through 3 as follows:

0: symptom is not present,

1: symptom is mild,

2: symptom is moderate,

3: symptom is severe.

There were 21 symptoms considered for this evaluation (see Table 1). The symptom score was obtained by adding the

Diego saporta MD. Allergy symptom assessment Starting date:

Name: DOB AGE SEX

Symptoms	PRE-RX								
Date SLIT/SCIT									
Sneezing									
Runny nose									
Nasal obstruction									
Post nasal drip									
Watery eyes									
Itchy eyes									
Itchy ears									
Itchy nose									
Itchy throat									
Itchy skin									
Hives-rash									
Sorethroat									
Clogged ears									
Facial pain/pressure									
Headaches intensity									
*Headaches frequency									
Dizziness									
Tinnitus (noise in ears)									
Diag ASTHMA Y N									
Cough									
Sensation of tight chest									
Wheezing									
SOB									
Exercise-cough									
Exercise-SOB									
Exercise-wheezing									
*Wake at night w/symptom									
*Bronchodilators(Pro/Max Albut/Xopen/Prove/Vent/Alup)									
Last time used									
*ICS (advair//pulmic/asmanex)									
*Allergy pills									
*Intranasal sprays									
Fatigue									
Poor sleep									
Snoring									
Difficulty concentrating									
Problems/complications									
FEV 1									
PFM									
SNR									

Symptoms:
0: Never,
1: Mild,
2: Moderate,
3: Severe;

Frequency (*):
0: Never,
1: 1 time/wk (or less),
2: 2-3 times per week,
3: 4 or more per week.

Figure 1

values of each symptom (0–3) in each patient; the maximum total symptom score was 63. The total number of symptoms also was considered for monitoring clinical response.

When considering clinical response to immunotherapy, the use of medications is monitored as well. Patients that do not exhibit a decrease in medication use with the administration of immunotherapy are reassessed. For this evaluation we considered use of allergy pills (antihistamines or leukotriene receptor antagonists), intranasal topical steroids, and short acting bronchoagonists. No patient in this study was on oral or systemic steroids or decongestants.

For evaluation of medication use we use a similar numerical scale as follows:

0: medication is not being used,

1: medication is being used once a week or less,

2: medication is being used 2 to 3 times per week,

3: medication is being used 4 or more times per week.

The maximum total medication score was 9 and the maximum total number of medications was 3. When immunotherapy is successful, medication use will decrease regardless of the type of medication. In the asthmatic patient, asthma inhalers will be used less frequently or even discontinued entirely in the more successful outcomes.

The value of the PFM determination is used as the parameter to be recorded during each patient's visit.

The sample included adults and children with or without asthma treated with subcutaneous injection immunotherapy (SCIT) or sublingual immunotherapy (SLIT). Antigens were mixed according to results of an intradermal dilutional test. The data presented here was not analyzed according to test results. Patients on SCIT were treated according to American Academy of Otolaryngic Allergy (AAOA) guidelines [19]. Patients on SLIT were treated according to a previously published protocol [20]. The formulation of both injectable and oral allergy-vaccines was the same; based on allergy test results, all positive allergens were included in the treatment mixture.

Asthma symptoms considered included cough, sensation of tight chest, wheezing, shortness of breath (SOB), exercise-induced symptoms (cough, SOB, wheezing), or waking up at night with any of those symptoms. Presence of asthma symptoms in patients who considered themselves "nonasthmatic" was also analyzed.

Sixty charts that met inclusion conditions were identified. They were analyzed using the same criteria regardless if patients were treated with SCIT or SLIT. For each patient we evaluated total symptoms score, total number of symptoms, medication score, total number of medications used, and the PF value, which was obtained before treatment initiation and at least once more at the time of data collection.

The changes of the above parameters during the administration of immunotherapy were evaluated. In determining the results between two or more groups of patients, an ANOVA (Analysis of Variance) was performed using the PF performance as the dependent variable. When determining significance between continuous variables, a bivariate correlation analysis was performed.

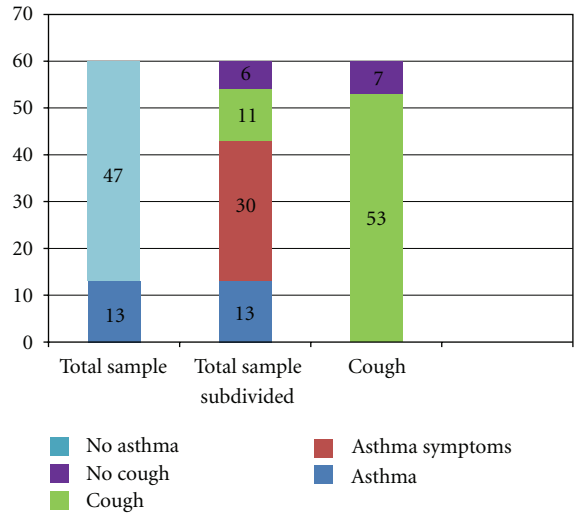

Figure 2: Total sample and subgroups. *Total sample*: 60 subjects, 13 report asthma, 47 do not. *Total sample subdivided*: 13 report asthma, Remainder 47: 30 have cough and other asthma symptoms, 11 have cough only, and 6 have no cough or other asthma symptoms. *Cough*: 53/60 subjects had cough.

3. Results

3.1. Demographics. The total sample included 60 subjects, ages 4 through 75 (mean 41.1 ± 17.5). Mean length of treatment was 22.9 ± 13.1 months. In 42 of 60 patients (70%), treatment extended for more than 12 months.

3.2. Asthma. A diagnosis of asthma was self-reported by 13 subjects (21% of the total sample), and only 1 of these subjects did not report cough as one of the symptoms. The remaining 47 subjects denied having asthma, although 30 reported asthma symptoms (30/47 = 63.8%) (see Figure 2).

The group of 47 patients that did not report asthma were divided into three subgroups (see Figure 2):

(a) patients with cough and other asthma symptoms: 30,

(b) patients with cough but no other asthma symptoms: 11,

(c) patients without cough (and no other asthma symptoms): 6.

3.3. Cough. Cough was reported by 53 out of 60 patients in this sample (88%). Eleven out of these 53 (subgroup b) had no other symptoms suggestive of lower airway inflammation. While it can be assumed that a patient with cough and other symptoms of lower airway inflammation has asthma, a patient with cough and no other symptoms is a different situation. Even though it is proven that cough can be the only symptom a patient with asthma may present, no further conclusions can be made without information on spirometric results or response to bronchodilator and/or anti-inflammatory therapy (see Figure 2).

Because all the information in this report comes from the symptom scoring sheet and not from office notes, we

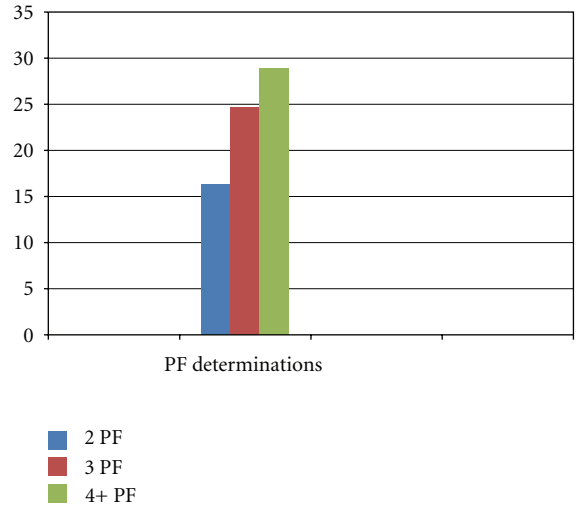

Figure 3: Percentage of PF value change in relation to the number of PF determinations. 2PF: two PF determinations. 3PF: three PF determinations. 4+PF: four or more PF determinations. The number of PF measurements is positively associated with the percentage of PF change ($r = 0.357$, $P < 0.01$).

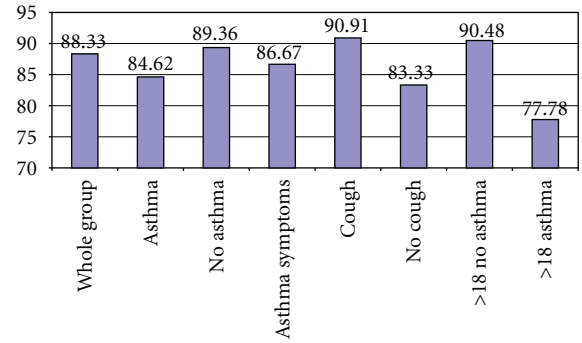

Figure 4: Number of subjects that had improved PF values in different groups. Whole group: 53/60; Asthma: Self-reported asthma patients: 11/13; No asthma: Patients that denied asthma: 42/47; Asthma Symptoms: Patients that denied asthma but had asthma symptoms: 26/30; Cough: Patients that denied asthma and had cough but no other asthma symptoms: 10/11; No cough: Patients that denied asthma and had NO cough and NO other asthma symptoms: 5/6. ANOVA showed no significant difference in average percentage of PF change of the aforementioned groups ($F = 0.975$, $P = N/S$). >18 No Asthma: Patients older than 18 years of age that did not report asthma: 38/42. >18 Asthma: Patients older than 18 years of age that reported asthma: 7/9.

decided to consider the group "cough but no other asthma symptoms" as a separate group (see Discussion).

Adding the number of patients with asthma to the number of patients with cough and other asthma symptoms (13 + 30 = 43) suggests that 72% (43/60 = 71.6%) of the patients in a group of nonselected allergy patients are indeed asthmatic, and this is, at best, a conservative number as some of patients with "cough only" could also be asthmatic.

3.4. PF Changes. Average PF change for all 60 patients during immunotherapy increased from 376.23 (±115.01) at the beginning of treatment to 472.65 (±127.47) at the time of data collection for an overall improvement of 25.63%. From the total sample, 53 out of the 60 subjects (88.33%) showed an improvement in PF value, 4 had a worsening of PF value, and 3 had no change. Of the 53 subjects that showed an improvement in PF value, 48 had a decrease in symptoms score and number of symptoms. Therefore a PF increase has a predictor value of 90.57% for clinical improvement of the patient.

While an increase in the PF value is strongly associated with a symptomatic improvement (48/53), a decrease in the PF value is not necessarily associated with clinical worsening: of the 4 subjects with a worse PF value, all still had a decreased symptom score and 3 had a decrease in the number of symptoms. Of the 3 subjects with no change in the PF value, 2 got better (decrease in symptoms scores and number of symptoms) and 1 got worse.

3.5. PF Changes in Relation to the Number of Measurements. In 11 patients there were 2 PF value measurements, one at the beginning of the treatment and the second one at the time of data collection (see Figure 3). Average PF change during immunotherapy for these patients ranged from 390.00 (±115.84) to 453.55 (±161.36), for an overall improvement of 16.29%.

In 13 patients there were 3 PF value measurements. Average PF change during immunotherapy for these patients ranged from 368.46 (±109.76) to 459.23 (±103.23), for an overall improvement of 24.63%.

In 36 patients there were 4 or more PF value measurements. Average PF change during immunotherapy for these patients spanned from 374.83 (±119.37) to 483.33 (±126.49), for an overall improvement of 28.95%.

A bivariate correlation analysis showed that the number of PF measurements is positively associated with the percentage of PF change ($r = 0.357$, $P < 0.01$). Therefore when more PF measurements are obtained, it is more likely to obtain a greater improvement in the PF value.

3.6. PF Value in Relation to Length of Treatment. There is a positive correlation between the change in the PF value and the number of months that the patient was treated ($r = 0.253$, $P < 0.05$). In other words, the longer a patient receives immunotherapy the more likely the PF value will increase.

3.7. PF Changes in Different Groups. PF changes were evaluated in patients that reported asthma and in the subgroups of the patients that did not report asthma (see Figure 4).

Patients That Reported Asthma. In the 13 patients who self-reported having asthma, the average PF value increased from 315.69 (±124.85) to 385.38 (±85.40), for an overall improvement of 18.08%. Eleven out of the 13 patients had an improvement in the PF value (84.62%). Two got worse (15.38%).

Patients That Did Not Report Asthma. In the group of 47 patients that did not report asthma, 42 (89.36%) had an improvement in the PF value, 3 did not improve (6.38%), and 2 decreased (4.26%). This group of 47 patients can be divided in 3 subgroups.

(a) For the thirty patients that did not report asthma but had asthma symptoms, the average PF value increased from 398.33 (±114.11) to 492.30 (±139.40), for an overall improvement of 23.59%. Twenty-six out of the 30 patients had an improvement in the PF value (86.67%). Three patients did not show any improvement (10.00%), and 1 got worse (3.33%).

(b) For the eleven patients that had cough but no other symptoms suggestive of lower airway inflammation, the average PF value increased from 376.36 (±109.39) to 510.91 (±105.49), for an overall improvement of 35.75%. Ten out of the 11 patients had an improvement in the PF value (90.91%). In 1 patient the PF value decreased (9.09%).

(c) For the six patients that had no cough or any other symptom suggestive of lower airway inflammation, the average PF value increased from 396.67 (±79.16) to 493.33 (±115.87) for an overall improvement of 24.37%. Five out of 6 patients had an improvement in the PF value (83.33%). In 1 patient the PF value decreased (16.67%).

An ANOVA showed that there is no significant difference among the average percentage of PF change of the aforementioned groups ($F = 0.975$, $P = N/S$).

3.8. Age. Because children can grow during a multiyear treatment (e.g., children between 3 and 13 years of age grow approximately 2 inches per year, and 2 inches can determine an increase of 15 to 25 points in PF value depending on the patient's sex), the same calculations were done excluding patients younger than 18 years of age. There were 51 patients older than 18 years of age.

The average PF value in these 51 patients (see Figure 4) changed from 395.76 (±110.01) to 495.49 (±120.97), for an overall improvement of 25.20%. In this group there were 9 patients (17.65%) that reported asthma and 42 patients (82.35%) that did not report asthma.

In the 42 patients that did not report asthma the PF value improved from 404.29 (±106.84) to 515.48 (±118.02), for an overall improvement of 27.50%. Thirty-eight out of the 42 showed an improvement in PF value (90.48%). The PF value did not change in 2 patients (4.76%) and decreased in 2 others (4.76%).

In the 9 patients that reported asthma, the PF value improved from 356.00 (±122.38) to 402.22 (±90.52), for an overall improvement of 12.98%. In 7 out of the 9 patients the PF value showed an improvement (77.78%). The PF value decreased in 2 (22.22%).

3.9. Pattern of PF Change. It was observed that patients could be divided into 2 groups according to the way the PF value changed during the administration of immunotherapy: in the first group the PF determination increased each time it was obtained, and in the second group the PF value fluctuated during the course of immunotherapy even though the value at the time of data collection was generally higher than the initial value.

Thirty-six patients (36/60: 60%) belonged to the first group (sustained improvement), and twenty-one patients (21/60: 35%) belonged to the second group (PF value cycled up and down). Three patients did not have any changes in the PF value (3/60: 5%).

In the first group 35 out of 36 patients (97%) had a decrease in the symptoms score, and 34 out of 36 patients (94%) had a decrease in the number of symptoms (coincident with an increase in the PF value).

For patients that consistently improved (35 of 60), the difference in symptom scores for those that had 5 or more PF value measurements was statistically higher than that for those that had fewer than 5 determinations ($F = 5.02$, $P < 0.05$). Number of symptoms also exhibited a similar pattern when comparing the same 2 groups ($F = 6.42$, $P < 0.05$).

4. Discussion

Patients with symptoms suggestive of lower airway inflammation may not consider themselves as asthmatic, but it is likely they are. A patient that only exhibits cough could be asthmatic, but unless an improvement in spirometric values after administration of bronchodilators is demonstrated or symptom improvement occurs after administration of asthma drugs, it is not possible to establish that patient as asthmatic.

Our sample consisted of 60 patients with allergies that were not screened for the presence of asthma. Of those patients, 13 self-reported having asthma and 30 reported experiencing asthma symptoms but did not report asthma, meaning that an impressive 71.67% of our sample potentially could be asthmatic. If the patients with cough but no other asthma symptoms were also considered, then this percentage could be even higher. If this finding is extrapolated to all allergy sufferers, it can then be assumed that the majority of allergy sufferers consulting an allergy practice might be asthmatic. It is clear that asking a patient if he/she is asthmatic is not sufficient; rather the presence of each symptom of asthma needs to be addressed. Patients with asthma are often at higher risk for severe reactions not only during the administration of immunotherapy [21, 22] but also during testing, and these patients should be considered more sensitive [23]. Fatalities from immunotherapy, although rare, are more common in asthmatics [24, 25]. Given all this, it is important to establish if an allergy patient is asthmatic or not, as a diagnosis of asthma affects quality of life as well as morbidity, and it can also potentially impact the life expectancy of that patient.

There is a tendency to consider asthma and allergic rhinitis (AR) as two separate entities, but there is strong evidence that this is not so. The term rhinobronchitis has been proposed to help recognize the concept of chronic

inflammation throughout the entire airway in the patient with concurrent allergic rhinitis and asthma [26]. Up to 19% of hay fever sufferers develop asthma later in life [27]. Nasal challenge with environmental stimuli (such as cold air) leads to bronchoconstriction [26, 28]. Some AR patients with no perceived asthma develop bronchial hyperreactivity during AR exacerbation [26]. All this data supports the concept that the upper and lower airways are a unique entity impacted by a common, evolving inflammatory process. Therefore from an immunotherapeutic point of view, AR and asthma should be considered a single entity [29], and the results reported here support the concept of the Unified Airway [30]. If the allergic reaction influences the whole body, it is only logical that the bronchi will be involved in the widespread inflammation that affects the allergy sufferer.

There were 6 patients in the sample of 60 that were definitely not asthmatic. In these patients the average PF value improved 24.37%, and the PF value increased in 5 of 6 cases (83.33%). Both of these figures are in range with the other groups, which supports the idea that even nonasthmatic patients with nasal allergies still have lower airway inflammation. This explains why, even when a patient reports no asthma symptoms, the pulmonary function as assessed by the PF value improves with treatment. With this in mind, nasal allergies and asthma should be considered a continuum of the same disease that expresses itself differently in each patient; for certain individuals this means that the nose and the eyes will be more affected, and for others it means that the lower respiratory system will be more substantially impacted. However, it is a disease that at all times, even if to a minimal degree, probably involves all the organ systems.

This concept is difficult for patients to accept, and we find that when patients with hyperreactive airway are told they have asthma, the usual response is to deny the possibility. Still this is an important concept when treating patients with immunotherapy, since, as mentioned earlier, asthmatic patients are more likely to experience reactions [21, 22].

These results should contribute to raising awareness that potentially any patient presenting with allergy symptoms could also have lower airway hyperreactivity. As previously stated, the figure of 72% is possibly a conservative estimate; and if patients that had cough but no other asthma symptoms were considered as patients with airway hyperreactivity, the number could be even higher. In other words, in a sample of 60 nonselected allergy patients, it is possible that up to 90% ([13 + 30 + 11]/60) were asthmatic.

Having any symptoms suggestive of lower airway inflammation (and cough is one that is very frequently found) should lead to a work up to rule out the presence of a hyperreactive lower airway. We feel that if this was the case, the frequency of asthma-related diagnoses would markedly increase.

The fact that when more PF measurements are obtained the PF value will be higher could be related to the finding that the longer the number of months the patient is treated with immunotherapy the higher the PF value will be. This suggests that the longer the treatment, the better the results, regardless of the presence of asthma symptoms and age of the patient. In other words it appears that immunotherapy, when successful, leads to improvement of pulmonary function in all patients with allergies.

Immunotherapy is a treatment that can modify the immunological mechanisms that cause allergy symptoms [12]. It is an old treatment modality [31] that is proven to be effective [32–34]. With immunotherapy, it is expected that medication use will decrease, regardless of when the medication was started and which medication was used. When the treatment is fully successful, medications can be stopped. We only analyzed medication use, as the purpose of this study was not to address potential differences in effects of various medications, but to demonstrate that with immunotherapy medication use diminishes.

This is a retrospective study and, as such, it lacks the value of a prospective, randomized study with controls, which is very difficult to perform in private practice settings. The information reported in this study suggests that a PFM device could be useful in monitoring a patient's progress during immunotherapy, as changes in PF value can predict how a patient is doing from a clinical standpoint. The information it provides is available immediately, offering a quick assessment of patient's progress.

When PF value consistently improves, the predictor value of the PF change is very high. However, in a few cases these findings are relative: in some cases we observed that with an unchanged or decreased PF value, a patient can still experience some improvement (decreased symptom/medication score). We also found that with a better PF value, the patient can have clinical worsening (increased symptom/medication score). Overall, it appears that following a patient's PF value during immunotherapy is a good indicator of how he/she is responding to the treatment: a decrease in PF value can be used to predict that a patient is not doing well, and an increase in PF value can be used to predict that a patient is doing well, particularly if the increase in PF value is sustained over time.

We hope that this information will serve as a stimulus for authors in research facilities to plan a randomized prospective study with a control group where the usefulness of a PFM can be more properly evaluated.

5. Conclusion

Patients with nasal allergies that deny having asthma often have asthma symptoms. In our study, this occurred in at least 72% of the patients that denied having asthma. Therefore when taking a patient's history, it is critical to ask about the presence of each symptom of asthma, regardless of the patient's perception of asthmatic status.

If it is accepted that the allergic condition affects the whole organism, then it is only logical that the lower airway of an allergy sufferer will be involved. While the degree of involvement varies according to the individual, it appears from this data that the proportion of individuals with affectation of the lower airway is staggering. Perhaps then it is time to reassess the definition of asthma. A "looser" term such as lower airway inflammation or airway hyperreactivity would be more inclusive of all patients with asthma

symptoms. Even the term rhinobronchitis [26] should be considered, as patients often reject the label of asthmatic. Regardless, it appears that lower airway inflammation is common and treatment of this inflammation either by anti-inflammatory inhaled corticosteroids, immunotherapy, or detoxifying interventions [35] could not only treat the present condition but perhaps, more importantly, prevent the development of irreversible lower airway remodeling.

Change in PF value can give a rapid assessment of how a patient is responding to treatment. When PF value increases, it is likely that a patient will improve in the majority of cases. In cases where the PF value is better in each determination, the PF has a 94% to 97% predictor value that the patient is doing well.

When PF value improves during the administration of immunotherapy, these changes are independent of asthmatic condition, the presence of any or no asthma symptoms, or the patient's age. Only length of treatment and number of PF measurements are related to an improved PF value.

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References

[1] J. H. Krouse, "Seasonal and perennial rhinitis," in *Allergy and Immunology. An Otolaryngic Approach*, Williams & Wilkins, Philadelphia, Pa, USA, 2002.

[2] C. E. Baena-Cagnani and P. Patel, "Efficacy and long-term safety of mometasone furoate nasal spray in children with perennial allergic rhinitis," *Current Medical Research and Opinion*, vol. 26, no. 9, pp. 2047–2055, 2010.

[3] "Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma," http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.pdf, Full Report 2007.

[4] E. Boyd, "Patient history," in *Allergy and Immunology. An Otolaryngic Approach*, Williams & Wilkins, Philadelphia, Pa, USA, 2002.

[5] D. E. Stull, E. O. Meltzer, J. H. Krouse et al., "The congestion quantifier five-item test for nasal congestion: refinement of the congestion quantifier seven-item test," *American Journal of Rhinology and Allergy*, vol. 24, no. 1, pp. 34–38, 2010.

[6] J. P. Corey, S. M. Houser, and B. A. Ng, "Nasal congestion: a review of its etiology, evaluation, and treatment," *Ear, Nose and Throat Journal*, vol. 79, no. 9, pp. 690–693, 2000.

[7] R. J. Soose, "Role of allergy in sleep-disordered breathing," *Otolaryngologic Clinics of North America*, vol. 44, no. 3, pp. 625–635, 2011.

[8] S. Tregear, J. Reston, K. Schoelles, and B. Phillips, "Obstructive sleep apnea and risk of motor vehicle crash: systematic review and meta-analysis," *Journal of Clinical Sleep Medicine*, vol. 5, no. 6, pp. 573–581, 2009.

[9] G. C. Gauchard, J. M. Mur, C. Touron et al., "Determinants of accident proneness: a case-control study in railway workers," *Occupational Medicine*, vol. 56, no. 3, pp. 187–190, 2006.

[10] T. Verse and W. Pirsig, "Impact of impaired nasal breathing on sleep-disordered breathing," *Sleep and Breathing*, vol. 7, no. 2, pp. 63–76, 2003.

[11] J. C. Posnick and N. Agnihotri, "Consequences and management of nasal airway obstruction in the dentofacial deformity patient," *Current Opinion in Otolaryngology and Head and Neck Surgery*, vol. 18, no. 4, pp. 323–331, 2010.

[12] H. S. Nelson, "Allergen immunotherapy: where is it now?" *Journal of Allergy and Clinical Immunology*, vol. 119, no. 4, pp. 769–777, 2007.

[13] M. Akdis and C. A. Akdis, "Mechanisms of allergen-specific immunotherapy," *Journal of Allergy and Clinical Immunology*, vol. 119, no. 4, pp. 780–789, 2007.

[14] R. C. Haydon and B. R. Gordon, "Aeroallergen immunotherapy," in *Allergy and Immunology. An Otolaryngic Approach*, Williams & Wilkins, Philadelphia, Pa, USA, 2002.

[15] E. F. Juniper, G. H. Guyatt, R. S. Epstein, P. J. Ferrie, R. Jaeschke, and T. K. Hiller, "Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials," *Thorax*, vol. 47, no. 2, pp. 76–83, 1992.

[16] M. A. Pynnonen, H. M. Kim, and J. E. Terrell, "Validation of the sino-nasal outcome test 20 (SNOT-20) domains in nonsurgical patients," *American Journal of Rhinology and Allergy*, vol. 23, no. 1, pp. 40–45, 2009.

[17] W. R. Fell, R. L. Mabry, and C. S. Mabry, "Quality of life analysis of patients undergoing immunotherapy for allergic rhinitis," *Ear, Nose and Throat Journal*, vol. 76, no. 8, pp. 528–536, 1997.

[18] G. Ciprandi, C. Klersy, I. Cirillo, and G. L. Marseglia, "Quality of life in allergic rhinitis: relationship with clinical, immunological, and functional aspects," *Clinical and Experimental Allergy*, vol. 37, no. 10, pp. 1528–1535, 2007.

[19] R. C. Haydon and B. R. Gordon, "Aeroallergen immunotherapy," in *Allergy and Immunology, an Otolaryngic Approach*, J. H. Krouse, S. J. Chadwick, B. R. Gordon, and M. J. Derebery, Eds., pp. 151–182, Williams & Wilkins, Philadelphia, Pa, USA, 2002.

[20] D. Saporta and A. B. McDaniel, "Efficacy comparison of multiple-antigen subcutaneous injection immunotherapy and multiple-antigen sublingual immunotherapy," *Ear, Nose and Throat Journal*, vol. 86, no. 8, pp. 493–497, 2007.

[21] W. E. Davis, P. R. Cook, M. S. McKinsey, and J. R. Templer, "Anaphylaxis in immunotherapy," *Otolaryngology: Head and Neck Surgery*, vol. 107, no. 1, pp. 78–83, 1992.

[22] A. T. Borchers, C. L. Keen, and M. E. Gershwin, "Fatalities following allergen immunotherapy," *Clinical Reviews in Allergy and Immunology*, vol. 27, no. 2, pp. 147–158, 2004.

[23] B. R. Gordon, "Prevention and management of office allergy emergencies," *Otolaryngologic Clinics of North America*, vol. 25, no. 1, pp. 119–134, 1992.

[24] R. F. Lockey, L. M. Benedict, P. C. Turkeltaub, and S. C. Bukantz, "Fatalities from immunotherapy (IT) and skin testing (ST)," *Journal of Allergy and Clinical Immunology*, vol. 79, no. 4, pp. 660–677, 1987.

[25] D. I. Bernstein, M. Wanner, L. Borish, G. M. Liss, and Immunotherapy Committee, American Academy of Allergy, Asthma and Immunology, "Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990–2001," *Journal of Allergy and Clinical Immunology*, vol. 113, no. 6, pp. 1129–1136, 2004.

[26] F. E. R. Simons, "Allergic rhinobronchitis: the asthma-allergic rhinitis link," *Journal of Allergy and Clinical Immunology*, vol. 104, no. 3 II, pp. 534–540, 1999.

[27] O. Linna, J. Kokkonen, and M. Lukin, "A 10-year prognosis for childhood allergic rhinitis," *Acta Paediatrica*, vol. 81, no. 2, pp. 100–102, 1992.

[28] S. S. Braman, A. A. Barrows, and B. A. DeCotiis, "Airway hyperresponsiveness in allergic rhinitis. A risk factor for asthma," *Chest*, vol. 91, no. 5, pp. 671–674, 1987.

[29] J. Bousquet, P. Demoly, and F. B. Michel, "Specific immunotherapy in rhinitis and asthma," *Annals of Allergy, Asthma and Immunology*, vol. 87, no. 1, pp. 38–42, 2001.

[30] J. H. Krouse, R. W. Brown, S. M. Fineman et al., "Asthma and the unified airway," *Otolaryngology: Head and Neck Surgery*, vol. 136, no. 5, pp. S75–S106, 2007.

[31] C. S. Smith, "Allergen immunotherapy practice parameter update," *The Journal of the Kentucky Medical Association*, vol. 102, no. 2, pp. 53–56, 2004.

[32] L. Cox, "Allergen immunotherapy and asthma: efficacy, safety, and other considerations," *Allergy and Asthma Proceedings*, vol. 29, no. 6, pp. 580–589, 2008.

[33] Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology, "Allergen immunotherapy: a practice parameter second update," *Journal of Allergy and Clinical Immunology*, vol. 120, supplement 3, pp. S25–S85, 2007.

[34] J. L. Broek, J. Bousquet, C. E. Baena-Cagnani et al., "Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision," *Journal of Allergy and Clinical Immunology*, vol. 126, no. 3, pp. 466–476, 2010.

[35] S. J. Genuis, "Sensitivity-related illness: the escalating pandemic of allergy, food intolerance and chemical sensitivity," *Science of the Total Environment*, vol. 408, no. 24, pp. 6047–6061, 2010.

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Review Article

Environmental Determinants of Chronic Disease and Medical Approaches: Recognition, Avoidance, Supportive Therapy, and Detoxification

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The World Health Organization warns that chronic, noncommunicable diseases are rapidly becoming epidemic worldwide. Escalating rates of neurocognitive, metabolic, autoimmune and cardiovascular diseases cannot be ascribed only to genetics, lifestyle, and nutrition; early life and ongoing exposures, and bioaccumulated toxicants may also cause chronic disease. Contributors to ill health are summarized from multiple perspectives—biological effects of classes of toxicants, mechanisms of toxicity, and a synthesis of toxic contributors to major diseases. Healthcare practitioners have wide-ranging roles in addressing environmental factors in policy and public health and clinical practice. Public health initiatives include risk recognition and chemical assessment then exposure reduction, remediation, monitoring, and avoidance. The complex web of disease and environmental contributors is amenable to some straightforward clinical approaches addressing multiple toxicants. Widely applicable strategies include nutrition and supplements to counter toxic effects and to support metabolism; as well as exercise and sweating, and possibly medication to enhance excretion. Addressing environmental health and contributors to chronic disease has broad implications for society, with large potential benefits from improved health and productivity.

1. Introduction

Common chronic conditions include cardiovascular and cerebrovascular disease, cancer, diabetes, metabolic syndrome, and obesity, neurocognitive disorders, and immune dysfunction such as autoimmune disease. These are leading causes of morbidity and mortality in developed countries, and are increasingly prevalent in developing nations [1–3].

While average life spans lengthened through recent history, rising rates of noncommunicable chronic diseases in younger people mean that escalating numbers are spending an increasing proportion of their life coping with sickness, rather than enjoying health [4]. Indeed, chronic diseases associated with obesity may even turn the tide of improvements in average lifespans [5], previously gained from diverse advancements in public health and medicine such as in maternal and neonatal care and improvements in management of infectious diseases, trauma, and cardiovascular events.

Chronic disease is crippling some economies as countries struggle to develop [3] in the face of rising healthcare costs, pervasive individual suffering, beleaguered families caring for afflicted loved ones, and truncated opportunities as workers fall ill during what should be their most productive years.

It was recently conservatively estimated that costs in the United States of environmental disease in children alone amounted to a staggering $76.6 billion in 2008, just from "lead poisoning, prenatal methylmercury exposure, childhood cancer, asthma, intellectual disability, autism, and attention deficit hyperactivity disorder" [6]. The wide-spread implications are vividly illustrated by considering neurocognitive disorders, with a small IQ decrement across society. As abilities and intellect are reduced among the best and brightest, we lose potential leaders and innovators, while

simultaneously costs mount for continuing care needed by larger numbers at the bottom end of the IQ and abilities spectrum [7].

Searching for reasons for increased chronic diseases in the young, their ailments cannot be ascribed to reduced mortality from infectious disease. Similarly, genetics may predispose individuals to chronic disease, but this cannot account for rapidly increasing prevalence within a generation or two. This leaves us with pervasive environmental factors [1, 3]. While research centers and international health organizations devote considerable time and attention to the issue of toxicant exposure and bioaccumulation of xenobiotics within the human body [8], direct connections to prevalent chronic disease are rarely made, and initiatives to tackle chronic disease may not even mention environmental or occupational exposures to toxicants, as was the case for a 2010 report prepared for the World Health Organization, "Tackling Chronic Disease in Europe" [9].

Other prominent authorities such as the U.S. President's Cancer Panel decry that chemical assessment, regulation, and enforcement are woefully inadequate to protect public health and that environmental and occupational exposures are rarely suspected or queried in the differential diagnosis of disease [10].

In this paper we provide a brief overview of toxicants and associated mechanisms that may be contributing to chronic disease, discuss how links between toxic exposures and adverse outcomes are explored and regulated, and outline measures that may be adopted by health care practitioners and individuals to improve health. These measures include reducing exposures, counteracting effects, and enhancing metabolism and excretion of toxicants.

2. Causes of Chronic Disease

Some chronic diseases may be initiated in susceptible individuals by single "germs," such as acquired immune deficiency syndrome (AIDS) from human immune deficiency virus (HIV), ulcers from *Helicobacter pylori,* or Lyme disease from *Borrelia burgdorferi*, but most major chronic diseases do not have a single cause.

Modifiable factors such as smoking, alcohol, lifestyle (e.g., exercise), and nutrition top lists of contributors to cardiovascular disease, cancer, and obesity/metabolic syndrome/diabetes [1]. The view is weakening that genetic makeup predestines one to disease, as understanding grows of potential remediation of biochemical abnormalities underlying genetic predisposition to ill health [11]. Indeed, a small, shrinking fraction of chronic disease is attributed directly to genetic makeup, as recently discussed for cancer [10] and autism [12]. Instead, gene-environment interactions are very plausibly postulated, such as Apolipoprotein-E 4 interactions with heavy metals associated with Alzheimer's disease [13] or expression of glutathione genes and mercury body burden [14]. Such observations led to development of the field of toxicogenomics, with Judith Stern of the University of California at Davis stating, "genetics loads the gun, but environment pulls the trigger" [15]. In this context, George Church of Harvard Medical School commented that the conversation has evolved from, "Here is your destiny, get used to it!" to "Here is your destiny, and you can do something about it!" [16].

The related field of epigenetics is also coming into focus. Environmental factors ranging from stress to various chemicals may alter methylation of DNA, which in turn modifies DNA expression. Without altering the genetic code, individuals' morphology and predisposition to chronic disease are affected by exposures early in life or even exposures in previous generations, predating conception [17–19].

2.1. Environmental Contributors to Chronic Disease. In a recent, extensive review of associations between early exposures to various chemicals and chronic disease throughout life, Cooper et al. highlight the inadequacy of current scientific methods to tackle the causal puzzle for multiple related conditions (e.g., obesity, metabolic syndrome, cardiovascular disease, type 2 diabetes, and Alzheimer's and Parkinson's diseases), that may be associated with lower socioeconomic circumstances (with poorer diet and housing and increased stress) as well as with multiple potential contributory environmental factors (e.g., air pollution, heavy metals, and various endocrine disrupting chemicals) [20]. The importance of sowing the seeds of health early in life prompted Leiss and Kotch to issue a recent "wake-up call" regarding the importance of environmental health for mothers and children, to tackle intensive and extensive exposures involved in gene-environment interactions, impairing development in a multitude of ways [21]. They call for improved environmental regulation and control, better public education to combat avoidable exposures that are routinely occurring as a result of ignorance, more research, environmental justice, and coverage of environmental health in training of health care professionals.

With the caveat of noted limitations of reductionist approaches in examination of environmental causes of chronic disease, we summarize effects of identifiable groups of chemicals. The point of this summary is neither to depict the weight of evidence nor to be completely comprehensive but to illustrate the multiplicity and complexity of relationships between chemical exposures and health.

2.1.1. Toxic Elements. These elements such as arsenic [22], cadmium [23], lead [24], and mercury [25] are typically found in drinking water, foods, dust, fish, dental amalgams, consumer products, and old pesticides. They are probable or established carcinogens that bind with sulphydryl groups in proteins and inhibit enzyme function, accumulating in organs such as the brain, kidney, liver, and bone, where they cause neurological dysfunction, organ compromise, and skeletal fragility. Among other wide-ranging effects of these chemicals are endocrine disruption by lead and immune system impairment by mercury. There are many other naturally occurring elements of concern, including fluoride, aluminum, and uranium [26].

2.1.2. Naturally Occurring Substances. These include molds and their volatile metabolites, as well as animal, plant, and

food allergens. In this context, it is increasingly recognized that sensitization to a variety of substances does not necessarily involve classic acute reactions, but that sensitivities to diverse substances, including foods, may be playing a role in diseases such as autism [27]. In parallel, sensitivities may arise to diverse man-made chemicals, not commonly thought to be associated with naturally occurring environmental factors [28–30].

2.1.3. Pesticides. These (e.g., insecticides, herbicides, and fungicides) have diverse modes of action to kill, repel, or otherwise control pests (e.g., insects, weeds, and rot fungi). Pesticides are the only chemicals manufactured and spread in the environment specifically to be toxic, with a tiny fraction of broadcast/sprayed chemicals reaching their targets. In her famous book *Silent Spring*, Rachel Carson detailed ecosystem effects of pesticides following World War II, and subsequently poisoning of populations and military with herbicides used in the Vietnam War catapulted pesticides into the public eye. Some persistent pesticides now banned in developed countries continue to be used in underdeveloped countries, and residues are still in the environment and have accumulated towards the poles. Various present-day pesticides have been linked to cancers, and neurological, endocrine, developmental, reproductive, respiratory, and immunological disorders [31–33]. Pesticides produced in tissues of genetically modified crops, and also applied in large quantities to exploit crops' resistance to herbicides, were recently found in women and cord blood [34]. A popular means to reduce risk is to reserve most pesticides for the most essential uses, but to use least-toxic options for landscaping, in order to minimize children's exposure [35].

2.1.4. Persistent Organic Pollutants (POPs). These are a large group of diverse chemicals defined by their longevity in the environment and in the body. They include dioxins and furans (herbicide contaminants, incineration products, environmental product of antimicrobial "triclosan"), polychlorinated biphenyls and polybrominated diphenyl ethers (old and newer flame retardants), polyfluorinated stain repellents, antiwrinkle and nonstick compounds (e.g., Teflon), and organochlorine chemicals (old pesticides—e.g., hexachlorobenzene, DDT, and metabolite DDE). POPs generally have low solubilities in water and are lipophilic (or in the case of some polyfluorinated compounds are surfactants, concentrating at phase interfaces). POPs biomagnify up the food chain as they accumulate in adipose tissue, where they may represent a pool of toxicants with diverse health effects including carcinogenesis [36]. Many POPs with conjugated ring structures are endocrine disruptors as they interact with binding sites for hormones. POPs may also bind with the aryl hydrocarbon receptor (AhR), which elicits a cascade of "dioxin-like" toxic responses [37]. Knowledge of AhR binding toxicities led to regulation of chemicals on this basis, but "nondioxin-like" POPs are also linked to health outcomes such as diabetes [38]. Diverse POPs now present a complex picture in the context of various pervasive health conditions such as obesity, metabolic syndrome, diabetes, and endometriosis [39–45].

2.1.5. Volatile Organic Compounds (VOCs). These are another large group of chemicals, defined by their lower molecular weight and volatility [46]. They include solvents, fuels such as gasoline, and fragrance ingredients [46, 47]. They may interfere with cellular membranes and cause diverse neurological effects. Some such as formaldehyde, benzene, and synthetic musks are carcinogens, and some fragrance ingredients are also known sensitizers [29, 30, 48, 49].

2.1.6. Plastics. These are manufactured from monomers, that are chemically strung together to form polymers. Toxic concerns regarding various plastic vary widely, with vinyl standing out for toxicity of the monomer and additives, as well as challenges with disposal [50]. Unless plastic is degraded (with heat, ultraviolet radiation or chemical attack, such as the xenoestrogen bisphenol-A released from polycarbonate or epoxy liners in food containers), it is generally inert, but chemicals dissolved in the plastic may leach out. "Plasticizers" to improve flexibility or resiliency include endocrine disrupting phthalates, and stabilizers and dyes may contain toxic metals such as lead or cadmium (leading to recalls of children's items such as toys, etc.).

2.2. Mechanisms of Toxicity in Chronic Diseases. The web of effects and interactions with diverse toxicants affecting multiple metabolic and physiological pathways may seem to defy reductionist approaches to single-chemical toxicities and causation of conditions. Nevertheless, with large population studies and sophisticated analyses, effects of toxicants and interactions with toxic as well as beneficial chemicals/nutrients may be discerned. First, some common biochemical effects merit highlighting.

2.2.1. Oxidative Stress. This features in development and exacerbation of many chronic conditions [29, 51, 52], such as allergy and autoimmunity [53, 54], cancer [55], cardiovascular disease [56–58], diabetes [59], neurological compromise [60], lung disease, and sensitization and pain syndromes [28, 61]. Mitochondria are a particular focus of these effects [62].

2.2.2. Endocrine Disruption. This is apparent in altered puberty and sexual development as well as energy utilization, glucose sensitivity, and neurological development. Indeed, interrelated effects on insulin signalling, oxidative stress and vascular health have prompted Alzheimer's disease to be dubbed "diabetes of the brain" [63, 64].

2.2.3. Genotoxicity. This has been studied extensively for single chemicals but is now recognized as only one aspect of development of clinically relevant cancers. Immune surveillance, oxidative stress, and stimulation of growth by infections, inflammation or endocrine disruption may also contribute to carcinogenesis or mutagenesis. More common than changes to DNA sequence are epigenetic changes that alter expression of the genome, and thereby induce a disease state [18, 65].

2.2.4. Enzyme Inhibition. This is a direct effect of pesticides designed to bind with receptors, or of toxic metals that bind

with protein sulfhydryl groups, thereby inactivating a wide range of enzymes, with diverse adverse effects. For example, altered porphyrin profiles may be detected with metal toxicity due to enzyme inhibition [26].

2.2.5. Dysbiosis. Dysbiosis or disruption of the human microbiome has become an area of intense research and clinical interest with the recently initiated Human Microbiome Project by the US National Institutes of Health (http://commonfund.nih.gov/Hmp/). Infection and various toxicants including some heavy metals have the potential to alter gut flora, and thus modify various functions of the gastrointestinal tract including digestion, bioavailability/absorption, elimination, detoxification, and immune function [27, 66–69]. Conversely, gut microbes may transform toxicants such as arsenic or polyaromatic hydrocarbons, altering toxic effects [70, 71]. These phenomena elicit diverse health sequelae for the gastrointestinal tract as well as diverse systemic toxicities such as inflammation and neurological effects [72–74].

Other toxicant-related mechanisms of harm are increasingly being recognized, including immune dysregulation [29], autonomic nervous system impairment [75], biochemical binding (e.g., carbon monoxide supplanting oxygen on hemoglobin, or heavy metals supplanting zinc on glutathione), and overall a toxicant induced loss of tolerance (TILT) [30].

3. Major Chronic Diseases

3.1. Obesity/Metabolic Syndrome/Diabetes. These are linked in complex ways to diverse POPs, including polychlorinated biphenyls, dioxins and flame retardants [76]. These chemicals may interfere with thyroid function, and as xenobiotics stored in adipose tissue are released into the blood stream during weight loss, they may undermine efforts to lose further weight [77]. Other toxicants such as arsenic or cadmium that increase oxidative stress in the pancreas may also contribute to diabetes.

3.2. Vascular Disease. This manifests in cardiac, renal, cerebral and peripheral disorders. Strong connections with toxic metals have been established, with oxidative stress implicated as a central mechanism. Chelation of toxic elements such as lead may decrease renal impairment [78]. Unfortunately, a large chelation trial for vascular disease currently under way is not measuring toxic elements [79].

3.3. Cancer. This is an extensively studied endpoint, with many carcinogens identified in occupational settings. Carcinogenicity of lower exposures and with mixtures are sometimes less clearly defined, as discussed above. Nevertheless, there is consensus that there is no "risk-free" or threshold dose of genotoxic substances. Environmental chemicals may contribute to cancer by altering DNA or its expression, by stimulating rapid growth and confounding cell repair mechanisms in hormone-sensitive tissues or via inflammation, or by impairing immune surveillance.

3.4. Neurocognitive Impairment. This, including reduced IQ and aberrant behaviour, is linked to early life exposure to a wide range of environmental toxicants, including heavy metals, various POPs and pesticides [80]. Effects may be immediate (e.g., problems with learning, attention and aggression) or delayed (e.g., increased predisposition to Alzheimer's or Parkinson's disease). A few common mechanisms include endocrine disruption (e.g., polyhalogenated biphenyls mimic thyroid hormone), direct inhibition of neuronal growth by toxic metals (e.g., mercury), or interference with signal transmission by pesticides or toxic metals.

3.5. Multi-System Complaints. Patients significantly disabled with multisystem complaints are increasingly commonly presenting themselves to clinicians. Recent evidence suggests that TILT is a pervasive mechanism of illness involving several organ systems concurrently [29, 30].

4. Timing and Vulnerabilities

The exquisite vulnerability of the young and unborn was tragically clear when mothers in Minimata, Japan, who were coping with relatively mild symptoms from methylmercury in the fish they ate, gave birth to children with severe neurological damage [81]. Unique vulnerabilities of the fetus and child arise when chemicals interfere with windows of differentiation of tissues *in utero* and during development. Lead, pesticides, and other chemicals at doses that do not overtly impair the mother may harm her offspring, with immediately evident or delayed neurological, endocrine or other effects [82–85]. Indeed, the mother may impart bioaccumulated toxicants (e.g., lead or cadmium from her bones, or lipophilic pollutants from her adipose tissues) to her fetus through the placenta, or infant via breast milk. Nevertheless, breast milk is undoubtedly the best food for infants.

Duration of exposure is another aspect of timing. Haber's Rule and related mathematical treatments of toxicity model that when a toxicant is persistent (or quasipersistent with ongoing exposure to a ubiquitous toxicant such as bisphenol-A), or the chemical's effect is irreversible, then either a low, chronic dose or a high acute dose may both result in toxic effects [86]. For many toxicants, there is no "nontoxic" dose. The public health and regulatory response to this situation is often to strive for levels that are "as low as reasonably achievable," typically accompanied by arguments with commercial enterprises over the "reasonableness" of costs of remediation, abatement, and alternative processes and products.

5. Exposure and Dose

In common usage, "exposure" is sometimes equated with dosage, with toxicant levels in the environment, drinking water, foods and products subject to regulation and public health measures. Nevertheless, toxicologists and health care professionals parse exposure and doses. For instance, the presence of a chemical in the environment (e.g., house dust) does not necessarily mean that a child will crawl in it and get it on his fingers. Even if he licks his fingers and ingests the myriad chemicals in house dust, intestinal absorption varies according to the chemical, age and state of the child's

development, nutritional status, and other materials and foods in the intestine at the same time. The dosage to a target organ such as the brain will once again be modified by absorption into cells and across membranes, and by metabolism and excretion. Measurement of a toxicant in most target organs or tissues is not feasible in living human study participants, so surrogate levels may be measured in blood, urine, hair, sweat, feces, and even meconium, cord blood, nails and deciduous teeth [82, 87–90].

6. Responses to Toxicants

6.1. Recognition. A potential risk must be recognized before any response is possible. This includes the classic questions: what? (is the substance), where? (is a substance made/found), how? (does toxicity manifest), who? (may be affected), and why? (is it used, and are there alternatives that pose less risk).

Beyond prioritization and assessment of chemicals, measures to reduce environmental and individual exposures occupy considerable resources of government agencies and ministries, and international bodies such as the World Health Organization. Increasingly, emissions and product content data, as well as toxicology data necessary for chemical assessment and registration for use, are being required from industries, manufacturers and importers.

6.2. Chemical Assessment. Numerous, diverse environmental exposures merit scrutiny for health effects, including factors affecting chronic disease. As well as age-old toxic elements (mercury, lead, cadmium, arsenic, etc.), and allergens and organic chemicals such as mold metabolites, we and our offspring carry many chemicals in our bodies that mankind has only recently encountered [8]. Approximately 80,000 novel chemicals have been registered for use with the United States' Environmental Protection Agency since World War II, but the vast majority have not been assessed for human health or environmental effects [91].

Around the world, various approaches are being used to address the conundrums posed by such vast numbers of novel chemicals. Environment and health ministries and regulatory agencies in North America have for years been prioritizing lists of chemicals including pesticides, assessing the most urgent ones, and restricting uses or banning occasional chemicals for particular uses. Europe instituted the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation in 2007, administered by the European Chemicals Agency (ECHA), placing greater responsibility on industry to provide data and to manage risk [92].

Risk is typically initially screened on the basis of inherent toxicity (often acute), whether the chemical is persistent or bioaccumulative, and the potential for exposure. Toxicological risk assessment is carried out by chemical agencies, on the basis of animal toxicology research supplied by industrial applicants. Typically a dose level is determined that produces no (serious) observable adverse effect in an animal, which is then extrapolated to a lower exposure (e.g., level in air, water, food, or a product, or an application rate for a pesticide) that is said to pose a reasonable risk to humans. As long as the permissible exposure is well above levels observed in the real world, then the chemical or product may be registered for sale and use. This paradigm does not address multiple exposures with potential cumulative or synergistic effects on a particular system such as the nervous system [93], although efforts are evolving to assess small groups of chemicals commonly found together [94], and to carry out *in vitro* high throughput, rapid biochemical and cell system screening, *in lieu* of animal testing [95].

The catch with toxicological testing is that it would be illogical for an industry to conduct testing at environmentally relevant doses, because the regulatory framework means that observations of effects will preclude registration. A longstanding tenant of toxicology, that all effects at low doses are presaged by effects at higher doses, is captured as "The dose makes the poison," a common paraphrase of Paracelsus' writings from the 16th century. Research now contradicts this view that toxicity becomes apparent at higher doses and is not detectable below a certain level, as non-monotonic dose response curves are well recognized to be usually associated with endocrine effects [96]. Indeed, the American Chemical Society, with extensive membership among the chemical industry, recognizes that low-dose, endocrine disrupting effects are scientifically undermining the toxicological testing that underpins current chemical regulation [97].

Another obvious shortcoming of regulatory animal toxicology is that humans are not rodents. Human studies of toxicants are ethically intransigent, and thus necessarily subject to limitations of observational studies, but health researchers and professionals argue persuasively why and how epidemiology could and should be given greater weight in chemical assessment [98].

Faced with fundamental doubts overshadowing today's environmental standards and regulatory decisions, some countries such as Sweden now consider inherent safety, how essential a chemical or product is, and whether a less toxic, lower risk substitute is feasible; this is the Substitution Principle, a means to put into operation the Precautionary Principle [99]. The Substitution Principle is in part a pragmatic response to the enormity of the numbers and diversity of anthropogenic chemicals, as well as the logistical impossibility of scientifically assessing all chemicals, let alone combinations [99]. In essence, the least-toxic, and most environmentally sustainable options for a particular product or application are the ones that are permitted. Inclusion of the null option—that a particular product is not necessary for society—allows regulators to rule out a number of chemicals from those needing to be further assessed while providing a strong incentive for "green" chemistry.

7. Public Health

With rules laid out as to allowable uses and levels of toxicants, protection of public health falls into the purview of a variety of professionals, from environment, agriculture, natural resource, and health ministries to local public health.

7.1. Monitoring and Detection. Pollution and human exposure may be tracked on many levels, by governments monitoring and reporting toxicants from large scale industrial

Table 1: Selected websites for chemical information.

World Health Organization	http://www.who.int/
INCHEM (Chemical Safety Information from Intergovernmental Organizations)	http://www.inchem.org/
Concise International Chemical Assessment Documents (CICADs)	http://www.inchem.org/pages/cicads.html
Environmental Health Criteria (EHC) Monographs	http://www.inchem.org/pages/ehc.html
Health and Safety Guides (HSGs)	http://www.inchem.org/pages/hsg.html
International Agency for Research on Cancer (IARC)—Summaries and Evaluations	http://www.inchem.org/pages/iarc.html
International Chemical Safety Cards (ICSCs)	http://www.inchem.org/pages/icsc.html
IPCS/CEC Evaluation of Antidotes Series	http://www.inchem.org/pages/antidote.html
Joint Meeting on Pesticide Residues (JMPR)	http://www.inchem.org/pages/jmpr.html
KemI-Riskline	http://www.inchem.org/pages/kemi.html
Pesticide Documents (PDs)	http://www.inchem.org/pages/pds.html
Poisons Information Monographs (PIMs)	http://www.inchem.org/pages/pims.html
Screening Information Data Set (SIDS) for High Production Volume Chemicals	http://www.inchem.org/pages/sids.html
Centers for Disease Control and Prevention (USA)	http://www.cdc.gov/
Agency for Toxic Substances and Disease Registry (USA)	http://www.atsdr.cdc.gov/
National Institute for Occupational Safety and Health (USA)	http://www.cdc.gov/niosh/
Environmental Protection Agency (USA)	http://www.epa.gov/
National Institute for Environmental Health and Safety (USA)	http://niehs.nih.gov/
National Toxicology Program (USA)	http://ntp.niehs.nih.gov/
European Commission—Public Health	http://ec.europa.eu/health/index_en.htm/
European Commission—Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)	http://ec.europa.eu/health/scientific_committees/emerging/index_en.htm/
European Chemicals Agency	http://echa.europa.eu/

emissions; environmental levels in air, water, soil, and wildlife; individual exposures in foods, drinking water and consumer products; and levels in people themselves with population surveillance. Publicly available data may serve many purposes, from deciding to avoid vigorous exercise on the smoggiest days, to investigating contaminated sites and pollution sources in one's neighbourhood. Governments regularly publish online data such as daily air quality, and may announce product recalls (e.g., children's products with lead or cadmium, or baby formula with melamine). A list of some helpful websites addressing chemical assessment and monitoring is provided in Table 1 (many additional sites are available for individual countries). Nongovernmental organizations also carry on myriad public education campaigns such as recommending foods with lower levels of pesticides [100] or personal care products with lower levels of chemicals of concern [101], or even identifying houses at risk for child lead exposures [102].

Governmental regulation provides a bottom line for use of and exposure to potential toxicants, but chemicals have historically been assumed "innocent until proven guilty," and there is a long history of examples such as pesticides being banned after decades of use, and uses and limits for chemicals such as lead falling as scientific knowledge improves. Evolution of public health initiatives as risks are recognized may drive innovation; for instance, more stringent standards are possible as water and waste treatment technologies improve, and best practices for pest management change when the array of permitted chemicals is limited.

Individual choices (e.g., when shopping) will be affected by education and perception regarding risk, as well as the cost, feasibility, and identification (with honest, meaningful labelling) of alternatives. Medical practitioners themselves should be knowledgeable, and have the resources to educate and facilitate their patients making the best choices for their personal, family, patient and community health. On a broader scale, the voice of the medical community has excellent credibility in setting public policy to promote health.

8. Clinical Considerations Regarding Toxicants

The reality of the contemporary world is that toxicants are ubiquitous and, while avoidance is central to any management strategy, toxicants are not entirely avoidable. This has always been true for naturally occurring elements, but the recent onslaught of anthropogenic substances poses new challenges for an individual's biochemistry and hence for the clinician.

Although identifying the epidemiologic patterns of toxicant exposure and health sequelae has been one focus of

attention, some within the clinical world are asking, "So what do we do about it?" The realization that many throughout the world already possess a significant body burden of anthropogenic compounds [8, 103], juxtaposed with the emerging reality of serious potential health sequelae associated with toxicant accrual has inevitably led some scientists and clinicians to consider possible measures to reduce the toxicant burden in the human body in order to reduce the risks associated with accretion of diverse compounds.

Some toxicologists and clinicians, often untrained in environmental health sciences, consider strategies to eliminate toxicants from the body to be myths lacking in merit, assuming that all toxicants are metabolized and excreted naturally and concluding that "there's nothing that does anything to hasten the detoxification process" [104]. Emerging evidence, however, challenges this misconception. The contention that the body has an inherent ability to eliminate quickly all adverse chemical compounds is inaccurate, as many toxicants with long half-lives accrue in tissues or blood, thus maintaining long-term potential to inflict damage. Metals such as lead and cadmium, and many halogenated compounds (e.g., flame retardants, nonstick compounds, stain repellents, and organochlorine pesticides), are persistent human pollutants with extended half-lives. Unfolding research reveals ineluctable evidence that various interventions facilitate elimination of retained compounds [87, 105], with the objective of diminishing the risks associated with biologically stockpiled poisons. Although more extensive reviews of various modalities to eliminate toxicants can be found in other works [105], we present an overview of potential approaches that can be employed to facilitate the removal of accrued toxicants.

With a wide range of distinct chemical compounds, each with a unique chemical structure and a potentially distinct way of interfacing with human biochemistry, there is no single mechanism or pathway for the body to eliminate the whole spectrum of 21st century chemical toxicants. Thus, when attempting to detoxify the human body, it is first important to explore the specific accrued toxicants comprising the total chemical burden, and to employ effective methods to facilitate excretion of various components.

8.1. Determination of Body Burden of Toxicants. When a patient with evidence of potential toxicant-related health problems presents to a clinician trained in environmental health sciences, an attempt is generally made to identify which adverse chemicals are retained within the body, in order to employ specific interventions to address each of these compounds. With the vast array of toxicants that individuals are exposed to, how does one comprehensively determine which toxicants are present and then assess the extent of the total body burden?

As clinical laboratory methods to assess many toxicants are not extensively validated with meaningful reference ranges, there is limited ability at this time to investigate a broad range of specific chemicals. Blood and urine are most commonly sampled to assess levels of retained toxicants. Apart from difficulties interpreting results in the absence of population-specific reference-limits, these measurements may be significantly flawed as indicators of bioaccumulation because many compounds sequester in tissues; they do not remain in blood and may not be readily excreted in urine. Thus testing of whole blood or serum generally does not adequately detect toxicants that are being stored primarily in organs, bone, muscle or adipose tissues [26, 87]. As well, levels of toxicant compounds in blood and urine can also fluctuate rapidly as a result of nutrient or pharmaceutical use, caloric restriction, hydration, underlying nutrient status, thermal changes, or exercise [106–110].

For clinical purposes therefore, blood or urine testing to determine the total body load of toxicants may underestimate the level of accrual for many toxicants. Testing of other tissues and bodily excretions has also been explored, including salivary testing, hair analysis, stool sampling, perspiration testing, breath analysis, provocation testing, as well as biopsies of fat tissue. Recent evidence confirms, however, that there are limitations with each of these approaches. Hair samples, for example may only reflect selected toxicant levels in the blood stream for the last few weeks, while stool samples only assess what is being eliminated through the gastrointestinal tract. Fat biopsy research confirms that toxicants sequester differently within different fat compartments, with toxicant concentrations varying widely among adipose tissue sites [111]. Although selected testing techniques for some toxicants can be helpful as an indication of toxicant bioaccumulation, attempts to accurately and comprehensively delineate the accrued level of each toxicant compound are impractical clinically and prohibitively expensive. The results are imprecise at best, and are prone to false negatives in the sense that a low blood value, for instance, may simply not reflect high levels in the bone, or a vital organ such as the kidney or brain. Values in urine, hair and feces by definition reflect the ability to excrete rather than the body burden of a toxicant, and impaired excretion may result in greater accretion and potential adverse effects, leading to the paradoxical finding that body burden is apparently lower in a population whose health is in fact being affected by a toxin, as was seen in children with autism [112–114].

So what is a reasonable clinical approach to patients who appear to have been harmed by bioaccumulative toxicant exposures?

8.2. Pragmatic Clinical Management. With currently available knowledge and technologies, three fundamental clinical steps should be considered in the initial assessment and patient care planning for those with potential toxicant-related health problems:

(a) perform a comprehensive history to endeavour to identify past and present exposures [115],

(b) order selected toxicant testing as is feasible in each situation,

(c) use clinical judgement to institute low-risk elimination strategies directed towards the individual patient.

Rather than specialized treatments for each specific toxicant identified, in this paper, we present a general approach to detoxification. This is an ongoing area of research, and other works are available that provide further particulars in the management of specific toxicant categories and individual chemical exposures [87, 88, 105].

The clinical approach to human elimination of accrued compounds generally involves three successive stages:

(1) personal avoidance,

(2) securing efficacy of endogenous mechanisms for toxicant elimination,

(3) directed interventions to facilitate removal of accrued toxicants.

8.2.1. Avoidance. It is sometimes bantered about in environmental health science circles that the three most important principles in addressing the problem of widespread toxicant exposure are "avoid, avoid, and avoid." As previously discussed, through means of personal education, notification (e.g., labelling) and actions (e.g., remediation of living spaces and changes in diet) as well as governmental and industrial regulation with enforcement, minimization of further exposure is achieved. This is fundamental to any successful strategy to diminish the toxicant burden of individuals and populations.

From a clinical perspective, it is useful for the health provider to perform a detailed inventory of potential exposures. By means of a meticulous environmental health questionnaire [115], most common exposures can be identified. An inventory of the six routes of possible sources of exposure should be undertaken:

(i) ingestion,

(ii) breathing,

(iii) skin contact,

(iv) olfactory transmission via smell,

(v) vertical transmission (i.e., mother to fetus/infant),

(vi) penetration of body tissues through processes such as surgery, dentistry, injection, or vector routes.

By identifying exposures and apprising individual patients regarding where and how they are being contaminated, patients are empowered to avoid further chemical contamination. With ongoing exposures minimized, the human organism is able to devote resources and energies of detoxification physiology to metabolizing and excreting retained compounds, with less devoted to ongoing exposures.

8.2.2. Securing Efficacy of Endogenous Mechanisms for Toxicant Elimination. The human body has enormous potential to detoxify foreign compounds through various physiological mechanisms. Endogenous detoxification of metabolic waste products as well as foreign toxicants is a primary physiological function, that requires considerable energy. Major organs of detoxification include the liver, kidney, skin, and lungs. When toxicants are identified by physiological processes within the body, pathways of excretion are mobilized to diminish toxicity and to eliminate the xenobiotic compounds. The particular pathways used to excrete specific substances will depend on the chemical properties of the particular agent in question. Potential pathways include metabolism or conjugation to form water-soluble compounds for renal excretion, metabolism to less toxic forms (e.g., methylation of arsenic), conjugation with biochemicals such as glutathione for gastrointestinal elimination or intracellular metallothionein binding of heavy metals [26].

The ability to eliminate undesired compounds, however, depends completely on the physiological functioning and biochemical status of the individual. Anything that impairs full functioning of detoxification biochemistry, such as nutritional deficiencies, will preclude proper elimination of toxic substances. Accordingly, it is imperative that health providers understand the fundamentals of detoxification physiology and biochemistry to secure functioning of the organs of elimination [26].

Clinical history and physical examination can provide clues to the status of physiological function and potential causes for impairment. A history of substance use, for example alcohol or medications known to be hepatotoxic, can be helpful in assessing liver function. Nutritional biochemistry testing, urinary organic acid testing, and biochemical markers for function of organs such as liver and kidney, can be employed to assess physiological status and function. Any impediment to proper physiological functioning should be addressed.

Remediation of disordered nutritional biochemistry is a fundamental component of patient care. For example, the protein molecule glutathione is a prerequisite component of cellular detoxification as well as an essential pillar in hepatic conjugation biochemistry. Individuals with ongoing exposures or toxicant bioaccumulation often have diminished stores of glutathione, and thus require ongoing repletion. Optimal nutrition through dietary instruction, correction of disordered biochemistry and physiology, and use of directed supplementation as dictated by laboratory testing is required for efficient physiological functioning of elimination pathways.

It has also been observed that despite biochemical competency, the human organism is not able to excrete some chemical toxicants effectively. A major reason for the failure of some compounds to be eradicated effectively is because of recycling within the body through reabsorption in the enterohepatic circulation [116] or reuptake in kidney tubules [117]. Accordingly, some toxicants are conjugated and released from tissues into the bloodstream for excretion, but are then reabsorbed back into the body. Additionally, some retained compounds deposit in specific tissues such as bone, fat, and muscle, where they will bioaccumulate and alter physiological functioning within these tissues. Some compounds will also remain in blood to some degree, frequently bound to plasma proteins [88]. Interventions to enhance excretion of retained compounds can be invaluable in diminishing morbidity associated with toxicant accrual.

Environmental determinants such as toxicant bioaccumulation in some situations may interfere with normal

physiological function and thus necessitate intervention. For example, vitamin D (an essential biochemical that regulates genetic expression, and facilitates absorption of calcium from the gut) may potentiate absorption of toxic elements such as lead, aluminum, and cadmium, that in turn may impair metabolism of vitamin D [118]. Mercury contamination may alter gastrointestinal absorption of required nutrients resulting in deficiencies and thus precluding normal physiology. Some persistent organic pollutants released from adipose tissue during weight loss, caloric restriction or exercise may suppress thyroid function [77]. Adverse effects from toxicant bioaccumulation may cascade within the body—some toxicants, for example, may alter immune function, which may in turn spawn autoimmunity [119] and thus engender abnormal physiological functioning. In difficult cases, consultation with experienced environmental health specialists may be required.

8.2.3. Directed Interventions to Facilitate Removal of Accrued Toxicants. Empirical research has shown that various strategies can be employed to assist with the effective removal of some accrued toxicants. Research into such strategies, however, remains at an early stage in the continuum of clinical science as the problem of widespread toxicant bioaccumulation is a newly recognized phenomenon for clinical medicine. Accordingly, adequate evidence-based research to objectively confirm or refute the alleged efficacy of assorted "detox strategies" is often lacking. A few strategies and a general approach to clinical detoxification are highlighted here for consideration. A more detailed discussion of commonly employed strategies to facilitate detoxification can be found elsewhere [105].

8.2.4. Thermal Depuration (Sweating). The skin is a major organ of detoxification, and a vast array of toxicants are able to be excreted to differing degrees via perspiration [87, 105, 120]. Various researchers and clinicians have endeavoured to take advantage of this dermal mechanism to facilitate excretion of accrued compounds and toxicological biomonitoring has confirmed that body burdens of many toxicants diminish with therapy to induce sweating [105, 121, 122]. Some chemical agents such as perfluorinated compounds, however, do not seem to be readily excreted [88]. Despite much attention given to saunas with heaters emitting at specific electromagnetic frequencies, research to date suggests that there is no difference in toxicant excretion rates between perspiration that occurs through infrared sauna, dry or wet regular saunas, or exercise [87, 123].

8.2.5. Selected Medications. These perform a useful role in facilitating the elimination of some compounds. For example, judicious use of chelators, or agents which strongly bind to some toxic elements have been demonstrated to assist in the removal of such toxicants [124, 125]. Chelating agents such as dimercaptosuccinic acid (DMSA) are generally safe and effectively bind to metals such as lead and mercury to enhance excretion rates and to prevent enterohepatic reabsorption of these compounds [125]. Marked clinical improvement has been noted in metal contaminated patients who have been treated with use of such medications [126]. The use of concomitant strategies, such as foods and supplements to increase glutathione, to enhance mobilization of toxicants from tissue storage sites may significantly increase the rate of elimination from the body when used along with chelators.

Bile acid sequestrants such as cholestyramine have recently garnered increasing attention as compounds that bind to some persistent compounds in the gastrointestinal tract to prevent enterohepatic reabsorption [88]. These agents may be useful with such persistent agents as perfluorinated compounds [88] and have been clinically effective in patients with mycotoxin accrual after mold exposure [127]. As medical interventions to enhance elimination of toxicants is a newer area of clinical research, much remains to be studied in order to develop evidence-based medication protocols for the removal of some toxic compounds.

8.2.6. Selected Foods and Supplements. Direct evidence of specific benefits in humans of diet and dietary supplements is often of limited applicability beyond the subject population, because of interactions with factors such as regional environment (e.g., cadmium or selenium in the soil), lifestyle, dietary practices, levels of nutrients such as vitamin D from sun exposure, poverty, and alcohol and tobacco use. Some medical evidence exists, however, that nutritional status (e.g., calcium, zinc and iron repletion) modifies absorption of toxic elements such as lead, and that beneficial effects of omega-3 fatty acids and selenium in fish somewhat counteract toxicities of methylmercury and persistent organic pollutants [128, 129].

Research questions are often much more amenable to animal and tissue culture research, and this body of evidence has confirmed traditions that some foods and supplemental nutrients are enormously valuable in facilitating excretion and reducing biochemical toxicities of toxicants. Although by no means an exhaustive list, some supplements include curcumin in the spice turmeric [130], alliums [131], plant flavonoids such as quercetin [132], selenium [133], algal products *Parachlorella* [134, 135] and *Chlorella* [136, 137], naturally occurring organic acids [138], folate requisite minerals, and dietary fibre [139] as well as mixed antioxidants [140] appear to be of great value to reduce the damage associated with toxicant exposure. The mechanisms of action may include preventing absorption of toxicants, facilitating elimination of accrued toxic compounds, hindering enterohepatic recycling of some persistent compounds, and diminishing toxicity through protective mechanisms. Insoluble carbohydrate and other fibre consumed in the diet, for example, appears to act like a sponge and increases the removal of adverse agents such as mycotoxins and POPs, perhaps by diminishing reabsorption through the enterohepatic circulation, and thus increasing elimination.

One example is a supplemental product called *Chlorella*, an algae from the sea, that has recently garnered much research attention for its unique properties in facilitating detoxification and preventing absorption of adverse compounds [134–137]. Recent research papers reported animal results where *Chlorella* appears to induce the excretion of

mercury [135] and lead [134]. Ongoing study continues to elucidate the range of compounds that are bound and removed with *Chlorella* as well as with other assorted foods and supplemental nutrients. A caution, however, is that supplements may potentially contain toxicants that accrued as they grew, such as *Chlorella* and other biosorbents that are also noted for their ability to sequester toxic elements from their environment [141], fish oil that may contain POPs [142], or other products that may be contaminated for other reasons [143].

8.2.7. Other Detoxification Modalities. With increasing recognition of ubiquitous toxicant exposure and bioaccumulation [8], a plethora of treatments called "cleanses" have become commonplace. Furthermore, "detox" clinics have sprung up throughout the western world allegedly to help individuals rid their bodies of accumulated toxicants. Unfortunately, there is limited research on many of the programs and therapies that are commonly used and scientific evidence is often lacking to support the audacious claims frequently made to vulnerable, sick people. While some interventions such as exercise [144, 145], induced sweating [87, 120], selected medications [124, 146] (including in those children with autism who have elevated levels of heavy metals [113, 147]), plasmapheresis [148, 149], some foods and some supplements [137, 138, 150], and other modalities show credible evidence of efficacy in removing toxicants, much research remains to be done in order to yield consistent evidential support for the increasing plethora of "detox" interventions. Using data available to date, a basic approach that can be used clinically, that incorporates the three successive steps for detoxification (avoidance, support of endogenous detoxification, and directed interventions) is presented for consideration.

9. Seven Steps General Clinical Approach to Detoxification

(1) A detailed environmental history and exposure inventory, followed by patient education to effect adequate avoidance [115].

(2) Specific toxicological testing if indicated according to the patient history [151].

(3) Remediation of abnormal biochemistry, as identified by laboratory investigations [26].

(4) A combination of optimal diet and supplemental nutrients may be utilized to secure adequate nutrition and sufficient biochemical reserve for detoxification [152]. Some examples include (this is not a comprehensive list) the following:

   (a) glutathione [153] and sulfur-containing foods (e.g., eggs, brassicas and alliums) and supplements such as N-acetylcysteine, taurine or methylsulfonylmethane to support glutathione and metallothionein synthesis,

   (b) folate and related nutrients (e.g., for arsenic metabolism and excretion [154]),

   (c) lipoic acid therapy may assist in detoxification of some compounds including mycotoxins resulting from exposure to certain molds [150],

   (d) adequate minerals such as calcium, iron and zinc, to reduce absorption of heavy metals [128],

   (e) *Chlorella* to decrease absorption and enhance excretion of toxicants is generally well tolerated [134–136, 155],

   (f) fiber to reduce absorption and facilitate elimination [139].

(5) Regular sweating (with mineral repletion), with exercise or in a sauna, can facilitate transdermal excretion [87].

(6) Daily exercise will enhance eliminate of some toxicants [144, 145].

(7) Directed therapies for retained toxicants (as determined by laboratory investigations) may be implemented [88, 105].

9.1. In Summary. Health care professionals in government ministries, public health, research, and the clinic will only be successful against the onslaught of chronic, debilitating diseases once environmental contributors are recognized, researched and addressed. Clinical intervention to preclude further exposure and to detoxify the body of toxicants can be life changing for afflicted individuals [126]. In an epoch marred by the unleashing of numerous untested chemical toxicants, basic knowledge of environmental medicine should be provided in the training of all health care workers. History repeatedly demonstrates, however, that the translation of emerging scientific information with adoption of required clinical skills is usually not expeditious [156, 157]. Hopefully, in our modern era of rapid information transfer, the process of widespread problem recognition and solution implementation will be expedited to stem the tide of chronic disease that is said to be poised to bankrupt healthcare systems.

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References

[1] World Health Organization. Global status report on noncommunicable diseases, 2010, http://www.who.int.proxy.bib.uottawa.ca/nmh/publications/ncd_report2010/en/index.html.

[2] World Health Organization. Noncommunicable diseases Advocacy Docket, 2011, http://www.searo.who.int/en/Section1174/Section1459_16034.htm.

[3] World Health Organization. Global assessment of the state-of-the-science of endocrine disruptors, 2002, http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/.

[4] E. M. Crimmins and H. Beltrán-Sánchez, "Mortality and morbidity trends: is there compression of morbidity?" *Journals of Gerontology—Series B*, vol. 66, no. 1, pp. 75–86, 2011.

[5] S. T. Stewart, D. M. Cutler, and A. B. Rosen, "Forecasting the effects of obesity and smoking on U.S. life expectancy," *The New England Journal of Medicine*, vol. 361, no. 23, pp. 2252–2260, 2009.

[6] L. Trasande and Y. Liu, "Reducing the staggering costs of environmental disease in children, estimated at $76.6 billion in 2008," *Health Affairs*, vol. 30, no. 5, pp. 863–870, 2011.

[7] S. G. Gilbert and B. Weiss, "A rationale for lowering the blood lead action level from 10 to 2 μg/dL," *NeuroToxicology*, vol. 27, no. 5, pp. 693–701, 2006.

[8] Centers for Disease Control and Prevention. Department of Health and Human Services. Fourth National Report on Human Exposure to Environmental Chemicals plus updates, 2009, http://www.cdc.gov/exposurereport/.

[9] R. Busse, M. Blümel, D. Scheller-Kreinsen, and A. Zentner, Tackling chronic disease in Europe: strategies, interventions, and challenges. World Health Organization, on behalf of the European Observatory on Health Systems and Policies, 2010, http://www.cabdirect.org/abstracts/20103284845.html.

[10] S.H. Reuben for the President's Cancer Panel, US Department of Health and Human Services, National Institutes of Health, National Cancer Institute. Reducing Environmental Cancer Risk. What We Can Do Now, 2010, http://deainfo.nci.nih.gov/advisory/pcp/annualReports/pcp08-09rpt/PCP_Report_08-09_508.pdf.

[11] S. J. Genuis and R. A. Lobo, "Potential amelioration of morbidity in patients with chromosomal anomalies: relevance to Bardet-Biedl syndrome," *Clinical Genetics*, vol. 79, no. 5, pp. 482–488, 2011.

[12] J. Hallmayer, S. Cleveland, A. Torres et al., "Genetic heritability and shared environmental factors among twin pairs with autism," *Archives of General Psychiatry*, vol. 68, no. 11, pp. 1095–1102, 2011.

[13] J. Mutter, J. Naumann, C. Sadaghiani, R. Schneider, and H. Walach, "Alzheimer disease: mercury as pathogenetic factor and apolipoprotein E as a moderator," *Neuroendocrinology Letters*, vol. 25, no. 5, pp. 331–339, 2004.

[14] K. S. Engström, U. Strömberg, T. Lundh et al., "Genetic variation in glutathione-related genes and body burden of methylmercury," *Environmental Health Perspectives*, vol. 116, no. 6, pp. 734–739, 2008.

[15] W. A. Suk, K. Olden, and R. S. H. Yang, "Chemical mixtures research: significance and future perspectives," *Environmental Health Perspectives*, vol. 110, no. 6, pp. 891–892, 2002.

[16] R. Cotton and H. van Kranen, Human Variome Project, 2010, http://www.nugo.org/downloadattachment/40946/10898/henk_100121.pdf.

[17] Z. Hochberg, R. Feil, M. Constancia et al., "Child health, developmental plasticity, and epigenetic programming," *Endocrine Reviews*, vol. 32, no. 2, pp. 159–224, 2011.

[18] M. K. Skinner, M. Manikkam, and C. Guerrero-Bosagna, "Epigenetic transgenerational actions of environmental factors in disease etiology," *Trends in Endocrinology and Metabolism*, vol. 21, no. 4, pp. 214–222, 2010.

[19] S. J. Genuis, "Our genes are not our destiny: incorporating molecular medicine into clinical practice," *Journal of Evaluation in Clinical Practice*, vol. 14, no. 1, pp. 94–102, 2008.

[20] K. Cooper, L. Marshall, L. Vanderlinden, and F. Ursitti, Early Exposures to Hazardous Pollutants/Chemicals and Associations with Chronic Disease—A Scoping Review—Canadian Partnership for Children's Health and Environment, Canadian Environmental Law Association, Ontario College of Family Physicians, and the Environmental Health Institute of Canada, for the Canadian Partnership for Children's Health and Environment, 2011, http://www.healthyenvironmentforkids.ca/resources/EE-andCD-scoping-review.

[21] J. K. Leiss and J. B. Kotch, "The importance of children's environmental health for the field of maternal and child health: a wake-up call," *Maternal and Child Health Journal*, vol. 14, no. 3, pp. 307–317, 2010.

[22] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Arsenic, 2007, http://www.atsdr.cdc.gov/ToxProfiles/TP.asp?id=22&tid=3.

[23] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Cadmium, 2008, http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=48&tid=15.

[24] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Lead, 2007, http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=96&tid=22.

[25] Agency for Toxic Substances and Disease Registry. Toxicological Profile: Mercury. US Department of Health and Human Services. Public Health Service, 1999, http://www.atsdr.cdc.gov/ToxProfiles/TP.asp?id=115&tid=24.

[26] R. Lord and J. Bralley, *Laboratory Evaluations for Integrative and Functional Medicine*, Metametrix Institute, Duluth, Ga, USA, 2nd edition, 2008.

[27] S. J. Genuis and T. P. Bouchard, "Celiac disease presenting as autism," *Journal of Child Neurology*, vol. 25, no. 1, pp. 114–119, 2010.

[28] C. De Luca, M. G. Scordo, E. Cesareo et al., "Biological definition of multiple chemical sensitivity from redox state and cytokine profiling and not from polymorphisms of xenobiotic-metabolizing enzymes," *Toxicology and Applied Pharmacology*, vol. 248, no. 3, pp. 285–292, 2010.

[29] S. J. Genuis, "Sensitivity-related illness: the escalating pandemic of allergy, food intolerance and chemical sensitivity," *Science of the Total Environment*, vol. 408, no. 24, pp. 6047–6061, 2010.

[30] N. Ashford and C. Miller, *Chemical Exposure. Low Levels and High Stakes*, John Wiley & Sons, New York, NY, USA, 2nd edition, 1998.

[31] M. Sears, C. R. Walker, R. H. C. Van Der Jagt, and P. Claman, "Pesticide assessment: protecting public health on the home turf," *Paediatrics and Child Health*, vol. 11, no. 4, pp. 229–234, 2006.

[32] M. Sanborn, K. J. Kerr, L. H. Sanin, D. C. Cole, K. L. Bassil, and C. Vakil, "Non-cancer health effects of pesticides: systematic review and implications for family doctors," *Canadian Family Physician*, vol. 53, no. 10, pp. 1712–1720, 2007.

[33] K. L. Bassil, C. Vakil, M. Sanborn, D. C. Cole, J. S. Kaur, and K. J. Kerr, "Cancer health effects of pesticides: systematic review," *Canadian Family Physician*, vol. 53, no. 10, pp. 1705–1711, 2007.

[34] A. Aris and S. Leblanc, "Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada," *Reproductive Toxicology*, vol. 31, no. 4, pp. 528–533, 2011.

[35] David Suzuki Foundation, Équiterre. Pesticide Free? Oui! 2011 progress report: a comparison of provincial cosmetic pesticide bans, 2011, http://www.healthyenvironmentforkids.ca/resources/pesticide-free-oui-2011-progress-report-comparison-provincial-cosmetic-pesticide-bans.

[36] P. Irigaray, J. A. Newby, S. Lacomme, and D. Belpomme, "Overweight/obesity and cancer genesis: more than a biological link," *Biomedicine and Pharmacotherapy*, vol. 61, no. 10, pp. 665–678, 2007.

[37] T. D. Bradshaw and D. R. Bell, "Relevance of the aryl hydrocarbon receptor (AhR) for clinical toxicology," *Clinical Toxicology*, vol. 47, no. 7, pp. 632–642, 2009.

[38] D. H. Lee, I. K. Lee, K. Song et al., "A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999–2002," *Diabetes Care*, vol. 29, no. 7, pp. 1638–1644, 2006.

[39] B. Trabert, A. J. de Roos, S. M. Schwartz et al., "Non-dioxin-like polychlorinated biphenyls and risk of endometriosis," *Environmental Health Perspectives*, vol. 118, no. 9, pp. 1280–1285, 2010.

[40] T. L. Hectors, C. Vanparys, K. van der Ven et al., "Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function," *Diabetologia*, vol. 54, no. 6, pp. 1273–1290, 2011.

[41] M. E. Jørgensen, K. Borch-Johnsen, and P. Bjerregaard, "A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland Inuit," *Diabetologia*, vol. 51, no. 8, pp. 1416–1422, 2008.

[42] D. H. Lee, I. N. K. Lee, S. H. Jin, M. Steffes, and D. R. Jacobs, "Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002," *Diabetes Care*, vol. 30, no. 3, pp. 622–628, 2007.

[43] D. H. Lee, I. K. Lee, M. Porta, M. Steffes, and D. R. Jacobs, "Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002," *Diabetologia*, vol. 50, no. 9, pp. 1841–1851, 2007.

[44] J. S. Llm, D. H. Lee, and D. R. Jacobs, "Association of brominated flame retardants with diabetes and metabolic syndrome in the U.S. population, 2003-2004," *Diabetes Care*, vol. 31, no. 9, pp. 1802–1807, 2008.

[45] J. F. Heilier, J. Donnez, and D. Lison, "Organochlorines and endometriosis: a mini-review," *Chemosphere*, vol. 71, no. 2, pp. 203–210, 2008.

[46] US Environmental Protection Agency. Basic Information on Indoor Air Quality: Volatile Organic Compounds, 2011, http://www.epa.gov/iaq/voc.html .

[47] International Fragrance Association. Ingredients, 2011, http://www.ifraorg.org/en-us/Ingredients_2.

[48] B. Bridges, "Fragrance: emerging health and environmental concerns," *Flavour and Fragrance Journal*, vol. 17, no. 5, pp. 361–371, 2002.

[49] A. Schnuch, W. Uter, J. Geier, H. Lessmann, and P. J. Frosch, "Sensitization to 26 fragrances to be labelled according to current European regulation: results of the IVDK and review of the literature," *Contact Dermatitis*, vol. 57, no. 1, pp. 1–10, 2007.

[50] D. Lithner, A. Larsson, and G. Dave, "Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition," *Science of the Total Environment*, vol. 409, no. 18, pp. 3309–3324, 2011.

[51] S. E. Gomez-Mejiba, Z. Zhai, H. Akram et al., "Inhalation of environmental stressors & chronic inflammation: autoimmunity and neurodegeneration," *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*, vol. 674, no. 1-2, pp. 62–72, 2009.

[52] R. Franco, R. Sánchez-Olea, E. M. Reyes-Reyes, and M. I. Panayiotidis, "Environmental toxicity, oxidative stress and apoptosis: Ménage à Trois," *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*, vol. 674, no. 1-2, pp. 3–22, 2009.

[53] L. Klareskog, L. Padyukov, J. Rönnelid, and L. Alfredsson, "Genes, environment and immunity in the development of rheumatoid arthritis," *Current Opinion in Immunology*, vol. 18, no. 6, pp. 650–655, 2006.

[54] M. F. Prummel, T. Strieder, and W. M. Wiersinga, "The environment and autoimmune thyroid diseases," *European Journal of Endocrinology*, vol. 150, no. 5, pp. 605–618, 2004.

[55] S. Reuter, S. C. Gupta, M. M. Chaturvedi, and B. B. Aggarwal, "Oxidative stress, inflammation, and cancer: how are they linked?" *Free Radical Biology and Medicine*, vol. 49, no. 11, pp. 1603–1616, 2010.

[56] M. C. Houston, "The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction," *Alternative Therapies in Health and Medicine*, vol. 13, no. 2, pp. S128–S133, 2007.

[57] C. H. Tseng, "Cardiovascular disease in arsenic-exposed subjects living in the arseniasis-hyperendemic areas in Taiwan," *Atherosclerosis*, vol. 199, no. 1, pp. 12–18, 2008.

[58] J. A. Araujo, "Particulate air pollution, systemic oxidative stress, inflammation, and atherosclerosis," *Air Quality, Atmosphere and Health*, vol. 4, no. 1, pp. 79–93, 2010.

[59] A. Navas-Acien, E. K. Silbergeld, R. Pastor-Barriuso, and E. Guallar, "Arsenic exposure and prevalence of type 2 diabetes in US adults," *JAMA*, vol. 300, no. 7, pp. 814–822, 2008.

[60] C. de Burbure, J. P. Buchet, A. Leroyer et al., "Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels," *Environmental Health Perspectives*, vol. 114, no. 4, pp. 584–590, 2006.

[61] M. Pall, "The NO/ONOO—cycle as the cause of fibromyalgia and related illnesses: etiology, explanation and effective therapy," in *New Research in Fibromyalgia*, chapter 2, Nova Science, Huntington, NY, USA, 2006.

[62] S. Mena, A. Ortega, and J. M. Estrela, "Oxidative stress in environmental-induced carcinogenesis," *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*, vol. 674, no. 1-2, pp. 36–44, 2009.

[63] T. Valente, A. Gella, X. Fernàndez-Busquets, M. Unzeta, and N. Durany, "Immunohistochemical analysis of human brain suggests pathological synergism of Alzheimer's disease and diabetes mellitus," *Neurobiology of Disease*, vol. 37, no. 1, pp. 67–76, 2010.

[64] C. Hölscher, "Diabetes as a risk factor for Alzheimer's disease: insulin signalling impairment in the brain as an alternative model of Alzheimer's disease," *Biochemical Society Transactions*, vol. 39, no. 4, pp. 891–897, 2011.

[65] M. Szyf, "The dynamic epigenome and its implications in toxicology," *Toxicological Sciences*, vol. 100, no. 1, pp. 7–23, 2007.

[66] K. E. Fujimura, N. A. Slusher, M. D. Cabana, and S. V. Lynch, "Role of the gut microbiota in defining human health,"

Expert Review of Anti-Infective Therapy, vol. 8, no. 4, pp. 435–454, 2010.

[67] A. Spor, O. Koren, and R. Ley, "Unravelling the effects of the environment and host genotype on the gut microbiome," *Nature Reviews Microbiology*, vol. 9, no. 4, pp. 279–290, 2011.

[68] T. R. van de Wiele, A. G. Oomen, J. Wragg et al., "Comparison of five in vitro digestion models to in vivo experimental results: lead bioaccessibility in the human gastrointestinal tract," *Journal of Environmental Science and Health—Part A*, vol. 42, no. 9, pp. 1203–1211, 2007.

[69] S. D. Bilbo, G. A. Wray, S. E. Perkins, and W. Parker, "Reconstitution of the human biome as the most reasonable solution for epidemics of allergic and autoimmune diseases," *Medical Hypotheses*, vol. 77, no. 4, pp. 494–504, 2011.

[70] T. van de Wiele, C. M. Gallawa, K. M. Kubachka et al., "Arsenic metabolism by human gut microbiota upon in vitro digestion of contaminated soils," *Environmental Health Perspectives*, vol. 118, no. 7, pp. 1004–1009, 2010.

[71] T. van de Wiele, L. Vanhaecke, C. Boeckaert et al., "Human colon microbiota transform polycyclic aromatic hydrocarbons to estrogenic metabolites," *Environmental Health Perspectives*, vol. 113, no. 1, pp. 6–10, 2005.

[72] K. S. Betts, "A study in balance: how microbiomes are changing the shape of environmental health," *Environmental Health Perspectives*, vol. 119, pp. 340–346, 2011.

[73] C. Lupp, M. L. Robertson, M. E. Wickham et al., "Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of enterobacteriaceae," *Cell Host and Microbe*, vol. 2, no. 2, pp. 119–129, 2007.

[74] A. Gonzalez, J. Stombaugh, C. Lozupone, P. J. Turnbaugh, J. I. Gordon, and R. Knight, "The mind-body-microbial continuum," *Dialogues in Clinical Neuroscience*, vol. 13, no. 1, pp. 55–62, 2011.

[75] D. Liao, Y. Duan, E. A. Whitsel et al., "Association of higher levels of ambient criteria pollutants with impaired cardiac autonomic control: a population-based study," *American Journal of Epidemiology*, vol. 159, no. 8, pp. 768–777, 2004.

[76] M. Rönn, L. Lind, B. V. Bavel, S. Salihovic, K. Michaëlsson, and P. Monica Lind, "Circulating levels of persistent organic pollutants associate in divergent ways to fat mass measured by DXA in humans," *Chemosphere*, vol. 85, no. 3, pp. 335–343, 2011.

[77] O. Hue, J. Marcotte, F. Berrigan et al., "Increased plasma levels of toxic pollutants accompanying weight loss induced by hypocaloric diet or by bariatric surgery," *Obesity Surgery*, vol. 16, no. 9, pp. 1145–1154, 2006.

[78] D. T. Lin-Tan, J. L. Lin, T. H. Yen, K. H. Chen, and Y. L. Huang, "Long-term outcome of repeated lead chelation therapy in progressive non-diabetic chronic kidney diseases," *Nephrology Dialysis Transplantation*, vol. 22, no. 10, pp. 2924–2931, 2007.

[79] ClinicalTrials.gov. US National Institutes of Health. Trial to Assess Chelation Therapy (TACT), 2011, http://clinicaltrials.gov/ct2/show/NCT00044213?intr=%22EDTA+chelation+therapy%22&rank=1.

[80] A. Miodovnik, "Environmental neurotoxicants and developing brain," *Mount Sinai Journal of Medicine*, vol. 78, no. 1, pp. 58–77, 2011.

[81] M. Harada, "Minamata disease: methylmercury poisoning in Japan caused by environmental pollution," *Critical Reviews in Toxicology*, vol. 25, no. 1, pp. 1–24, 1995.

[82] H. Hu, M. M. Téllez-Rojo, D. Bellinger et al., "Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development," *Environmental Health Perspectives*, vol. 114, no. 11, pp. 1730–1735, 2006.

[83] R. Ronchetti, P. Van Den Hazel, G. Schoeters et al., "Lead neurotoxicity in children: is prenatal exposure more important than postnatal exposure?" *Acta Paediatrica, International Journal of Paediatrics*, vol. 95, no. 453, pp. 45–49, 2006.

[84] P. Grandjean and P. Landrigan, "Developmental neurotoxicity of industrial chemicals," *The Lancet*, vol. 368, no. 9553, pp. 2167–2178, 2006.

[85] M. S. Golub, "Adolescent health and the environment," *Environmental Health Perspectives*, vol. 108, no. 4, pp. 355–362, 2000.

[86] F. J. Miller, P. M. Schlosser, and D. B. Janszen, "Haber's rule: a special case in a family of curves relating concentration and duration of exposure to a fixed level of response for a given endpoint," *Toxicology*, vol. 149, no. 1, pp. 21–34, 2000.

[87] S. J. Genuis, D. Birkholz, I. Rodushkin, and S. Beesoon, "Blood, urine, and sweat (BUS) study: monitoring and elimination of bioaccumulated toxic elements," *Archives of Environmental Contamination and Toxicology*, vol. 61, no. 2, pp. 344–357, 2011.

[88] S. J. Genuis, D. Birkholz, M. Ralitsch, and N. Thibault, "Human detoxification of perfluorinated compounds," *Public Health*, vol. 124, no. 7, pp. 367–375, 2010.

[89] C. B. Jiang, C. Y. Yeh, H. C. Lee et al., "Mercury concentration in meconium and risk assessment of fish consumption among pregnant women in Taiwan," *Science of the Total Environment*, vol. 408, no. 3, pp. 518–523, 2010.

[90] J. B. Adams, J. Romdalvik, V. M. S. Ramanujam, and M. S. Legator, "Mercury, lead, and zinc in baby teeth of children with autism versus controls," *Journal of Toxicology and Environmental Health—Part A*, vol. 70, no. 12, pp. 1046–1051, 2007.

[91] Statement of John Stephenson, Director Natural Resources and the Environment. Chemical Regulation. Options for Enhancing the Effectiveness of the Toxic Substances Control Act. Testimony Before the Subcommittee on Commerce, Trade, and Consumer Protection,Committee on Energy and Commerce, House of Representatives. United States Government Accountability Office, 2009, http://www.gao.gov/new.items/d09428t.pdf.

[92] European Chemicals Agency. REACH Frequently Asked Questions, 2011, http://echa.europa.eu/reach/reach_faq_en.asp?topic=general&#general.

[93] D. A. Cory-Slechta, "Studying toxicants as single chemicals: does this strategy adequately identify neurotoxic risk?" *NeuroToxicology*, vol. 26, no. 4, pp. 491–510, 2005.

[94] Agency for Toxic Substances and Disease Registry. Interaction Profiles Home Page, 2010, http://www.atsdr.cdc.gov/interactionprofiles/index.asp.

[95] D. Biello, Robot Allows High-Speed Testing of Chemicals. Scientific American, 2011, http://www.scientificamerican.com/article.cfm?id=robot-allows-high-speed-chemical-testing.

[96] J. P. Myers, R. T. Zoeller, and F. S. vom Saal, "A clash of old and new scientific concepts in toxicity, with important implications for public health," *Environmental Health Perspectives*, vol. 117, no. 11, pp. 1652–1655, 2009.

[97] American Chemical Society. Testing for Endocrine Disruption. Public Policy Statement 2009–2012, 2009, http://portal.acs.org/portal/PublicWebSite/policy/publicpolicies/promote/endocrinedisruptors/CNBP_023441.

[98] K. E. Nachman, M. A. Fox, M. C. Sheehan, T. A. Burke, J. V. Rodricks, and T. J. Woodruff, "Leveraging epidemiology

to improve risk assessment," *The Open Epidemiology Journal*, vol. 4, pp. 3–29, 2011.

[99] Swedish Chemicals Agency (KEMI). The Substitution Principle, Stockholm, Sweden, 2007, http://www.kemi.se/upload/Trycksaker/Pdf/Rapporter/Report8_07_The_Substitution_Principle.pdf.

[100] Environmental Working Group. EWG's Shopper's Guide to Pesticides, 2011, http://www.ewg.org/foodnews/.

[101] David Suzuki Foundation. "Dirty Dozen" cosmetic chemicals to avoid, 2011, http://www.davidsuzuki.org/issues/health/science/toxics/dirty-dozen-cosmetic-chemicals/.

[102] The First Six Years. Renfrew County House Lead Risk Search, 2011, http://www.first6years.org/?q=node/14.

[103] S. J. Genuis, "Nowhere to hide: chemical toxicants and the unborn child," *Reproductive Toxicology*, vol. 28, no. 1, pp. 115–116, 2009.

[104] New York Press. The Rundown on Scientology's Purification Rundown, 2011, http://www.nypress.com/article-16488-the-rundown-on-scientologys-purification-rundown.html.

[105] S. J. Genuis, "Elimination of persistent toxicants from the human body," *Human and Experimental Toxicology*, vol. 30, no. 1, pp. 3–18, 2011.

[106] Y. Alcaraz-Contreras, L. Garza-Ocañas, K. Carcaño-Díaz, and X. S. Ramírez-Gómez, "Effect of glycine on lead mobilization, lead-induced oxidative stress, and hepatic toxicity in rats," *Journal of Toxicology*, vol. 2011, Article ID 430539, 7 pages, 2011.

[107] D. Nandi, R. C. Patra, and D. Swarup, "Effect of cysteine, methionine, ascorbic acid and thiamine on arsenic-induced oxidative stress and biochemical alterations in rats," *Toxicology*, vol. 211, no. 1-2, pp. 26–35, 2005.

[108] P. Hoet, J. P. Buchet, L. Decerf, B. Lavalleye, V. Haufroid, and D. Lison, "Clinical evaluation of a lead mobilization test using the chelating agent dimercaptosuccinic acid," *Clinical Chemistry*, vol. 52, no. 1, pp. 88–96, 2006.

[109] W. J. Crinnion, "The benefits of pre- and post-challenge urine heavy metal testing: part 1," *Alternative Medicine Review*, vol. 14, no. 1, pp. 3–8, 2009.

[110] R. J. Jandacek, N. Anderson, M. Liu, S. Zheng, Q. Yang, and P. Tso, "Effects of yo-yo diet, caloric restriction, and olestra on tissue distribution of hexachlorobenzene," *American Journal of Physiology*, vol. 288, no. 2, pp. G292–G299, 2005.

[111] G. W. Yu, J. Laseter, and C. Mylander, "Persistent organic pollutants in serum and several different fat compartments in humans," *Journal of Environmental and Public Health*, vol. 2011, Article ID 417980, 8 pages, 2011.

[112] J. B. Adams, J. Romdalvik, K. E. Levine, and L. W. Hu, "Mercury in first-cut baby hair of children with autism versus typically-developing children," *Toxicological and Environmental Chemistry*, vol. 90, no. 4, pp. 739–753, 2008.

[113] J. B. Adams, M. Baral, E. Geis et al., "The severity of autism is associated with toxic metal body burden and red blood cell glutathione levels," *Journal of Toxicology*, vol. 2009, Article ID 532640, 7 pages, 2009.

[114] D. A. Geier, P. G. King, L. K. Sykes, and M. R. Geier, "A comprehensive review of mercury provoked autism," *Indian Journal of Medical Research*, vol. 128, no. 4, pp. 383–411, 2008.

[115] L. Marshall, Taking An Exposure History, 2004, http://www.ocfp.on.ca/docs/public-policy-documents/taking-an-exposure-history.pdf?Status=Master#d14b9fba-3da2-4216-a446-980b43dbd82e.

[116] R. J. Jandacek and P. Tso, "Enterohepatic circulation of organochlorine compounds: a site for nutritional intervention," *Journal of Nutritional Biochemistry*, vol. 18, no. 3, pp. 163–167, 2007.

[117] M. E. Andersen, H. J. Clewell, Y. M. Tan, J. L. Butenhoff, and G. W. Olsen, "Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys-Probing the determinants of long plasma half-lives," *Toxicology*, vol. 227, no. 1-2, pp. 156–164, 2006.

[118] J. Moon, "The role of vitamin D in toxic metal absorption: a review," *Journal of the American College of Nutrition*, vol. 13, no. 6, pp. 559–564, 1994.

[119] A. Vojdani, "Antibodies as predictors of complex autoimmune diseases and cancer," *International Journal of Immunopathology and Pharmacology*, vol. 21, no. 3, pp. 553–566, 2008.

[120] N. Fucci, N. De Giovanni, and S. Scarlata, "Sweat testing in addicts under methadone treatment: an Italian experience," *Forensic Science International*, vol. 174, no. 2-3, pp. 107–110, 2008.

[121] D. W. Schnare and M. G. Shields, "Body burden reductions of PCBs, PBBs and chlorinated pesticides in human subjects," *Ambio*, vol. 13, no. 5-6, pp. 378–380, 1984.

[122] J. Dahlgren, M. Cecchini, H. Takhar, and O. Paepke, "Persistent organic pollutants in 9/11 world trade center rescue workers: reduction following detoxification," *Chemosphere*, vol. 69, no. 8, pp. 1320–1325, 2007.

[123] S. Genuis, S. Beesoon, D. Birkholz, and R. Lobo, "Human excretion of bisphenol-A: blood, urine and sweat (BUS) study," *Journal of Environmental and Public Health*. In press.

[124] M. Blanuša, V. M. Varnai, M. Piasek, and K. Kostial, "Chelators as antidotes of metal toxicity: therapeutic and experimental aspects," *Current Medicinal Chemistry*, vol. 12, no. 23, pp. 2771–2794, 2005.

[125] H. V. Aposhian, R. M. Maiorino, D. Gonzalez-Ramirez et al., "Mobilization of heavy metals by newer, therapeutically useful chelating agents," *Toxicology*, vol. 97, no. 1–3, pp. 23–38, 1995.

[126] S. J. Genuis, "Toxic causes of mental illness are overlooked," *NeuroToxicology*, vol. 29, no. 6, pp. 1147–1149, 2008.

[127] R. C. Shoemaker and D. E. House, "Sick building syndrome (SBS) and exposure to water-damaged buildings: time series study, clinical trial and mechanisms," *Neurotoxicology and Teratology*, vol. 28, no. 5, pp. 573–588, 2006.

[128] P. Grandjean and K. T. Herz, "Methylmercury and brain development: imprecision and underestimation of developmental neurotoxicity in humans," *Mount Sinai Journal of Medicine*, vol. 78, no. 1, pp. 107–118, 2011.

[129] N. V. C. Ralston and L. J. Raymond, "Dietary selenium's protective effects against methylmercury toxicity," *Toxicology*, vol. 278, no. 1, pp. 112–123, 2010.

[130] S. Daniel, J. L. Limson, A. Dairam, G. M. Watkins, and S. Daya, "Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain," *Journal of Inorganic Biochemistry*, vol. 98, no. 2, pp. 266–275, 2004.

[131] K. F. Ola-Mudathir, S. M. Suru, M. A. Fafunso, U. E. Obioha, and T. Y. Faremi, "Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats," *Food and Chemical Toxicology*, vol. 46, no. 12, pp. 3604–3611, 2008.

[132] S. Milton Prabu, K. Shagirtha, and J. Renugadevi, "Quercetin in combination with vitamins (C and E) improves oxidative stress and renal injury in cadmium intoxicated rats,"

European Review for Medical and Pharmacological Sciences, vol. 14, no. 11, pp. 903–914, 2010.

[133] I. Messaoudi, J. El Heni, F. Hammouda, K. Saïd, and A. Kerkeni, "Protective effects of selenium, zinc, or their combination on cadmium-induced oxidative stress in rat kidney," *Biological Trace Element Research*, vol. 130, no. 2, pp. 152–161, 2009.

[134] T. Uchikawa, Y. Kumamoto, I. Maruyama, S. Kumamoto, Y. Ando, and A. Yasutake, "The enhanced elimination of tis- sue methylmercury in Parachlorella beijerinckii-fed mice," *Journal of Toxicological Sciences*, vol. 36, no. 1, pp. 121–126, 2011.

[135] T. Uchikawa, A. Yasutake, Y. Kumamoto, I. Maruyama, S. Kumamoto, and Y. Ando, "The influence of Parachlorella beyerinckii CK-5 on the absorption and excretion of methylmercury (MeHg) in mice," *Journal of Toxicological Sciences*, vol. 35, no. 1, pp. 101–105, 2010.

[136] K. Morita, M. Ogata, and T. Hasegawa, "Chlorophyll derived from Chlorella inhibits dioxin adsorption from the gastrointestinal tract and accelerates dioxin excretion in rats," *Environmental Health Perspectives*, vol. 109, no. 3, pp. 289–294, 2001.

[137] H. Takekoshi, G. Suzuki, H. Chubachi, and M. Nakano, "Effect of Chlorella pyrenoidosa on fecal excretion and liver accumulation of polychlorinated dibenzo-p-dioxin in mice," *Chemosphere*, vol. 59, no. 2, pp. 297–304, 2005.

[138] J. L. Domingo, M. Gomez, and J. M. Llobet, "Citric, malic and succinic acids as possible alternatives to deferoxamine in aluminum toxicity," *Journal of Toxicology—Clinical Toxicology*, vol. 26, no. 1-2, pp. 67–79, 1988.

[139] C. C. Lim, L. R. Ferguson, and G. W. Tannock, "Dietary fibres as "prebiotics": implications for colorectal cancer," *Molecular Nutrition and Food Research*, vol. 49, no. 6, pp. 609–619, 2005.

[140] V. Eybl, D. Kotyzova, and J. Koutensky, "Comparative study of natural antioxidants—curcumin, resveratrol and melatonin—in cadmium-induced oxidative damage in mice," *Toxicology*, vol. 225, no. 2-3, pp. 150–156, 2006.

[141] C. White, S. C. Wilkinson, and G. M. Gadd, "The role of microorganisms in biosorption of toxic metals and radionuclides," *International Biodeterioration and Biodegradation*, vol. 35, no. 1–3, pp. 17–40, 1995.

[142] J. A. Bourdon, T. M. Bazinet, T. T. Arnason, L. E. Kimpe, J. M. Blais, and P. A. White, "Polychlorinated biphenyls (PCBs) contamination and aryl hydrocarbon receptor (AhR) agonist activity of Omega-3 polyunsaturated fatty acid supplements: implications for daily intake of dioxins and PCBs," *Food and Chemical Toxicology*, vol. 48, no. 11, pp. 3093–3097, 2010.

[143] J. C. P. Mattos, M. Hahn, P. R. Augusti et al., "Lead content of dietary calcium supplements available in Brazil," *Food Additives and Contaminants*, vol. 23, no. 2, pp. 133–139, 2006.

[144] V. Nadeau, G. Truchon, M. Brochu, and R. Tardif, "Effect of physical exertion on the biological monitoring of exposure of various solvents following exposure by inhalation in human volunteers: I. Toluene," *Journal of Occupational and Environmental Hygiene*, vol. 3, no. 9, pp. 481–489, 2006.

[145] R. Tardif, V. Nadeau, G. Truchon, and M. Brochu, "Effect of physical exertion on the biological monitoring of exposure to various solvents following exposure by inhalation in human volunteers: II. n-Hexane," *Journal of Occupational and Environmental Hygiene*, vol. 4, no. 7, pp. 502–508, 2007.

[146] H. V. Aposhian, "Mobilization of mercury and arsenic in humans by sodium 2,3-dimercapto-1-propane sulfonate (DMPS)," *Environmental Health Perspectives*, vol. 106, supplement 4, pp. 1017–1025, 1998.

[147] J. B. Adams, M. Baral, E. Geis et al., "Safety and efficacy of oral DMSA therapy for children with autism spectrum disorders: part B—behavioral results," *BMC Clinical Pharmacology*, vol. 9, article 17, 2009.

[148] D. Hastings, B. Patel, A. S. Torioni et al., "Plasmapheresis therapy for rare but potentially fatal reaction to rituximab," *Journal of Clinical Apheresis*, vol. 24, no. 1, pp. 28–31, 2009.

[149] M. Emet, S. Aslan, Z. G. Cakir et al., "Plasmapheresis is useful in phenprobamate overdose," *American Journal of Emergency Medicine*, vol. 27, no. 5, pp. 626.e1–626.e2, 2009.

[150] S. A. Rogers, "Lipoic acid as a potential first agent for protection from mycotoxins and treatment of mycotoxicosis," *Archives of Environmental Health*, vol. 58, no. 8, pp. 528–532, 2003.

[151] S. Genuis, "What's out there making us sick?" *Journal of Environmental and Public Health*, vol. 2012, 10 pages, 2012.

[152] S. David and M. D. Jones, Eds., *Textbook of Functional Medicine*, Institute for Functional Medicine, Gig Harbor, Wash, USA, 3rd edition, 2010.

[153] H. W. Gil, E. J. Kang, K. H. Lee, J. O. Yang, E. Y. Lee, and S. Y. Hong, "Effect of glutathione on the cadmium chelation of EDTA in a patient with cadmium intoxication," *Human and Experimental Toxicology*, vol. 30, no. 1, pp. 79–83, 2011.

[154] J. E. Heck, M. V. Gamble, Y. Chen et al., "Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh," *American Journal of Clinical Nutrition*, vol. 85, no. 5, pp. 1367–1374, 2007.

[155] S. Nakano, T. Noguchi, H. Takekoshi, G. Suzuki, and M. Nakano, "Maternal-fetal distribution and transfer of dioxins in pregnant women in Japan, and attempts to reduce maternal transfer with Chlorella (Chlorella pyrenoidosa) supplements," *Chemosphere*, vol. 61, no. 9, pp. 1244–1255, 2005.

[156] S. K. Genuis and S. J. Genuis, "Exploring the continuum: medical information to effective clinical practice∗. Paper I: the translation of knowledge into clinical practice," *Journal of Evaluation in Clinical Practice*, vol. 12, no. 1, pp. 49–62, 2006.

[157] S. Doherty, "History of evidence-based medicine. Oranges, chloride of lime and leeches: barriers to teaching old dogs new tricks," *EMA*, vol. 17, no. 4, pp. 314–321, 2005.

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Review Article

A Safe Protocol for Amalgam Removal

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Today's environment has different impacts on our body than previous generations. Heavy metals are a growing concern in medicine. Doctors and individuals request the removal of their amalgam (silver mercury) restorations due to the high mercury content. A safe protocol to replace the silver mercury filling will ensure that there is minimal if any absorption of materials while being removed. Strong alternative white composite and lab-processed materials are available today to create a healthy and functioning mouth. Preparation of the patient prior to the procedure and after treatment is vital to establish the excretion of the mercury from the body.

1. Introduction

In dentistry, there is a lot of controversy about the topic of silver mercury fillings; are they safe or not safe? There are many articles written on the pros and cons of these types of fillings. It is difficult to quantify and to assess the effects in each individual. It is not easy to identify silver mercury fillings as the cause if illness presents or if the fillings contributed to illness, except in extreme toxicity cases. Refer to the beginning sections of this review paper concerning the science and mechanism of how mercury interconnects with body tissues and functions.

Environmental doctors investigate heavy metal toxicity as part of their overall wellness regiment to help their patients with health concerns. These doctors look at sources of metals when the patient's lab reports/diagnostic tests show high levels of mercury and other metals. They investigate what sources are contributing and how to reduce the burden on the body. The doctor may prescribe the safe removal of silver mercury fillings so as not to create an additional burden on the body and to help their patient heal. Thus, when removing amalgams, additional steps help ensure that the patient is protected.

2. Introduction of Amalgam in Dentistry

Dental amalgam restorations, also called silver mercury fillings, were introduced to North America in the 1830s and have been the standard restorative filling for our molars and premolars. At that time there was a lot of controversy about its intraoral use. Silver mercury fillings began to take over the cast gold and gold foil restorations. These were excellent and lasted for years; however they were labour intensive and the cast gold required a lab process that centrifuged gold into a wax pattern to fit the tooth accurately. This was a two-appointment process with added expense. Gold foil restorations were often traumatic to the pulp of the tooth, creating necrosis and requiring root canal. The addition of amalgams as a restorative filling was a welcomed opportunity to offer at a substantial cost reduction as the mercury was triturated with a pellet containing silver, copper, tin, and zinc. This created a substance that could be placed into the cleaned out tooth structure where decay had been present. It was packed, condensed, and allowed to harden within a few minutes and then carved intraoral chairside. Today the extra, unused amalgam is placed in a container for safe disposal. This restoration is easily burnished to tooth structure to recreate the tooth to its original shape and size. The onset of amalgam allowed people to keep their teeth, rather than having them extracted if money did not allow for gold restorations. Keeping teeth enabled people to have better digestion and supported a more balanced quality of life.

Today, with the increase of chemicals such as pesticides, preservatives, processed ingredients in food, and diverse contaminants in our environment; sensitivities, allergies, and

other illnesses are increasing rapidly. *The Brain Wash* postulates that the toxins in our society are not additive but synergistic. For example, the average apple contains residue of eleven different neurotoxins and is sprayed with pesticides seventeen times prior to being picked from a tree [1]. Our food intake of many pesticides and additives is most often unknown. The level of materials such as mercury that our bodies could tolerate several decades ago may not be what we can sustain today.

3. Amalgam and Composite Fillings

Silver mercury amalgam restorations are comprised of 50% mercury, with the balance being silver, copper, tin, and zinc [2]. Over time the exposed surface changes. The fillings corrode, and surface texture becomes rough. People who chew gum create a smooth, shiny surface on their fillings. Mercury vapor is released by chewing grains, nuts, seeds, and gum, as detected using mercury vapor analyzers [3]. A study in 2010 looked at the wearability of composite (white) restorations compared to amalgams. It showed that over 12 years, the group of patients that were not prone to decay, with resin/composite-filled restorations, were better off than the group of patients with silver amalgam restorations [4]. Today with awareness of diet, home care, and education, the majority of people who seek preventative dental care are less prone to decay. The author has worked with alternative restorations for over 27 years.

The advantage of white composite restorations is that composite binds to composite and the base of the tooth rarely needs to be disturbed once the amalgams are removed. Dental restorative materials have various components, and individual Material Safety Data Sheets (MSDSs) are available from the manufacturer. If an individual has concerns or is sensitive to materials, one can refer to these reference sheets. For example, there are many composites and bonds available today without bisphenol A. Psychological benefits are also a positive factor for patients. People feel that they now have a mouth without the "scars" of the past. They are no longer self-conscious when smiling, laughing, and singing.

With the introduction of composite restorations, many modifications have been made with the materials and applications due to the extensive ongoing technology and research. The concerns with good marginal seals and prevention of recurrent decay have been diminished. Wear and polishability of the composite materials with nanohybrid particulates can withstand stronger chewing forces. Composites are technique sensitive, and various aids can be used to ensure a proper seal of the restorative material to the tooth structure and to create tight contacts to the adjacent tooth to prevent food impaction between teeth. Today we aim for minimally invasive dentistry to maintain integrity of the tooth structure, and white composite materials are ideal for these restorations.

4. Considerations prior to Amalgam Removal

When examining a patient for amalgam removal upon request, many factors must be looked at including the rate of wear/attrition on their teeth, pressures exerted, type of diet consumed on a daily basis, their oral hygiene, and other metals in their mouth. Often amalgam restorations exist under crowns and amalgam tattoos (discoloration along the gum) are noted. Amalgams have also been used to seal the apex of root canal treated teeth. If heavy pressures are exerted by an individual or there is evidence of grinding and clenching, then the longevity of a composite restoration may be compromised. The size of the restoration will also influence the choice of materials. Tooth cusps often fracture over time, as well as with excessive pressure, requiring an indirect restoration to be fabricated by a lab. Today the increasing trend is to work with a computer-generated restoration to secure/repair the tooth in the long term. Bite plates to prevent grinding and clenching help preserve these new restorations from excessive wear and pressure.

When the patient is seen for an initial exam, a thorough medical and dental history is taken. Records including radiographs and intraoral pictures are taken, and a comprehensive exam follows. Previous films are requested or brought in by the patient. Lengthy conversations ensue to make sure that the patient is properly prepared and that we are working with their physician, in a timely manner, to complement the detoxification process that their doctor has prescribed and is administering. The physician evaluates the overall health of the body and the ability of the individual to eliminate toxins. For example, if a patient has a leaky gut, physicians restore this prior to removal as it is difficult to flush out toxins [5]. If a woman is pregnant or breast feeding, amalgam removal does not occur until she has completed breast feeding her child [6]. It has been reported that the mercury concentration in the blood of the fetus can be thirty times greater than the mother's blood [7]. Supplements are helpful and are prescribed on an individual basis by the physician. Vitamin C intake is recommended, often with other supplements, prior to and following amalgam removal. Once the amalgam restorations have been removed, the physician continues to work with the patient to help with the detoxification of mercury that is stored in the body.

5. Chairside Procedures

The following steps are taken when removing silver mercury fillings, to ensure minimal if any absorption sublingually, or through the mucosal tissues, and to minimize mercury vapor absorption through the blood/brain barrier [8–10].

In office, the patient is prepared as follows, prior to amalgam removal:

(i) the patient is draped with a plastic apron under the dental bib to cover their clothing;

(ii) a dental dam ("raincoat") is customized to fit the existing tooth/teeth to prevent particulates from contacting the oral mucosa;

(iii) underneath the dam, activated charcoal or chlorella is placed, along with a cotton roll and gauze. This helps to intercept particles and to chelate dissolved metals that seep under the dam. Often the particles are

found on the sublingual tissues and lateral borders of the tongue. This must be prevented as this is the fastest absorption route into the body;

(iv) the patient's face is draped under the dam, with a liner;

(v) goggles for the eyes and hair cap or bonnet protection are placed;

(vi) oxygen is supplied to the patient with a nasal mask and the mercury vapor ionizer is turned on. The vapor ionizer is a specialized air filtration system that is used to bind mercury vapors that are attached by the negative ion flow and are then carried to a positively charged ionizer plate at the opposite end of the room.

The operators also protect themselves with a filtered mask, eye and hair protection, and face shields.

The removal of amalgam commences as follows:

(i) a new dental bur is used in the handpiece to ensure easy removal;

(ii) high volume suction and a continual addition of water spray are supplied to the site where the amalgam is being extracted;

(iii) if possible, the amalgam restoration is sectioned and then scooped out to eliminate as much mercury vapor release as possible [11]. The vitality of the tooth is always a concern and the less trauma to the tooth, the healthier the pulp, which supplies blood vessels and nerve supply to the tooth. The deeper the restoration, the greater the chance of pulpal degeneration, causing necrosis and subsequent abscess at the apex of the tooth, as well as bone loss.

Once the amalgam is removed completely,

(i) the oxygen and protective coverings are taken away;

(ii) an immediate inspection under the dental dam occurs. The gauze, cotton roll and activated charcoal/chlorella are wiped away. Gauze is then used to inspect the floor of the mouth and tongue to make sure no particulates seeped under the dam;

(iii) once all mucosal tissues are fully inspected and cleaned, the mouth is flushed with copious amounts of water, again to ensure no ingestion or absorption of amalgam particulates.

The tooth is then restored to a healthy state of form and function. Materials are taken into consideration as discussed previously on an individual need. Often environmental healthcare providers give direction on the preferred choice of materials to be used through biocompatibility testing. It is the dentist's ultimate responsibility to advise the patient about the strengths and limitations, if they cannot tolerate some materials. It has been the author's experience that once the amalgam materials have been removed and the patient detoxes under the supervision of their physician, the range and variety of materials increase, allowing the dentist to create the best prognosis for the tooth.

Dentists by law in Ontario [12] and elsewhere in Canada must have a certified amalgam separator on the wastewater lines in dental offices in their practices and must use a certified hazardous waste carrier for the recycling and disposing of amalgam waste.

6. After Amalgam Removal

A 2011 Norwegian study showed a 3-year followup after amalgam removal with precautions in a treatment group compared to a reference group. It showed significant reductions in intraoral and general health complaints [13].

The following is a list of outcomes that I repeatedly hear from my patients over the years. Although I have not scientifically collected them, after amalgam removal and detoxification, they have also been reported in the literature. Comments include that

(a) patients no longer have a metallic taste in their mouth;

(b) patients feel as if they have more energy;

(c) patients are able to concentrate better and make decisions easier (the "brain fog" is gone);

(d) their body responds better to other treatments, as if a barrier has been lifted.

To achieve effective results one must include an integrative approach with a physician and health care team with attention to detoxification and diet over several months, with laboratory tests to monitor progress.

Disclosure

Dr. D. G. Colson is a D.D.S. at Dr. Dana Colson & Associates as well as the author of "*Your Mouth: The Gateway to a Healthier You*."

References

[1] M. S. Cook, *The Brain Wash: A Powerful, All-Natural Program to Protect Your Brain Against Alzheimer's, Chronic Fatigue Syndrome, Depression, Parkinson's, and other Diseases*, John Wiley & Sons, Mississauga, Canada, 2007.

[2] B. M. Eley, "The future of dental amalgam: a review of the literature part 1 : dental amalgam structure and corrosion," *British Dental Journal*, vol. 182, no. 7, pp. 247–249, 1997.

[3] G. M. Richardson, R. Wilson, D. Allard, C. Purtill, S. Douma, and J. Gravière, "Mercury exposure and risks from dental amalgam in the US population, post-2000," *The Science of the Total Environment*, vol. 409, no. 20, pp. 4257–4268, 2011.

[4] N. J. M. Opdam, E. M. C. Bronkhorst, B. A. Loomans, and M.-C Huysmans, "12-year survival of composite vs. amalgam restorations," *Journal of Dental Research*, vol. 89, no. 10, pp. 1063–1067, 2010.

[5] D. Hollander, "Intestinal permability, leaky gut, and intestinal disorders," *Current Gastroenterology Reports*, vol. 1, no. 5, pp. 410–416, 1999.

[6] The Safety of Dental Amalgam, *Health Canada*, Department of Supply and Services Canada, 1996.

[7] B. J. Koos and L. D. Longo, "Mercury toxicity in the pregnant woman, fetus, and newborn infant. A review," *American Journal of Obstetrics and Gynecology*, vol. 126, no. 3, pp. 390–409, 1976.
[8] M. Nylander, L. Friberg, and B. Lind, "Mercury concentrations in the human brain and kidneys in relation to exposure from dental amalgam fillings," *Swedish Dental Journal*, vol. 11, no. 5, pp. 179–187, 1987.
[9] T. W. Clarkson, "Metal toxicity in the central nervous system," *Environmental Health Perspectives*, vol. 75, pp. 59–64, 1987.
[10] F. L. Lorscheider, M. J. Vimy, and A. O. Summers, "Mercury exposure from "silver" tooth fillings: emerging evidence questions a traditional dental paradigm," *The FASEB Journal*, vol. 9, no. 7, pp. 504–508, 1995.
[11] S. M. Koral, *IAOMT Safe Removal of Amalgam Fillings*, International Academy of Oral Medicine & Toxicology, 2007.
[12] Ontario. Service Ontario, *Dentistry Act*, chapter 24, 1991.
[13] T. T. Sjursen, G. B. Lygre, K. Dalen et al., "Changes in health complaints after removal of amalgam fillings," *Journal of Oral Rehabilitation*, vol. 38, no. 11, pp. 835–848, 2011.

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Clinical Study

Combination of Micronutrients for Bone (COMB) Study: Bone Density after Micronutrient Intervention

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Along with other investigations, patients presenting to an environmental health clinic with various chronic conditions were assessed for bone health status. Individuals with compromised bone strength were educated about skeletal health issues and provided with therapeutic options for potential amelioration of their bone health. Patients who declined pharmacotherapy or who previously experienced failure of drug treatment were offered other options including supplemental micronutrients identified in the medical literature as sometimes having a positive impact on bone mineral density (BMD). After 12 months of consecutive supplemental micronutrient therapy with a combination that included vitamin D_3, vitamin K_2, strontium, magnesium and docosahexaenoic acid (DHA), repeat bone densitometry was performed. The results were analyzed in a group of compliant patients and demonstrate improved BMD in patients classified with normal, osteopenic and osteoporotic bone density. According to the results, this combined micronutrient supplementation regimen appears to be at least as effective as bisphosphonates or strontium ranelate in raising BMD levels in hip, spine, and femoral neck sites. No fractures occurred in the group taking the micronutrient protocol. This micronutrient regimen also appears to show efficacy in individuals where bisphosphonate therapy was previously unsuccessful in maintaining or raising BMD. Prospective clinical trials are required to confirm efficacy.

1. Introduction

Disordered bone health is an age-related illness that affects an increasing proportion of the population in many western nations. Throughout much of the developed world, the fastest growing segment of the current population is the baby-boomer generation, the group born during the post-WWII baby boom that is rapidly approaching retirement. According to the Statistics Canada 2006 Census, for example, baby-boomers account for one-third of the country's 32 million people, 20% of which are in the 55–64 age class and soon to leave the workforce [1]. Older patients with low bone density are at high risk for falls and fragility fractures [2], which in turn cause considerable morbidity and subsequent mortality as well as exerting an enormous financial burden on public health care systems [3]. With an aging population, prevention of age-related diseases including osteoporosis and related fragility fractures will continue to play an important role in the sustainability and implementation of good personal and public health care.

As improved bone mineral density (BMD) has been associated with a diminished risk of fragility fractures, preferred BMD status in greater proportions of the population would deliver not only improved quality of life but also significant cost savings. With escalating rates of osteoporosis in various jurisdictions over the last decade, it would be desirable if primary prevention strategies to obviate the development of compromised bone health could be instituted as well as nontoxic interventions to restore bone strength in those with deficient BMD.

Recent clinical practice guidelines for the diagnosis and management of impaired bone health have primarily focused on pharmacologic therapy and lifestyle modifications to prevent fragility fractures and their adverse sequelae [4–6].

Pharmaceutical interventions to address abnormal bone density have focused to a great degree on antiresorptive bisphosphonates. Other medications considered in select situations may include other antiresorptive agents such as a human monoclonal antibody RANK ligand inhibitor (Denosumab), a bone forming analog to parathyroid hormone (Teriparatide), strontium ranelate, calcitonin, and hormonal replacement therapy or a selective estrogen receptor modulator for postmenopausal women [4, 7].

A recent study has shown, however, that lack of compliance with current osteoporosis protocols is putting the elderly at increased risk for fragility fractures and associated morbidity and mortality [8]. Moreover, increasing numbers of patients decline osteoporosis pharmacotherapy because of media attention to potential adverse effects and legal proceedings related to outcomes alleged to be connected with some osteoporosis medications. For example, recent concerns about long-term hormone replacement therapy [9, 10] and media reports about atypical fractures [11], osteonecrosis [12], atrial fibrillation [13], and esophageal cancer [14] allegedly associated with bisphosphonate use has led some patients to pursue other approaches to ameliorate bone health despite the fact that the link between these medications and all the purported adverse side effects still remains controversial [13, 15].

Various micronutrients have recently been identified in the scientific and biochemistry literature as integral to the proper development, physiology, and maintenance of bone. Depletion of essential nutrients for bone health because of inadequate intake, impaired digestion, malabsorption, or disordered assimilation may result in deficient biochemistry, disordered biology, and resultant bone health compromise. Thus far, however, assessment and maintenance of nutritional adequacy in relation to the spectrum of essential compounds required for proper bone function has been limited, as micronutrient strategies have focused almost exclusively on calcium and vitamin D supplementation [4]. Recent guidelines have not yet incorporated the fact that some patients with osteoporosis may be malnourished in relation to other essential bone nutrients.

Recent research suggests that remediation of nutritional insufficiency and repletion of various biochemicals integral to healthy bone physiology may ameliorate bone health status [16, 17]. This retrospective cohort study, approved by the Health Ethics Research Board at the University of Alberta, assesses the value of the use of a combination of micronutrients on BMD status.

2. Methods

A review of the medical and scientific literature was undertaken to identify micronutrient elements associated with bone health status [16] by assessing available medical and scientific literature from MEDLINE/PubMed, as well as by reviewing numerous books, nutrition journals, and health periodicals, conference proceedings, and government publications. References cited in identified publications were also examined for additional relevant writings. The evidence base to support the role of specific essential micronutrients in bone status ranges from scant to very firm, depending on the compound [18]. Multiple studies have demonstrated that micronutrients (and drugs derived from nutrients) beyond just calcium and vitamin D have an impact on bone health. Vitamin K_2 [19, 20], strontium [16, 21–25], magnesium [26], and DHA [27–30] have all been implicated in improving the status of bones, but to our knowledge, none of these individual micronutrients have been assessed when given in combination.

The first author practices environmental medicine, where many referred patients present with long-term chronic disease. With the view that comprehensive fracture risk assessment should be a routine part of patient care, and the observation that patients with chronic disease have higher rates of bone compromise and frequently do not receive therapy to prevent fractures [31–33], bone health determination was established as a component of the overall clinical assessment in chronically ill patients. Starting in 2006, patients found to have suboptimal BMDs were provided with options for management, including micronutrient therapy. As well as discussion related to lifestyle and standard-of-care pharmaceutical interventions, the potential consequences of not intervening, and the scientific literature on the published efficacy of micronutrient interventions were presented to patients along with information on recommended clinical practice guidelines for compromised bone health.

Some patients adamantly refused to use pharmaceutical therapies while others reported they had previously discontinued such therapy because of continued loss of BMD. A portion of these individuals indicated interest in supplemental nutrients linked in the scientific literature to improved bone health status. As patients presenting to environmental health specialists often have chemical sensitivities [34], the reluctance expressed to using pharmaceuticals was sometimes related to a sensitivity to medications or excipients commonly used within dispensed drugs.

Patients wishing to explore micronutrient use were given medical literature detailing the benefits purported in various studies. Eager to ameliorate their bone health status if possible, some individuals chose to use micronutrients rather than using pharmacologic therapies or not using any intervention at all. After 12 months of consecutive micronutrient therapy, repeat BMDs were performed to assess for evidence of change. A retrospective review of the outcomes was undertaken to gather data for analysis.

2.1. Demographics and Inclusion Criteria. The population of patients for the study came from an environmental medicine clinic in Edmonton, Alberta, Canada. Out of 219 patients assessed, 16 were still in the process of taking supplements (data not yet complete) and 126 were excluded. Exclusion criteria included patients with recognized bone compromising medical conditions or those on medications known to potentially affect bone health. (e.g., 1 with anorexia nervosa and 1 on chemotherapy) as well as patients who had repeat BMD measurements inadvertently performed on different machines from the original (5 patients)—making comparison inaccurate. In addition, 37 patients were excluded

because they did not comply with therapy (the micronutrients were taken inconsistently—self-reported at less than half the time), 6 patients commencing the protocol changed their mind about taking the nutrients (for financial reasons), and 71 patients did not return at the end of the time period for follow-up BMD assessment or decided they did not want a repeat BMD and therefore had incomplete data. Two patients died during the course of the study, unrelated to the intervention (1 from ALS and the other from a motor vehicle accident). The final sample of 77 patients taking the micronutrient combination most or all of the time was included with complete data for analysis. Table 2 shows the demographics for the study group.

2.2. Protocol and Rationale. Each patient in the analysis who chose to use the micronutrient intervention followed a suggested daily protocol of supplemental nutrient consumption and exercise as described in Table 1. It was hypothesized that perhaps bone compromise might be related to nutritional insufficiency in some patients and that remediation of nutritional biochemistry may be of assistance in restoring bone health. Also, both DHA and vitamin D are involved in genetic regulation of many genes and restoration of optimal levels has been associated with improved bone strength [35–38]. Given the debate about the efficacy of calcium supplementation for reducing fractures [39, 40] and the potential risks associated with high-dose supplementation including renal calculi and cardiovascular events [40, 41], patients were advised to obtain calcium from dietary sources including vegetables such as Brussels sprouts or broccoli rather than calcium supplements. Patients were also instructed to commence and maintain a regimen of daily impact exercises such as jumping jacks or skipping where possible as impact has been associated with prevention of bone density loss [42, 43].

As BMD is a major determinant among several risk factors for predicting fragility fractures, BMD follow-up measurement was therefore used as an intervention outcome along with fall surveillance. The main areas analyzed for bone density included the lumbar spine, the femoral neck, and the femoral trochanter. In this study, we evaluated comparative differences in the femoral neck, total hip, and total spine in relation to previous studies. We also investigated change at the lowest hip and lowest spine sites to determine whether there was improvement in the areas that were the least dense and potentially the most vulnerable.

2.3. Statistics. Statistics were calculated with SPSS 18.0 (IBM Corporation, USA). Pre- and postintervention bone densities were compared using a one-way ANOVA with a P value threshold of 0.05. Changes were also evaluated using mean percentage change over one year to compare treatment effect with the bisphosphonates and strontium ranelate. In the analyses, z-scores were used in order to avoid any age-related bias in some other BMD scores.

In addition, a post hoc analysis of the noncompliant group ($N = 37$) was carried out to evaluate whether adherence to the combined micronutrient strategy was beneficial. One-way ANOVA with a P value threshold of 0.05 was again used to determine if this group showed a significant difference over the course of the one year period. Moreover, the percentage change at each site in this group was compared to the percentage change in the intervention group.

Table 1: Combination of micronutrients (COMB) Protocol for Bone Health.

COMB protocol for bone health
(1) Docosahexanoic acid or DHA (from Purified Fish Oil): 250 mg/day
(2) Vitamin D_3: 2000 IU/day
(3) Vitamin K_2 (non-synthetic MK_7 form): 100 ug/day
(4) Strontium citrate: 680 mg/day
(5) Elemental magnesium: 25 mg/day
(6) Dietary sources of calcium recommended
(7) Daily impact exercising encouraged

Table 2: Distribution of bone density diagnosis in the sample.

	Total (77)	Females (72)	Males (5)
Postmenopausal	—	58 (81%)	—
Normal BMD	19 (25%)	16 (22%)	3 (60%)
Reduced BMD	4 (5%)	3 (4%)	1 (20%)
Osteopenia	32 (42%)	32 (44%)	0
Osteoporosis	22 (29%)	21 (29%)	1 (20%)

Nineteen patients (25%) were classified in the normal category despite having low bone mass or suboptimal levels when age-matched to general population standards. According to the interpretation of the reports provided, the "bone quality in younger individuals differs from that of older people" and thus "absolute fracture risk has not been determined in this population." As such, diminished bone health with levels considerably lower than the mean are still placed in a "normal" diagnostic category. BMD testing was done on various younger patients as many of these individuals presented with chronic illness and had evidence of irregularities on biochemical nutritional status testing, or had other factors that might predispose them to bone health compromise.

3. Results

The population included predominantly women (94%) who were mostly postmenopausal (81%). Of these patients, 29 (38%) reported lack of success with previous use of bisphosphonates and 48 (62%) declined standard drug therapy. The distribution of bone densities in the sample is summarized in Table 2.

After treatment, there was a significant improvement in bone density (z-scores) in the femoral neck, total spine as well as lowest hip and spine scores in the overall group (Table 3). Improvement was observed in the total hip scores, but this change was not significant.

Overall percentage change after one year is presented in Figure 1 and comparisons with published results for selected

Table 3: Pre and Posttreatment bone density.

	Pretreatment result (Mean ± SD)	Posttreatment result (Mean ± SD)	P value
Femoral neck (z-score)	−0.51 ± 0.74	−0.24 ± 0.81	0.03*
Total hip (z-score)	−0.27 ± 0.82	−0.06 ± 0.84	0.12
Lowest hip site (z-score)	−0.61 ± 0.71	−0.27 ± 0.81	0.006*
L1–L4 spine (z-score)	−0.85 ± 0.98	−0.39 ± 1.07	0.006*
Lowest spine site (z-score)	−1.40 ± 0.95	−0.67 ± 1.07	<0.001*

* Significant value.

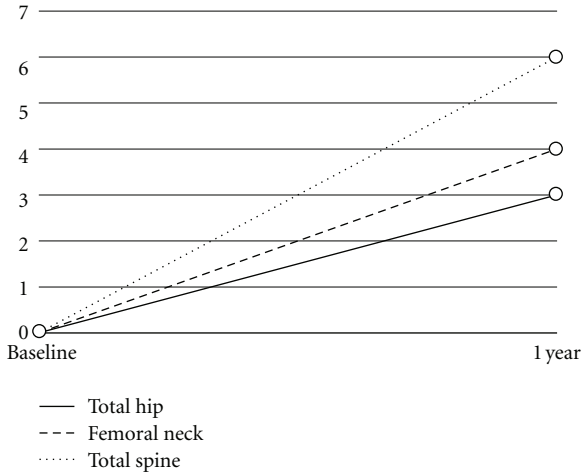

Figure 1: Mean percent change in bone density from baseline in the intervention group.

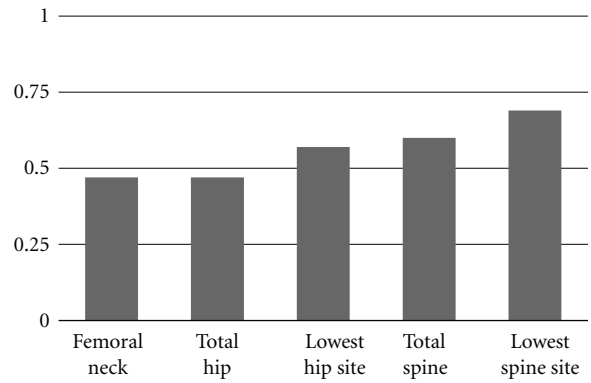

Figure 2: Proportion of patients showing >3% change in the various sites within the first year.

pharmaceutical interventions are presented in Table 4. The percent changes in the entire group as shown in Table 4 were the same when the whole group (males and females, all ages) was analyzed as when only postmenopausal females were analyzed. Isolating only the postmenopausal females with osteopenia and osteoporosis also revealed the same percentage changes with the exception of the lowest hip site improving 5% with this subgroup rather than 4% for the overall group. In the five males, there was an even greater percentage change: 10% in the femoral neck, 8% in the total hip, 10% in the lowest hip site, 10% in the total spine, and 16% in the lowest spine site. Table 4 compares the results in the current sample of patients using combination of micronutrients to strontium ranelate alone [21], as well as to published bisphosphonate trials of Alendronate [44] and Risedronate [45].

The BMD change in one year was more pronounced in the hip (femoral neck and lowest hip site) among those who reported lack of success with previous bisphosphonate therapy (Table 5) and more pronounced in the lowest spine site among those who had chosen to decline primary bisphosphonate therapy. Over the course of the study period, there were no fractures from ground level falls in any of the participants. Finally, Figure 2 summarizes the proportion of patients who experienced a BMD change of greater than 3% within the first year of following the COMB protocol, suggesting rapid onset of BMD improvement for many participants.

Compliance issues have become evident in this study. Many patients did not complete the 12 month course consistently, but took the intervention sporadically. In this group of patients ($N = 37$), a post hoc analysis was carried out to determine whether sporadic supplementation would be of benefit. There was no significant difference in z-scores at any of the sites after one year ($P > 0.05$). At one year, the percentage change in the femoral neck was −3%, the total hip was −1%, the total spine was −2%, the lowest hip site was −2%, and the lowest spine site was −1%. Figure 3 shows the percentage change after one year in the noncompliant group (compared to Figure 1 in the compliant group).

4. Discussion

Diminished BMD is an important indicator of compromised bone health and has been established as a determinant associated with fragility fractures. Integrative approaches for preventing fragility fractures will be essential in addressing the health concerns in our aging baby boomer population. In selected patients with diminished bone health, combined micronutrient therapy may be a promising alternative to pharmaceutical strategies in order to prevent bone compromise as well as to maintain or to improve BMD. In this study, we observe that an expanded micronutrient combination alone can improve BMD in many patients who failed to achieve success with bisphosphonate medications as well as those who declined to start bisphosphonate therapy for reasons of choice or chemical sensitivity.

Table 4: One year of therapy with the COMB protocol compared to strontium ranelate and bisphosphonate medications.

Percent change	COMB protocol: one year whole group (postmenopausal females)	Comparison to Strontium Ranelate at one year [21]	Comparison to Alendronate at one year [44]	Comparison to Risedronate at one year [45]
Femoral neck	4%	2%	2%	2%
Total hip	3%	3-4%	2%	Not calculated
Lowest hip site	4%	Not calculated	Not calculated	Not calculated
Total spine	6%	5-6%	4%	4%
Lowest spine site	8%	Not calculated	Not calculated	Not calculated

Table 5: Comparison of outcomes between patients who commenced the COMB protocol for declined drug therapy and those who previously showed no improvement on bisphosphonates.

Percent change	Patients who declined therapy with bisphosphonate ($N = 48$)	Patients who reported failure with previous bisphosphonate therapy ($N = 29$)
Femoral neck	3%	5%
Total hip	3%	3%
Lowest hip site	4%	5%
Total spine	6%	6%
Lowest spine site	9%	8%

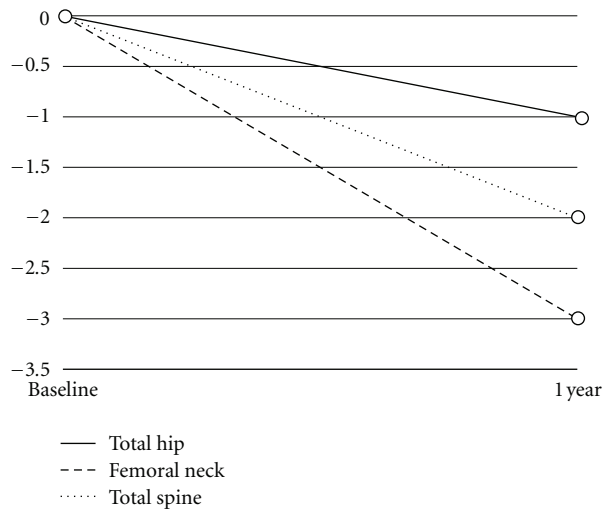

Figure 3: Mean percent change in bone density from baseline in noncompliant group ($N = 37$).

4.1. Limitations, Confounder, and Strengths. Limitations of this study include the small sample size and the lack of a blinded placebo-controlled group. Furthermore, given the multiple intervention nature of the combination micronutrient regimen, it is difficult to pinpoint which nutrients or nutrient groups were ultimately responsible for the improved BMD in each case. As well, the one-year followup limits the ability to determine the long-term effects of this regimen on BMD measurements and sustained prevention of fragility fractures.

There is also marked selection bias in this study group which might potentially lead to an underestimate of the full potential of these interventions in the general population. Some of these patients, for example, have unsuccessfully tried pharmacologic therapies for many years and thus represent a skewed portion of the population. Furthermore, many of these patients have multisystem health problems that may inhibit normal physical activity or may be associated with other pathophysiologic mechanisms impairing proper bone physiology.

Each micronutrient in the regimen has prior published data to suggest effectiveness in improving bone health, but this is the first study to our knowledge that examines these micronutrients in combination. While it is impossible to determine which component or components of the micronutrient combination were able to achieve the benefit realized, the issue of isolating the individual effective component(s) of the COMB protocol is more of a theoretical than practical concern. Biochemicals in their natural physiological state as produced in foods or gut microbiota do not work in isolation. Combinations of nutrients are known to be required for normal biochemical function. For example, both vitamin D and magnesium are required for proper calcium deposition and bone development [26]. In addition, emerging evidence suggests that other nutrients including some phytochemicals may contribute to the constellation of factors involved in healthy bone biochemistry [46]. Using single supplemental biochemicals in isolation may not be successful, whereas using them in combination may be efficacious.

The contention that a combination intervention is less credible, and that a traditional prospective clinical trial isolating individual variables to determine independent efficacy compared to controls is required to demonstrate benefit and to recommend micronutrient therapies, is debatable. With the emergence of molecular medicine and the Human Genome Project (HGP) recently identifying each person as biochemically unique, it may not be valid to say that any single therapy used broadly will work in a similar fashion for individual patients as genomic variability already introduces multivariables. The HGP has demonstrated that genomic solitary nucleotide polymorphisms (SNPs) regulate genomic function and affect the function of enzymes they code for, thus raising the question of what constitutes a proper control group [47, 48]. Most clinical trials to date have controls based on race, age, and sex, but genomic variables with SNP variability may be just as significant as race, sex, and age as determinants of physiological outcome. Emerging evidence

shows that SNP variability is enormous within race and sex groups and many published clinical trials, including osteoporosis research, which omit relevant genomic information may not have proper controls and are, at best, anecdotal.

The rapidly emerging field of genomics is increasingly supplanting knowledge gleaned from broad-based clinical trials in many branches of medicine [49–51] and has ushered in the expansion of pharmacogenomics and nutrigenomics to specifically assess and treat individuals according to their unique biochemistry [52]. Furthermore, the Human Microbiome Project has recently uncovered the individual nature of the gut microflora [53], creating further evidence of individual biochemistry, a unique gut microbiome, and resultant unique physiology. Broad-based research without genomic controls, identifying a single pharmacologic agent as a widespread therapy for osteoporosis or any other condition may be judged to have inadequate experimental design and thus scientifically unreliable. Accordingly, reproducible clinical interventions which yield positive clinical outcomes, as in this study, definitely have limitations but may have at least comparable merit to traditional trials when considering benefit.

4.2. Strontium Citrate and Strontium Ranelate. It has repeatedly been documented in the literature that pharmacologic therapy with strontium ranelate is associated with an elevation in BMD as well as reduction in fragility fractures [16, 21–25]. Since strontium is a metal in the same group of periodic elements as calcium, it has been recognized that strontium in high concentrations may displace and replace calcium in bone by heteroionic exchange [54], a phenomenon which has elicited disparaging regard for strontium therapy among some bone specialists. Rather than an increased BMD, however, this physiochemical process in the presence of excessive strontium ultimately results in decreased bone calcium content [55], dissolution of mineralized bone [56], disruption of bone architecture [57], and lower BMD [58]. This phenomenon only appears to be the consequence of disproportionately high doses of strontium intake, not regular supplemental levels at low dose.

At low supplemental doses of strontium, in fact, there is evidence of an increase in both the bone formation rate and the trabecular bone density related to a strontium-induced stimulation of osteoblastic activity [58]. Furthermore, at low doses, strontium is not associated with any mineralization defect or any increase in the number of active bone-resorbing cells [59, 60]. In addition, it has recently been found that the mechanism of strontium benefit may also involve a calcium preservation effect as the rate of calcium release was almost halved after strontium treatment was assessed in recent research on teeth [61]. Finally, strontium supplementation, unlike use of calcium supplementation, shows ability to recalcify osteopenic areas in pathological bone conditions characterized by accelerated bone loss and extensive demineralization [58, 62].

Strontium is increasingly being recognized as a trace mineral which may be essential to the normal biology of bone and teeth and it is yet undetermined if strontium deficiency, like iodine deficiency, results in physiological malfunction [63]. It has been recently reported that commercial foods grown on fields using synthetic fertilizers, pesticides, and herbicides have appreciably lower levels of strontium than organic food counterparts [64]. Thus the restoration of adequate strontium levels to individuals may simply represent the normal homeostatic requirement for strontium, and normal healthy bone may require some level of strontium to prevent calcium loss [61]. Most importantly, treatment to elevate strontium levels has repeatedly been shown to demonstrate safe and remarkable efficacy at diminishing fractures in hip, vertebral as well as peripheral sites [21–25].

Studies to date have predominantly focused on strontium ranelate rather than the readily available strontium citrate supplement as used in this study. The results of this study, however, demonstrate that the micronutrient combination including strontium citrate is at least as effective in BMD change as strontium ranelate with suggestion of preferred efficacy of the former therapy at improving femoral neck outcomes. Furthermore, the ranelic acid salt is a purely synthetic molecular compound, while citrate is naturally occurring. It appears to be the strontium portion of the molecules which exerts most or all of the positive effect on bone. When consuming the strontium ranelate, for example, the compound splits into two strontium ions and one molecule of ranelic acid, with each absorbed separately. There is little evidence that the ranelic acid portion of the strontium ranelate compound contributes to the effect of strontium on skeletal tissue, and of the small amount of ranelic acid that is absorbed into the body, almost all is excreted within a week without ever being metabolized. All forms of strontium have bioavailabilities in the 25–30% range, but gastric tolerance appears to be better with the ranelate and citrate forms.

With the mounting concern about the safety profile of some standard medical interventions for bone compromise, strontium is very well tolerated and has shown remarkably little in the way of side effects or long-term adverse sequelae. An increased risk of thrombosis has been noted with strontium ranelate, an effect not reported (to our knowledge) with strontium citrate [16].

4.3. Mechanism of Action of Micronutrients. Unlike pharmacologic interventions, it is hypothesized that micronutrient strategies do not work by altering physiological parameters such as osteoclast function, but rather function by remediating underlying nutritional deficiencies which then permit restoration of inherent physiological processes. It is increasingly documented that nutritional deficiency continues to be an unrecognized and undertreated problem in clinical practice [65, 66]. Compromised nutritional status has recently been correlated with diminished quality of life and increased morbidity and mortality [67, 68]. It is well established that adequate weight and BMI, oft assumed to indicators of nutritional sufficiency, underestimate nutritional status and risk [69]. Investigation and management of malnutrition, often found in those with chronic disease, should become standard practice in clinical medicine [65, 70].

4.4. Relative Cost of COMB Protocol. An important factor to consider in evaluating this combination of micronutrients

for bone health is the cost to patients, given that supplements are generally not covered by public formularies while many pharmaceuticals used for osteoporosis receive coverage. The current COMB protocol was evaluated at $2.26 (CDN) per day, amounting to $67.80 per month or $824.90 per year. Bisphosphonates, on the other hand, range from $0.90 (least expensive generic preparation) per day to $12.96 (brand name) per day for Risedronate and $1.10 per day (least expensive generic) to $5.58 per day (brand name) for Alendronate (according to Blue Cross coverage for Alberta, Canada). A small percentage of the group discontinued the micronutrient intervention because they felt it was too expensive to purchase the nutrients, which were not covered by their drug plans. Given the potential cost saving in maximizing bone health, it would be prudent for government formulary administrators to consider funding such a protocol in appropriate patients.

4.5. Public Health Considerations. From a public health perspective, a number of fundamental questions need to be addressed.

(i) Why is there an epidemic of impaired bone health?

(ii) Why is the incidence and prevalence of osteoporosis increasing?

(iii) Why is there disparity in the geographic distribution of osteoporosis?

(iv) Why does osteoporosis frequently occur in individuals with no family history of bone compromise?

Genetics have not changed in the last three decades but lifestyle and environmental factors influencing bone health have. While use of pharmaceuticals may diminish risk of fracture in individual cases, they do not address the etiology or underlying cause of bone compromise; osteoporosis is not a bisphosphonate-deficiency disease. Accordingly, any prevention strategy must investigate and address lifestyle, nutritional and environmental determinants that have contributed to the rise in bone health compromise. The marked improvement in BMD with simple micronutrients in this study raises the question as to whether nutrient deficiency is a widespread phenomenon and a major determinant of this public health problem. Comprehensive research on nutritional status of patients with osteoporosis needs to be undertaken to determine if nutritional deficiency is a factor.

It has been well documented that vitamin D insufficiency is a widespread reality and a determinant of myriad health problems including bone compromise [71]. A challenge with the consideration of nutritional status assessment, however, is that levels of some essential nutrients for bone metabolism such as strontium and vitamin K_2 are not yet available in most laboratories. Accordingly, clinical suspicion, laboratory testing where possible, and repletion of nutrients required for normal bone physiology may represent the best that can be done with regards to nutritional management at the current time.

5. Concluding Thoughts

Osteoporosis has become a serious personal health issue for countless individuals as well as a disturbing public health problem for many countries as it now affects up to 1 in 2 women and 1 in 5 men over the age of 50 in some population groups [5]. Fragility fractures associated with impaired bone health account for widespread morbidity and, in the case of hip and vertebral fractures, undue rates of mortality [4]. Public expenditures associated with the management of osteoporotic fractures and their complications are staggering [3]. Left untreated, impaired bone health often has debilitating sequelae for individuals and profound implications for public health care.

The current practice standard for making a diagnosis of impaired bone health involves bone density measurement in conjunction with determination of clinical risk factors. Based on this combined assessment, clinical decisions to intervene with treatment are routinely made. The objective of any treatment to improve bone health, medications or otherwise, is to reduce the risk of fragility fractures in the future. It has been repeatedly established that those individuals with deficient bone mineral density, as measured by densitometry testing, are at increased risk for fragility fractures [72, 73]. It has been found that timely and effective management of compromised bone health, as diagnosed in part by suboptimal BMD measurements, can reduce fracture risk [6]. Measures which are successful in improving BMD measurements have been found to diminish the risk of fragility fractures [21, 74].

Interventions to improve BMD usually include the use of bisphosphonate or other pharmacologic options including teriparatide, strontium ranelate, raloxifene, hormone therapy, or calcitonin. However, there are some individuals who do not tolerate these medications, some that have not experienced improved BMD with these treatments, and some who decline to take these therapies because of reluctance to use medication in general, or because of increasing media attention to potential adverse effects associated with some osteoporosis drugs. Accordingly, some authors have recommended that nonpharmacologic strategies to improve or maintain bone health be included in discussion of options for bone preservation and therapy [6].

In this study, we introduce the use of a combination of micronutrients, each of which has previously been shown individually in the medical and scientific literature to benefit BMD outcomes. To assess the value of any therapy for compromised bone strength, one should ask if it fulfills the following criteria:

(i) protection from fragility fractures at multiple skeletal sites;

(ii) rapid onset of action in order to provide benefit as soon as possible;

(iii) minimal side effects for maximum tolerability;

(iv) long-term safety;

(v) patient acceptability.

It appears that the COMB strategy may fulfill many of these criteria. The protection from fragility fractures was suggested by the occurrence of no fractures in the group taking the intervention as well as a notable increase in BMD at femur, hip, and spine sites on the BMD testing. A major proportion of the patients had an increase in BMD of more than 3% within the first year of therapy alone. There were no reported side effects with the use of this therapy among those taking the intervention for the year and the literature suggests long-term safety with each of these agents—this might contribute to greater compliance with the subgroup of patients who are reluctant to use pharmacologic therapies. For those who completed the course of therapy, the acceptability was high.

In response to these findings, two questions arise:

(i) How does nutritional supplementation work for disordered bone strength?

(ii) Does micronutrient therapy have any role in mainstream medical practice?

A scientific approach to illness necessitates exploring the source etiology of health problems when possible and addressing causative determinants, including biochemical deficiencies [75]. From the results of this study it is hypothesized that osteoporosis in some cases may be related to nutritional deficiency of selected nutrients. Nutrient biochemicals are the fundamental building blocks of the human body, including the skeletal system; deficiency of required nutrients results in disordered biology and disease. Repletion of such nutrients may spontaneously correct and perhaps cure bone compromise in both young and mature patients. Just as restoring gestational folic acid to prevent open neural tube defects or supplementing with iron to ameliorate iron-deficiency anemia are recognized as credible and indicated nutritional interventions, remediation of essential biochemicals to restore and maintain bone strength is both evidence-based and science-based medicine. Further research of micronutrient strategies with longer followup will be needed to explore the effectiveness of this approach to disorders of bone health, but these preliminary results are encouraging indeed.

Conflict of Interests

There are no conflicts of interest. No funding has been received for any part of this work.

References

[1] Canada Statistics, "Portrait of the Canadian Population in 2006, by Age and Sex, 2006 Census," *Catalogue*, no. 97-551-XIE, 2006.

[2] H. A. Fink, K. E. Ensrud, D. B. Nelson et al., "Disability after clinical fracture in postmenopausal women with low bone density: the Fracture Intervention Trial (FIT)," *Osteoporosis International*, vol. 14, no. 1, pp. 69–76, 2003.

[3] N. Harvey, E. Dennison, and C. Cooper, "Osteoporosis: impact on health and economics," *Nature Reviews Rheumatology*, vol. 6, no. 2, pp. 99–105, 2010.

[4] A. Papaioannou, S. Morin, A. M. Cheung et al., "2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary," *CMAJ*, vol. 182, no. 17, pp. 1864–1873, 2010.

[5] R. Keen, "Osteoporosis: strategies for prevention and management," *Best Practice and Research: Clinical Rheumatology*, vol. 21, no. 1, pp. 109–122, 2007.

[6] F. Bonura, "Prevention, screening, and management of osteoporosis: an overview of the current strategies," *Postgraduate Medicine*, vol. 121, no. 4, pp. 5–17, 2009.

[7] S. J. Gallacher and T. Dixon, "Impact of treatments for postmenopausal osteoporosis (bisphosphonates, parathyroid hormone, strontium ranelate, and denosumab) on bone quality: a systematic review," *Calcified Tissue International*, vol. 87, no. 6, pp. 469–484, 2010.

[8] D. Huas, F. Debiais, F. Blotman et al., "Compliance and treatment satisfaction of post menopausal women treated for osteoporosis," *BMC Women's Health*, vol. 10, article 26, 2010.

[9] S. Wassertheil-Smoller, S. L. Hendrix, M. Limacher et al., "Effect of estrogen plus progestin on stroke in postmenopausal women—the women's health initiative: a randomized trial," *Journal of the American Medical Association*, vol. 289, no. 20, pp. 2673–2684, 2003.

[10] S. A. Shumaker, C. Legault, S. R. Rapp et al., "Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women—the women's health initiative memory study: a randomized controlled trial," *Journal of the American Medical Association*, vol. 289, no. 20, pp. 2651–2662, 2003.

[11] D. E. Sellmeyer, "Atypical fractures as a potential complication of long-term bisphosphonate therapy," *Journal of the American Medical Association*, vol. 304, no. 13, pp. 1480–1484, 2010.

[12] C. A. Migliorati, M. M. Schubert, D. E. Peterson, and L. M. Seneda, "Bisphosphonate-associated osteonecrosis of mandibular and maxillary bone: an emerging oral complication of supportive cancer therapy," *Cancer*, vol. 104, no. 1, pp. 83–93, 2005.

[13] P. A. Howard, B. J. Barnes, J. L. Vacek, W. Chen, and S. M. Lai, "Impact of bisphosphonates on the risk of atrial fibrillation," *American Journal of Cardiovascular Drugs*, vol. 10, no. 6, pp. 359–367, 2010.

[14] J. Green, G. Czanner, G. Reeves, J. Watson, L. Wise, and V. Beral, "Oral bisphosphonates and risk of cancer of oesophagus, stomach, and colorectum: case-control analysis within a UK primary care cohort," *BMJ*, vol. 341, Article ID c4444, 2010.

[15] C. R. Cardwell, C. C. Abnet, M. M. Cantwell, and L. J. Murray, "Exposure to oral bisphosphonates and risk of esophageal cancer," *Journal of the American Medical Association*, vol. 304, no. 6, pp. 657–663, 2010.

[16] S. J. Genuis and G. K. Schwalfenberg, "Picking a bone with contemporary osteoporosis management: nutrient strategies to enhance skeletal integrity," *Clinical Nutrition*, vol. 26, no. 2, pp. 193–207, 2007.

[17] J. W. Nieves, "Osteoporosis: the role of micronutrients," *American Journal of Clinical Nutrition*, vol. 81, no. 5, pp. 1232S–1239S, 2005.

[18] K. D. Cashman, "Diet, nutrition, and bone health," *Journal of Nutrition*, vol. 137, no. 11, pp. 2507S–2512S, 2007.

[19] D. Feskanich, P. Weber, W. C. Willett, H. Rockett, S. L. Booth, and G. A. Colditz, "Vitamin K intake and hip fractures in women: a prospective study," *American Journal of Clinical Nutrition*, vol. 69, no. 1, pp. 74–79, 1999.

[20] M. Ryan-Harshman and W. Aldoori, "Bone health: new role for vitamin K?" *Canadian Family Physician*, vol. 50, pp. 993–997, 2004.

[21] P. J. Meunier, C. Roux, E. Seeman et al., "The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis," *New England Journal of Medicine*, vol. 350, no. 5, pp. 459–468, 2004.

[22] P. J. Marie, P. Ammann, G. Boivin, and C. Rey, "Mechanisms of action and therapeutic potential of strontium in bone," *Calcified Tissue International*, vol. 69, no. 3, pp. 121–129, 2001.

[23] S. Ortolani and S. Vai, "Strontium ranelate: an increased bone quality leading to vertebral antifracture efficacy at all stages," *Bone*, vol. 38, no. 2, pp. S19–S22, 2006.

[24] J. Y. Reginster, R. Deroisy, M. Dougados, I. Jupsin, J. Colette, and C. Roux, "Prevention of early postmenopausal bone loss by strontium ranelate: the randomized, two-year, double-masked, dose-ranging, placebo-controlled PREVOS trial," *Osteoporosis International*, vol. 13, no. 12, pp. 925–931, 2002.

[25] R. Cesareo, C. Napolitano, and M. Iozzino, "Strontium ranelate in postmenopausal osteoporosis treatment: a critical appraisal," *International Journal of Women's Health*, vol. 2, no. 1, pp. 1–6, 2010.

[26] R. K. Rude and H. E. Gruber, "Magnesium deficiency and osteoporosis: animal and human observations," *Journal of Nutritional Biochemistry*, vol. 15, no. 12, pp. 710–716, 2004.

[27] L. A. Weiss, E. Barrett-Connor, and D. Von Mühlen, "Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo study," *American Journal of Clinical Nutrition*, vol. 81, no. 4, pp. 934–938, 2005.

[28] M. C. Kruger and D. F. Horrobin, "Calcium metabolism, osteoporosis and essential fatty acids: a review," *Progress in Lipid Research*, vol. 36, no. 2-3, pp. 131–151, 1997.

[29] P. Albertazzi and K. Coupland, "Polyunsaturated fatty acids. Is there a role in postmenopausal osteoporosis prevention," *Maturitas*, vol. 42, no. 1, pp. 13–22, 2002.

[30] P. Salari, A. Rezaie, B. Larijani, and M. Abdollahi, "A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis," *Medical Science Monitor*, vol. 14, no. 3, pp. RA37–RA44, 2008.

[31] L. Bessette, L. G. Ste-Marie, S. Jean et al., "Recognizing osteoporosis and its consequences in Quebec (ROCQ): background, rationale, and methods of an anti-fracture patient health-management programme," *Contemporary Clinical Trials*, vol. 29, no. 2, pp. 194–210, 2008.

[32] A. Papaioannou, L. Giangregorio, B. Kvern, P. Boulos, G. Ioannidis, and J. D. Adachi, "The osteoporosis care gap in Canada," *BMC Musculoskeletal Disorders*, vol. 5, article no. 11, 2004.

[33] A. Papaioannou, C. C. Kennedy, G. Ioannidis et al., "The osteoporosis care gap in men with fragility fractures: the Canadian Multicentre Osteoporosis Study," *Osteoporosis International*, vol. 19, no. 4, pp. 581–587, 2008.

[34] S. J. Genuis, "Sensitivity-related illness: the escalating pandemic of allergy, food intolerance and chemical sensitivity," *Science of the Total Environment*, vol. 408, no. 24, pp. 6047–6061, 2010.

[35] D. Feskanich, W. C. Willett, and G. A. Colditz, "Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women," *American Journal of Clinical Nutrition*, vol. 77, no. 2, pp. 504–511, 2003.

[36] D. P. Trivedi, R. Doll, and K. T. Khaw, "Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial," *British Medical Journal*, vol. 326, no. 7387, pp. 469–472, 2003.

[37] M. F. Holick, "Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis," *American Journal of Clinical Nutrition*, vol. 79, no. 3, pp. 362–371, 2004.

[38] B. Y. Y. Lau, W. E. Ward, J. X. Kang, and D. W. L. Ma, "Fat-1 gene modulates the fatty acid composition of femoral and vertebral phospholipids," *Applied Physiology, Nutrition and Metabolism*, vol. 35, no. 4, pp. 447–455, 2010.

[39] B. M. Tang, G. D. Eslick, C. Nowson, C. Smith, and A. Bensoussan, "Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis," *Lancet*, vol. 370, no. 9588, pp. 657–666, 2007.

[40] I. R. Reid, M. J. Bolland, and A. Grey, "Effect of calcium supplementation on hip fractures," *Osteoporosis International*, vol. 19, no. 8, pp. 1119–1123, 2008.

[41] M. J. Bolland, A. Avenell, J. A. Baron et al., "Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis," *BMJ*, vol. 341, Article ID c3691, 2010.

[42] K. Niu, R. Ahola, H. Guo et al., "Effect of office-based brief high-impact exercise on bone mineral density in healthy premenopausal women: the Sendai Bone Health Concept Study," *Journal of Bone and Mineral Metabolism*, vol. 28, no. 5, pp. 568–577, 2010.

[43] R. Korpelainen, S. Keinänen-Kiukaanniemi, J. Heikkinen, K. Väänänen, and J. Korpelainen, "Effect of impact exercise on bone mineral density in elderly women with low BMD: a population-based randomized controlled 30-month intervention," *Osteoporosis International*, vol. 17, no. 1, pp. 109–118, 2006.

[44] D. M. Black, S. R. Cummings, D. B. Karpf et al., "Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures," *Lancet*, vol. 348, no. 9041, pp. 1535–1541, 1996.

[45] S. T. Harris, N. B. Watts, H. K. Genant et al., "Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial," *Journal of the American Medical Association*, vol. 282, no. 14, pp. 1344–1352, 1999.

[46] B. H. Arjmandi, C. D. Johnson, S. C. Campbell, S. Hooshmand, S. C. Chai, and M. P. Akhter, "Combining fructooligosaccharide and dried plum has the greatest effect on restoring bone mineral density among select functional foods and bioactive compounds," *Journal of Medicinal Food*, vol. 13, no. 2, pp. 312–319, 2010.

[47] P. H. Sudmant, J. O. Kitzman, F. Antonacci et al., "Diversity of human copy number variation and multicopy genes," *Science*, vol. 330, no. 6004, pp. 641–646, 2010.

[48] D. L. Altshuler, R. M. Durbin, G. R. Abecasis et al., "A map of human genome variation from population-scale sequencing," *Nature*, vol. 467, no. 7319, pp. 1061–1073, 2010.

[49] G. E. Palomaki, S. Melillo, L. Neveux et al., "Use of genomic profiling to assess risk for cardiovascular disease and identify individualized prevention strategies—a targeted evidence-based review," *Genetics in Medicine*, vol. 12, no. 12, pp. 772–784, 2010.

[50] S. Lambrecht, K. Tilleman, D. Elewaut, and D. Deforce, "Proteomics in rheumatology: the beginning of a fairy tale?" *Proteomics—Clinical Applications*, vol. 2, no. 3, pp. 411–419, 2008.

[51] K. H. Brettingham-Moore, C. P. Duong, A. G. Heriot, R. J. S. Thomas, and W. A. Phillips, "Using gene expression profiling to predict response and prognosis in gastrointestinal

cancers—the promise and the perils," *Annals of Surgical Oncology*, vol. 18, no. 5, pp. 1484–1491, 2011.

[52] N. Malandrino and R. J. Smith, "Personalized medicine in diabetes," *Clinical Chemistry*, vol. 57, no. 2, pp. 231–240, 2011.

[53] D. V. Dimitrov, "The human gutome: nutrigenomics of the host-microbiome interactions," *OMICS*, vol. 15, no. 7-8, pp. 419–430, 2011.

[54] A. G. Mattar and B. A. Diefel, "Bone tracers: radionucleotide imaging and related techniques," in *Skeletal Research: An Experimental Approach*, D. J. Simmons and A. S. Kustrin, Eds., pp. 455–486, Academic Press, New York, NY, USA, 1979.

[55] A. R. Johnson, "The influence of strontium on characteristic factors of bone," *Calcified Tissue Research*, vol. 11, no. 3, pp. 215–221, 1973.

[56] J. Christoffersen, M. R. Christoffersen, N. Kolthoff, and O. Bärenholdt, "Effects of strontium ions on growth and dissolution of hydroxyapatite and on bone mineral detection," *Bone*, vol. 20, no. 1, pp. 47–54, 1997.

[57] E. Eisenberg, "The biological metabolism of strontium," in *Biological Mineralization*, I. Zipkin, Ed., pp. 435–442, John Wiley & Sons, New York, NY, USA, 1973.

[58] W. E. Cabrera, I. Schrooten, M. E. De Broe, and P. C. D'Haese, "Strontium and bone," *Journal of Bone and Mineral Research*, vol. 14, no. 5, pp. 661–668, 1999.

[59] M. D. Grynpas and P. J. Marie, "Effects of low doses of strontium on bone quality and quantity in rats," *Bone*, vol. 11, no. 5, pp. 313–319, 1990.

[60] P. J. Marie, M. T. Garba, M. Hott, and L. Miravet, "Effect of low doses of stable strontium on bone metabolism in rats," *Mineral and Electrolyte Metabolism*, vol. 11, no. 1, pp. 5–13, 1985.

[61] M. Riyat and D. C. Sharma, "An experimental study of the effect of strontium pre-treatment on calcium release from carious and non-carious teeth," *Biological Trace Element Research*, vol. 133, no. 3, pp. 251–254, 2010.

[62] S. C. Skoryna, "Metabolic aspects of the pharmacologic uses of trace elements in human subjects with specific references to stable strontium," in *Trace Substances in Environmental Health-XVIII*, pp. 3–23.

[63] J. D. Kirshmann, *Nutrition Almanac*, McGraw-Hill, New York, NY, USA, 6th edition, 2007.

[64] W. J. Rea, *Chemical Sensitivity: Tools of Diagnosis and Methods of Treatment*, vol. 4, Lewis Publishers, Boca Raton, Fla, USA, 1997.

[65] U. G. Kyle and J. A. Coss-Bu, "Nutritional assessment and length of hospital stay," *CMAJ*, vol. 182, no. 17, pp. 1831–1832, 2010.

[66] S. J. Genuis, "Nutritional transition: a determinant of global health," *Journal of Epidemiology and Community Health*, vol. 59, no. 8, pp. 615–617, 2005.

[67] J. Sorensen, J. Kondrup, J. Prokopowicz et al., "EuroOOPS: an international, multicentre study to implement nutritional risk screening and evaluate clinical outcome," *Clinical Nutrition*, vol. 27, no. 3, pp. 340–349, 2008.

[68] T. Wakahara, M. Shiraki, K. Murase et al., "Nutritional screening with Subjective Global Assessment predicts hospital stay in patients with digestive diseases," *Nutrition*, vol. 23, no. 9, pp. 634–639, 2007.

[69] U. G. Kyle, L. Genton, and C. Pichard, "Hospital length of stay and nutritional status," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 8, no. 4, pp. 397–402, 2005.

[70] S. J. Genuis and T. P. Bouchard, "Celiac disease presenting as autism," *Journal of Child Neurology*, vol. 25, no. 1, pp. 114–119, 2010.

[71] S. J. Genuis, G. K. Schwalfenberg, M. N. Hiltz, and S. A. Vaselenak, "Vitamin D status of clinical practice populations at higher latitudes: analysis and applications," *International Journal of Environmental Research and Public Health*, vol. 6, no. 1, pp. 151–173, 2009.

[72] S. R. Cummings, D. M. Black, M. C. Nevitt et al., "Bone density at various sites for prediction of hip fractures," *Lancet*, vol. 341, no. 8837, pp. 72–75, 1993.

[73] L. J. Melton III, E. J. Atkinson, W. M. O'Fallon, H. W. Wahner, and B. L. Riggs, "Long-term fracture prediction by bone mineral assessed at different skeletal sites," *Journal of Bone and Mineral Research*, vol. 8, no. 10, pp. 1227–1233, 1993.

[74] P. J. Meunier, "Evidence-based medicine and osteoporosis: a comparison of fracture risk reduction data from osteoporosis randomised clinical trials," *International Journal of Clinical Practice*, vol. 53, no. 2, pp. 122–129, 1999.

[75] S. J. Genuis, "Medical practice and community health care in the 21st Century: a time of change," *Public Health*, vol. 122, no. 7, pp. 671–680, 2008.

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Review Article

Earthing: Health Implications of Reconnecting the Human Body to the Earth's Surface Electrons

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Environmental medicine generally addresses environmental factors with a negative impact on human health. However, emerging scientific research has revealed a surprisingly positive and overlooked environmental factor on health: direct physical contact with the vast supply of electrons on the surface of the Earth. Modern lifestyle separates humans from such contact. The research suggests that this disconnect may be a major contributor to physiological dysfunction and unwellness. Reconnection with the Earth's electrons has been found to promote intriguing physiological changes and subjective reports of well-being. Earthing (or grounding) refers to the discovery of benefits—including better sleep and reduced pain—from walking barefoot outside or sitting, working, or sleeping indoors connected to conductive systems that transfer the Earth's electrons from the ground into the body. This paper reviews the earthing research and the potential of earthing as a simple and easily accessed global modality of significant clinical importance.

1. Introduction

Environmental medicine focuses on interactions between human health and the environment, including factors such as compromised air and water and toxic chemicals, and how they cause or mediate disease. Omnipresent throughout the environment is a surprisingly beneficial, yet overlooked global resource for health maintenance, disease prevention, and clinical therapy: the surface of the Earth itself. It is an established, though not widely appreciated fact, that the Earth's surface possesses a limitless and continuously renewed supply of free or mobile electrons. The surface of the planet is electrically conductive (except in limited ultradry areas such as deserts), and its negative potential is maintained (i.e., its electron supply replenished) by the global atmospheric electrical circuit [1, 2].

Mounting evidence suggests that the Earth's negative potential can create a stable internal bioelectrical environment for the normal functioning of all body systems. Moreover, oscillations of the intensity of the Earth's potential may be important for setting the biological clocks regulating diurnal body rhythms, such as cortisol secretion [3].

It is also well established that electrons from antioxidant molecules neutralize reactive oxygen species (ROS, or in popular terms, free radicals) involved in the body's immune and inflammatory responses. The National Library of Medicine's online resource PubMed lists 7021 studies and 522 review articles from a search of "antioxidant + electron + free radical" [3]. It is assumed that the influx of free electrons absorbed into the body through direct contact with the Earth likely neutralize ROS and thereby reduce acute and

chronic inflammation [4]. Throughout history, humans mostly walked barefoot or with footwear made of animal skins. They slept on the ground or on skins. Through direct contact or through perspiration-moistened animal skins used as footwear or sleeping mats, the ground's abundant free electrons were able to enter the body, which is electrically conductive [5]. Through this mechanism, every part of the body could equilibrate with the electrical potential of the Earth, thereby stabilizing the electrical environment of all organs, tissues, and cells.

Modern lifestyle has increasingly separated humans from the primordial flow of Earth's electrons. For example, since the 1960s, we have increasingly worn insulating rubber or plastic soled shoes, instead of the traditional leather fashioned from hides. Rossi has lamented that the use of insulating materials in post-World War II shoes has separated us from the Earth's energy field [6]. Obviously, we no longer sleep on the ground as we did in times past.

During recent decades, chronic illness, immune disorders, and inflammatory diseases have increased dramatically, and some researchers have cited environmental factors as the cause [7]. However, the possibility of modern disconnection with the Earth's surface as a cause has not been considered. Much of the research reviewed in this paper points in that direction.

In the late 19th century, a back-to-nature movement in Germany claimed many health benefits from being barefoot outdoors, even in cold weather [8]. In the 1920s, White, a medical doctor, investigated the practice of sleeping grounded after being informed by some individuals that they could not sleep properly "unless they were on the ground or connected to the ground in some way," such as with copper wires attached to grounded-to-Earth water, gas, or radiator pipes. He reported improved sleeping using these techniques [9]. However, these ideas never caught on in mainstream society.

At the end of the last century, experiments initiated independently by Ober in the USA [10] and K. Sokal and P. Sokal [11] in Poland revealed distinct physiological and health benefits with the use of conductive bed pads, mats, EKG- and TENS-type electrode patches, and plates connected indoors to the Earth outside. Ober, a retired cable television executive, found a similarity between the human body (a bioelectrical, signal-transmitting organism) and the cable used to transmit cable television signals. When cables are "grounded" to the Earth, interference is virtually eliminated from the signal. Furthermore, all electrical systems are stabilized by grounding them to the Earth. K. Sokal and P. Sokal, meanwhile, discovered that grounding the human body represents a "universal regulating factor in Nature" that strongly influences bioelectrical, bioenergetic, and biochemical processes and appears to offer a significant modulating effect on chronic illnesses encountered daily in their clinical practices.

Earthing (also known as grounding) refers to contact with the Earth's surface electrons by walking barefoot outside or sitting, working, or sleeping indoors connected to conductive systems, some of them patented, that transfer the energy from the ground into the body. Emerging scientific research supports the concept that the Earth's electrons induce multiple physiological changes of clinical significance, including reduced pain, better sleep, a shift from sympathetic to parasympathetic tone in the autonomic nervous system (ANS), and a blood-thinning effect. The research, along with many anecdotal reports, is presented in a new book entitled *Earthing* [12].

2. Review of Earthing Papers

The studies summarized below involve indoor-testing methods under controlled conditions that simulate being barefoot outdoors.

2.1. Sleep and Chronic Pain. In a blinded pilot study, Ober recruited 60 subjects (22 males and 28 females) who suffered from self-described sleep disturbances and chronic muscle and joint pain for at least six months [10]. Subjects were randomly divided for the month-long study in which both groups slept on conductive carbon fiber mattress pads provided by Ober. Half the pads were connected to a dedicated Earth ground outside each subject's bedroom window, while the other half were "sham" grounded—not connected to the Earth. Results are presented in Table 1.

Most grounded subjects described symptomatic improvement while most in the control group did not. Some subjects reported significant relief from asthmatic and respiratory conditions, rheumatoid arthritis, PMS, sleep apnea, and hypertension while sleeping grounded. These results indicated that the effects of earthing go beyond reduction of pain and improvements in sleep.

2.2. Sleep, Stress, Pain, and Cortisol. A pilot study evaluated diurnal rhythms in cortisol correlated with changes in sleep, pain, and stress (anxiety, depression, and irritability), as monitored by subjective reporting [13]. Twelve subjects with complaints of sleep dysfunction, pain, and stress were grounded to Earth during sleep in their own beds using a conductive mattress pad for 8 weeks.

In order to obtain a baseline measurement of cortisol, subjects chewed Dacron salvettes for 2 minutes and then placed them in time-labeled sampling tubes that were stored in a refrigerator. Self-administered sample collections began at 8 AM and were repeated every 4 hours. After 6 weeks of being grounded, subjects repeated this 24-hour saliva test. The samples were processed using a standard radioimmunoassay. A composite of the results is shown in Figure 1.

Subjective symptoms of sleep dysfunction, pain, and stress were reported daily throughout the 8-week test period. The majority of subjects with high- to out-of-range nighttime secretion levels experienced improvements by sleeping grounded. This is demonstrated by the restoration of normal day-night cortisol secretion profiles.

Eleven of 12 participants reported falling asleep more quickly, and all 12 reported waking up fewer times at night. Grounding the body at night during sleep also appears to

Table 1: Subjective sleep, pain, and well-being feedback.

Categories	Test subjects*		Control subjects**	
	Same	Improved	Same	Improved
Time to fall asleep	4 = 15%	23 = 85%	20 = 87%	3 = 13%
Quality of sleep	2 = 7%	25 = 93%	20 = 87%	3 = 13%
Wake feeling rested	0 = 0%	27 = 100%	20 = 87%	3 = 13%
Muscles stiffness and pain	5 = 18%	22 = 82%	23 = 100%	0 = 0%
Chronic back and/or joint pain	7 = 26%	20 = 74%	23 = 100%	0 = 0%
General well-being	6 = 22%	21 = 78%	20 = 87%	3 = 13%

*Reports not received from three participants.
**Reports not received from seven participants.

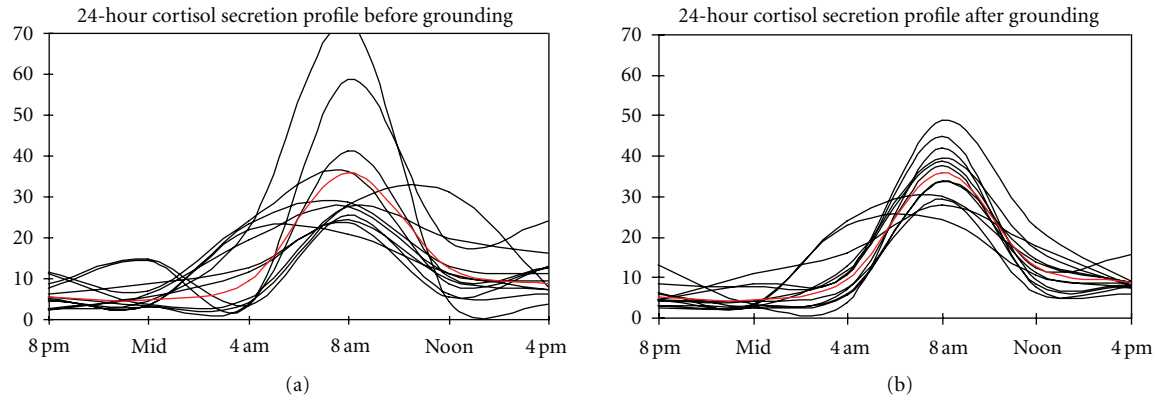

Figure 1: Cortisol levels before and after grounding. In unstressed individuals, the normal 24-hour cortisol secretion profile follows a predictable pattern: lowest around midnight and highest around 8 a.m. Graph (a) illustrates the wide variation of patterns among study participants prior to grounding, while (b) shows a realignment and normalization trend of patterns after six weeks of sleeping grounded.

positively affect morning fatigue levels, daytime energy, and nighttime pain levels.

About 30 percent of the general American adult population complain of sleep disruption, while approximately 10 percent have associated symptoms of daytime functional impairment consistent with the diagnosis of insomnia. Insomnia often correlates with major depression, generalized anxiety, substance abuse, dementia, and a variety of pain and physical problems. The direct and indirect costs of chronic insomnia have been estimated at tens of billions of dollars annually in the USA alone [14]. In view of the burdens of personal discomfort and health care costs, grounding the body during sleep seems to have much to offer.

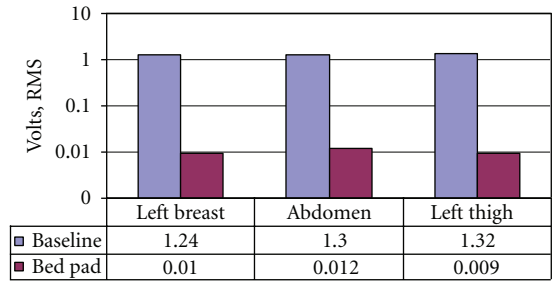

Figure 2: Effect of bed pad grounding on 60 Hz mode.

2.3. Earthing Reduces Electric Fields Induced on the Body. Voltage induced on a human body from the electrical environment was measured using a high-impedance measurement head. Applewhite, an electrical engineer and expert in the design of electrostatic discharge systems in the electronic industry, was both subject and author of the study [15]. Measurements were taken while ungrounded and then grounded using a conductive patch and conductive bed pad. The author measured the induced fields at three positions: left breast, abdomen, and left thigh.

Each method (patch and sheet) immediately reduced the common alternating current (AC) 60 Hz ambient voltage induced on the body by a highly significant factor of about 70 on average. Figure 2 shows this effect.

The study showed that when the body is grounded, its electrical potential becomes equalized with the Earth's electrical potential through a transfer of electrons from the Earth to the body. This, in turn, prevents the 60 Hz mode from producing an AC electric potential at the surface of the body and from producing perturbations of the electric charges of the molecules inside the body. The study confirms the "umbrella" effect of earthing the body explained by Nobel Prize winner Richard Feynman in his lectures on electromagnetism [16]. Feynman said that when the body potential is the same as the Earth's electric potential (and

thus grounded), it becomes an extension of the Earth's gigantic electric system. The Earth's potential thus becomes the "working agent that cancels, reduces, or pushes away electric fields from the body."

Applewhite was able to document changes in the ambient voltage induced on the body by monitoring the voltage drop across a resistor. This effect clearly showed the "umbrella effect" described above. The body of the grounded person is not subject to the perturbation of electrons and electrical systems.

Jamieson asks whether the failure to appropriately ground humans is a factor contributing to the potential consequences of electropollution in office settings [17]. Considerable debate exists on whether electromagnetic fields in our environment cause a risk to health [18], but there is no question that the body reacts to the presence of environmental electric fields. This study demonstrates that grounding essentially eliminates the ambient voltage induced on the body from common electricity power sources.

2.4. Physiological and Electrophysiological Effects

2.4.1. Reductions in Overall Stress Levels and Tension and Shift in ANS Balance. Fifty-eight healthy adult subjects (including 30 controls) participated in a randomized double-blind pilot study investigating earthing effects on human physiology [19]. Earthing was accomplished with a conductive adhesive patch placed on the sole of each foot. A biofeedback system recorded electrophysiological and physiological parameters. Experimental subjects were exposed to 28 minutes in the unearthed condition followed by 28 minutes with the earthing wire connected. Controls were unearthed for 56 minutes.

Upon earthing, about half the subjects showed an abrupt, almost instantaneous change in root mean square (rms) values of electroencephalograms (EEGs) from the left hemisphere (but not the right hemisphere) at all frequencies analyzed by the biofeedback system (beta, alpha, theta, and delta).

All grounded subjects presented an abrupt change in rms values of surface electromyograms (SEMGs) from right and left upper trapezius muscles. Earthing decreased blood volume pulse (BVP) in 19 of 22 experimental subjects (statistically significant) and in 8 of 30 controls (not significant). Earthing the human body showed significant effects on electrophysiological properties of the brain and musculature, on the BVP, and on the noise and stability of electrophysiological recordings. Taken together, the changes in EEG, EMG, and BVP suggest reductions in overall stress levels and tensions and a shift in ANS balance upon earthing. The results extend the conclusions of previous studies.

2.4.2. Confirming Shift from Sympathetic to Parasympathetic Activation. A multiparameter double-blind study was designed to reproduce and expand on previous electrophysiological and physiological parameters measured immediately after grounding with an improved methodology and state-of-the-art equipment [20]. Fourteen men and 14 women, in good health, ages 18–80, were tested while seated in a comfortable recliner during 2-hour grounding sessions, leaving time for signals to stabilize before, during, and after grounding (40 minutes for each period). Sham 2-hour grounding sessions were also recorded with the same subjects as controls. For each session, statistical analyses were performed on four 10-minute segments: before and after grounding (sham grounding for control sessions) and before and after ungrounding (sham ungrounding for control sessions). The following results were documented:

(i) an immediate decrease (within a few seconds) in skin conductance (SC) at grounding and an immediate increase at ungrounding. No change was seen for the control (sham grounding) sessions;

(ii) respiratory rate (RR) increased during grounding, an effect that lasted after ungrounding. RR variance increased immediately after grounding and then decreased;

(iii) blood oxygenation (BO) variance decreased during grounding, followed by a dramatic increase after ungrounding;

(iv) pulse rate (PR) and perfusion index (PI) variances increased toward the end of the grounding period, and this change persisted after ungrounding.

The immediate decrease in SC indicates a rapid activation of the parasympathetic nervous system and corresponding deactivation of the sympathetic nervous system. The immediate increase in SC at cessation of grounding indicates an opposite effect. Increased RR, stabilization of BO, and slight rise in heart rate suggest the start of a metabolic healing response necessitating an increase in oxygen consumption.

2.4.3. Immune Cell and Pain Responses with Delayed-Onset Muscle Soreness Induction. Pain reduction from sleeping grounded has been documented in previous studies [10, 13]. This pilot study looked for blood markers that might differentiate between grounded and ungrounded subjects who completed a single session of intense, eccentric exercise resulting in delayed-onset muscle soreness (DOMS) of the gastrocnemius [21]. If markers were able to differentiate these groups, future studies could be done in greater detail with a larger subject base. DOMS is a common complaint in the fitness and athletic world following excessive physical activity and involves acute inflammation in overtaxed muscles. It develops in 14 to 48 hours and persists for more than 96 hours [22]. No known treatment reduces the recovery period, but apparently massage and hydrotherapy [23–25] and acupuncture [26] can reduce pain.

Eight healthy men ages 20–23 were put through a similar routine of toe raises while carrying on their shoulders a barbell equal to one-third of their body weight. Each participant was exercised individually on a Monday morning and then monitored for the rest of the week while following a similar eating, sleeping, and living schedule in a hotel. The

group was randomly divided in half and either grounded or sham grounded with the use of a conductive patch placed at the sole of each foot during active hours and a conductive sheet at night. Complete blood counts, blood chemistry, enzyme chemistry, serum and saliva cortisol, magnetic resonance imaging and spectroscopy, and pain levels (a total of 48 parameters) were taken at the same time of day before the eccentric exercise and at 24, 48, and 72 hours afterwards. Parameters consistently differing by 10 percent or more, normalized to baseline, were considered worthy of further study.

Parameters that differed by these criteria included white blood cell counts, bilirubin, creatine kinase, phosphocreatine/inorganic phosphate ratios, glycerolphosphorylcholine, phosphorylcholine, the visual analogue pain scale, and pressure measurements on the right gastrocnemius.

The results showed that grounding the body to the Earth alters measures of immune system activity and pain. Among the ungrounded men, for instance, there was an expected, sharp increase in white blood cells at the stage when DOMS is known to reach its peak and greater perception of pain (see Figure 3). This effect demonstrates a typical inflammatory response. In comparison, the grounded men had only a slight decrease in white blood cells, indicating scant inflammation, and, for the first time ever observed, a shorter recovery time. Brown later commented that there were "significant differences" in the pain these men reported [12].

2.4.4. Heart Rate Variability. The rapid change in skin conductance reported in an earlier study led to the hypothesis that grounding may also improve heart rate variability (HRV), a measurement of the heart's response to ANS regulation. A double-blind study was designed with 27 participants [27]. Subjects sat in a comfortable reclining chair. Four transcutaneous electrical nerve stimulation (TENS) type adhesive electrode patches were placed on the sole of each foot and on each palm.

Participants served as their own controls. Each participant's data from a 2-hour session (40 minutes of which was grounded) were compared with another 2-hour sham-grounded session. The sequence of grounding versus sham-grounding sessions was assigned randomly.

During the grounded sessions, participants had statistically significant improvements in HRV that went way beyond basic relaxation results (which were shown by the nongrounded sessions). Since improved HRV is a significant positive indicator on cardiovascular status, it is suggested that simple grounding techniques be utilized as a basic integrative strategy in supporting the cardiovascular system, especially under situations of heightened autonomic tone when the sympathetic nervous system is more activated than the parasympathetic nervous system.

2.4.5. Reduction of Primary Indicators of Osteoporosis, Improvement of Glucose Regulation, and Immune Response. K. Sokal and P. Sokal, cardiologist and neurosurgeon father and son on the medical staff of a military clinic in Poland, conducted a series of experiments to determine whether contact with the Earth via a copper conductor can affect physiological processes [11]. Their investigations were prompted by the question as to whether the natural electric charge on the surface of the Earth influences the regulation of human physiological processes.

Double-blind experiments were conducted on groups ranging from 12 to 84 subjects who followed similar physical activity, diet, and fluid intake during the trial periods. Grounding was achieved with a copper plate ($30\,\text{mm} \times 80\,\text{mm}$) placed on the lower part of the leg, attached with a strip so that it would not come off during the night. The plate was connected by a conductive wire to a larger plate ($60\,\text{mm} \times 250\,\text{mm}$) placed in contact with the Earth outside.

In one experiment with nonmedicated subjects, grounding during a single night of sleep resulted in statistically significant changes in concentrations of minerals and electrolytes in the blood serum: iron, ionized calcium, inorganic phosphorus, sodium, potassium, and magnesium. Renal excretion of both calcium and phosphorus was reduced significantly. The observed reductions in blood and urinary calcium and phosphorus directly relate to osteoporosis. The results suggest that Earthing for a single night reduces primary indicators of osteoporosis.

Earthing continually during rest and physical activity over a 72-hour period decreased fasting glucose among patients with non-insulin-dependent diabetes mellitus. Patients had been well controlled with glibenclamide, an antidiabetic drug, for about 6 months, but at the time of study had unsatisfactory glycemic control despite dietary and exercise advice and glibenclamide doses of 10 mg/day.

K. Sokal and P. Sokal drew blood samples from 6 male and 6 female adults with no history of thyroid disease. A single night of grounding produced a significant decrease of free tri-iodothyronine and an increase of free thyroxin and thyroid-stimulating hormone. The meaning of these results is unclear but suggests an earthing influence on hepatic, hypothalamus, and pituitary relationships with thyroid function. Ober et al. [12] have observed that many individuals on thyroid medication reported symptoms of hyperthyroid, such as heart palpitations, after starting grounding. Such symptoms typically vanish after medication is adjusted downward under medical supervision. Through a series of feedback regulations, thyroid hormones affect almost every physiological process in the body, including growth and development, metabolism, body temperature, and heart rate. Clearly, further study of earthing effects on thyroid function is needed.

In another experiment, the effect of grounding on the classic immune response following vaccination was examined. Earthing accelerated the immune response, as demonstrated by increases in gamma globulin concentration. This result confirms an association between earthing and the immune response, as was suggested in the DOMS study [21].

K. Sokal and P. Sokal conclude that earthing the human body influences human physiological processes, including increasing the activity of catabolic processes and may be "the primary factor regulating endocrine and nervous systems."

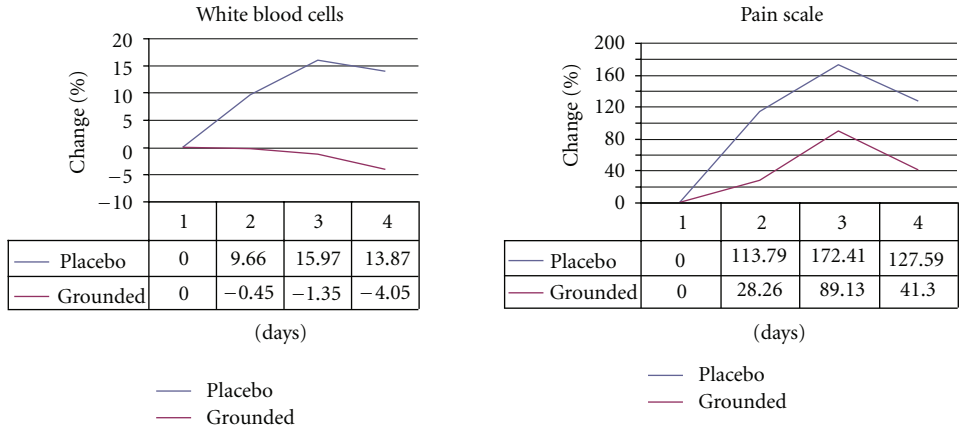

Figure 3: Delayed onset muscle soreness and grounding. Consistent with all measurements, ungrounded subjects expressed the perception of greater pain. Related to the pain finding was evidence of a muted white blood cell response indicating that a grounded body experiences less inflammation.

2.4.6. Altered Blood Electrodynamics. Since grounding produces changes in many electrical properties of the body [1, 15, 19, 28], a next logical step was to evaluate the electrical property of the blood. A suitable measure is the zeta potential of red blood cells (RBCs) and RBC aggregation. Zeta potential is a parameter closely related to the number of negative charges on the surface of an RBC. The higher the number, the greater the ability of the RBC to repel other RBCs. Thus, the greater the zeta potential the less coagulable is the blood.

Ten relatively healthy subjects participated in the study [29]. They were seated comfortably in a reclining chair and were grounded for two hours with electrode patches placed on their feet and hands, as in previous studies. Blood samples were taken before and after.

Grounding the body to the earth substantially increases the zeta potential and decreases RBC aggregation, thereby reducing blood viscosity. Subjects in pain reported reduction to the point that it was almost unnoticeable. The results strongly suggest that earthing is a natural solution for patients with excessive blood viscosity, an option of great interest not just for cardiologists, but also for any physician concerned about the relationship of blood viscosity, clotting, and inflammation. In 2008, Adak and colleagues reported the presence of both hypercoagulable blood and poor RBC zeta potential among diabetics. Zeta potential was particularly poor among diabetics with cardiovascular disease [30].

3. Discussion

Until now, the physiological significance and possible health effects of stabilizing the internal bioelectrical environment of an organism have not been a significant topic of research. Some aspects of this, however, are relatively obvious. In the absence of Earth contact, internal charge distribution will not be uniform, but instead will be subject to a variety of electrical perturbations in the environment. It is well known that many important regulations and physiological processes involve events taking place on cell and tissue surfaces. In the absence of a common reference point, or "ground," electrical gradients, due to uneven charge distribution, can build up along tissue surfaces and cell membranes.

We can predict that such charge differentials will influence biochemical and physiological processes. First, the structure and functioning of many enzymes are sensitive to local environmental conditions. Each enzyme has an optimal pH that favors maximal activity. A change in the electrical environment can alter the pH of biological fluids and the charge distribution on molecules and thereby affect reaction rates. The pH effect results because of critical charged amino acids at the active site of the enzyme that participate in substrate binding and catalysis. In addition, the ability of a substrate or enzyme to donate or accept hydrogen ions is influenced by pH.

Another example is provided by voltage-gated ion channels, which play critical biophysical roles in excitable cells such as neurons. Local alterations in the charge profiles around these channels can lead to electrical instability of the cell membrane and to the inappropriate spontaneous activity observed during certain pathological states [31].

Earthing research offers insights into the clinical potential of barefoot contact with the Earth, or simulated barefoot contact indoors via simple conductive systems, on the stability of internal bioelectrical function and human physiology. Initial experiments resulted in subjective reports of improved sleep and reduced pain [10]. Subsequent research showed that improved sleep was correlated with a normalization of the cortisol day-night profile [13]. The results are significant in light of the extensive research showing that lack of sleep stresses the body and contributes to many detrimental health consequences. Lack of sleep is often the result of pain. Hence, reduction of pain might be one reason for the benefits just described.

Pain reduction from sleeping grounded has been confirmed in a controlled study on DOMS. Earthing is the first intervention known to speed recovery from DOMS [21]. Painful conditions are often the result of various kinds of acute or chronic inflammation conditions caused

in part by ROS generated by normal metabolism and also by the immune system as part of the response to injury or trauma. Inflammation can cause pain and loss of range of motion in joints. Inflammatory swelling can put pressure on pain receptors (nocireceptors) and can compromise the microcirculation, leading to ischemic pain. Inflammation can cause the release of toxic molecules that also activate pain receptors. Modern biomedical research has also documented a close relationship between chronic inflammation and virtually all chronic diseases, including the diseases of aging, and the aging process itself. The steep rise in inflammatory diseases, in fact, has been recently called "inflamm-aging" to describe a progressive inflammatory status and a loss of stress-coping ability as major components of the aging process [32].

Reduction in inflammation as a result of earthing has been documented with infrared medical imaging [28] and with measurements of blood chemistry and white blood cell counts [21]. The logical explanation for the anti-inflammatory effects is that grounding the body allows negatively charged antioxidant electrons from the Earth to enter the body and neutralize positively charged free radicals at sites of inflammation [28]. Flow of electrons from the Earth to the body has been documented [15].

A pilot study on the electrodynamics of red blood cells (zeta potential) has revealed that earthing significantly reduces blood viscosity, an important but neglected parameter in cardiovascular diseases and diabetes [29], and circulation in general. Thus, thinning the blood may allow for more oxygen delivery to tissues and further support the reduction of inflammation.

Stress reduction has been confirmed with various measures showing rapid shifts in the ANS from sympathetic to parasympathetic dominance, improvement in heart rate variability, and normalization of muscle tension [19, 20, 27].

Not reported here are many observations over more than two decades by Ober et al. [12] and K. Sokal and P. Sokal [11] indicating that regular earthing may improve blood pressure, cardiovascular arrhythmias, and autoimmune conditions such as lupus, multiple sclerosis, and rheumatoid arthritis. Some effects of earthing on medication are described by Ober et al. [12] and at the website: http://www.earthinginstitute.net/. As an example, the combination of earthing and coumadin has the potential to exert a compounded blood thinning effect and must be supervised by a physician. Multiple anecdotes of elevated INR have been reported. INR (international normalized ratio) is a widely used measurement of coagulation. The influence of earthing on thyroid function and medication has been described earlier.

From a practical standpoint, clinicians could recommend outdoor "barefoot sessions" to patients, weather, and conditions permitting. Ober et al. [12] have observed that going barefoot as little as 30 or 40 minutes daily can significantly reduce pain and stress, and the studies summarized here explain why this is the case. Obviously, there is no cost for barefoot grounding. However, the use of conductive systems while sleeping, working, or relaxing indoors offer a more convenient and routine-friendly approach.

4. Conclusion

De Flora et al. wrote the following: "Since the late 20th century, chronic degenerative diseases have overcome infectious disease as the major causes of death in the 21st century, so an increase in human longevity will depend on finding an intervention that inhibits the development of these diseases and slows their progress" [33].

Could such an intervention be located right beneath our feet? Earthing research, observations, and related theories raise an intriguing possibility about the Earth's surface electrons as an untapped health resource—the Earth as a "global treatment table." Emerging evidence shows that contact with the Earth—whether being outside barefoot or indoors connected to grounded conductive systems—may be a simple, natural, and yet profoundly effective environmental strategy against chronic stress, ANS dysfunction, inflammation, pain, poor sleep, disturbed HRV, hypercoagulable blood, and many common health disorders, including cardiovascular disease. The research done to date supports the concept that grounding or earthing the human body may be an essential element in the health equation along with sunshine, clean air and water, nutritious food, and physical activity.

Disclosure

G. Chevalier, S. T. Sinatra, and J. L. Oschman are independent contractors for Earthx L. Inc., the company sponsoring earthing research, and own a small percentage of shares in the company.

References

[1] E. Williams and S. Heckman, "The local diurnal variation of cloud electrification and the global diurnal variation of negative charge on the Earth," *Journal of Geophysical Research*, vol. 98, no. 3, pp. 5221–5234, 1993.

[2] S. Anisimov, E. Mareev, and S. Bakastov, "On the generation and evolution of aeroelectric structures in the surface layer," *Journal of Geophysical Research D*, vol. 104, no. 12, pp. 14359–14367, 1999.

[3] J. L. Oschman, "Perspective: assume a spherical cow: the role of free or mobile electrons in bodywork, energetic and movement therapies," *Journal of Bodywork and Movement Therapies*, vol. 12, no. 1, pp. 40–57, 2008.

[4] J. L. Oschman, "Charge transfer in the living matrix," *Journal of Bodywork and Movement Therapies*, vol. 13, no. 3, pp. 215–228, 2009.

[5] D. Holiday, R. Resnick, and J. Walker, *Fundamentals of Physics, Fourth Edition*, John Wiley & Sons, New York, NY, USA, 1993.

[6] W. Rossi, *The Sex Life of the Foot and Shoe*, vol. 61, Wordsworth Editions, Hertfordshire, UK, 1989.

[7] R. Stein, *Is Modern Life Ravaging Our Immune Systems?* Washington Post, 2008.

[8] A. Just, *Return to Nature: The True Natural Method of Healing and Living and The True Salvation of the Soul*, B. Lust, New York, NY, USA, 1903.

[9] G. White, *The Finer Forces of Nature in Diagnosis and Therapy*, Phillips Printing Company, Los Angeles, Calif, USA, 1929.

[10] C. Ober, "Grounding the human body to neutralize bioelectrical stress from static electricity and EMFs," ESD Journal, http://www.esdjournal.com/articles/cober/ground.htm, January 2000.
[11] K. Sokal and P. Sokal, "Earthing the human body influences physiologic processes," *Journal of Alternative and Complementary Medicine*, vol. 17, no. 4, pp. 301–308, 2011.
[12] C. Ober, S. T. Sinatra, and M. Zucker, *Earthing: The Most Important Health Discovery Ever?* Basic Health Publications, Laguna Beach, Calif, USA, 2010.
[13] M. Ghaly and D. Teplitz, "The biologic effects of grounding the human body during sleep as measured by cortisol levels and subjective reporting of sleep, pain, and stress," *Journal of Alternative and Complementary Medicine*, vol. 10, no. 5, pp. 767–776, 2004.
[14] "NIH State-of-the-Science Conference on Manifestations and Management of Chronic Insomnia in Adults," http://consensus.nih.gov/2005/insomniastatement.htm, June 13-15, 2005.
[15] R. Applewhite, "The effectiveness of a conductive patch and a conductive bed pad in reducing induced human body voltage via the application of earth ground," *European Biology and Bioelectromagnetics*, vol. 1, pp. 23–40, 2005.
[16] R. Feynman, R. Leighton, and M. Sands, *The Feynman Lectures on Physics*, vol. II, Addison-Wesley, Boston, Mass, USA, 1963.
[17] K. S. Jamieson, H. M. ApSimon, S. S. Jamieson, J. N. B. Bell, and M. G. Yost, "The effects of electric fields on charged molecules and particles in individual microenvironments," *Atmospheric Environment*, vol. 41, no. 25, pp. 5224–5235, 2007.
[18] S. J. Genuis, "Fielding a current idea: exploring the public health impact of electromagnetic radiation," *Public Health*, vol. 122, no. 2, pp. 113–124, 2008.
[19] G. Chevalier, K. Mori, and J. L. Oschman, "The effect of Earthing (grounding) on human physiology," *European Biology and Bioelectromagnetics*, vol. 2, no. 1, pp. 600–621, 2006.
[20] G. Chevalier, "Changes in pulse rate, respiratory rate, blood oxygenation, perfusion index, skin conductance, and their variability induced during and after grounding human subjects for 40 minutes," *Journal of Alternative and Complementary Medicine*, vol. 16, no. 1, pp. 1–7, 2010.
[21] R. Brown, G. Chevalier, and M. Hill, "Pilot study on the effect of grounding on delayed-onset muscle soreness," *Journal of Alternative and Complementary Medicine*, vol. 16, no. 3, pp. 265–273, 2010.
[22] M. F. Bobbert, A. P. Hollander, and P. A. Huijing, "Factors in delayed onset muscular soreness of man," *Medicine and Science in Sports and Exercise*, vol. 18, no. 1, pp. 75–81, 1986.
[23] B. Tartibian, B. Maleki, and A. Abbasi, "The effects of ingestion of Omega-3 fatty acids on perceived pain and external symptoms of delayed onset muscle soreness in untrained men," *Clinical Journal of Sport Medicine*, vol. 19, no. 2, pp. 115–119, 2009.
[24] J. Vaile, S. Halson, N. Gill, and B. Dawson, "Effect of hydrotherapy on the signs and symptoms of delayed onset muscle soreness," *European Journal of Applied Physiology*, vol. 102, no. 4, pp. 447–455, 2008.
[25] Z. Zainuddin, M. Newton, P. Sacco, and K. Nosaka, "Effects of massage on delayed-onset muscle soreness, swelling, and recovery of muscle function," *Journal of Athletic Training*, vol. 40, no. 3, pp. 174–180, 2005.
[26] M. Hübscher, L. Vogt, M. Bernhörster, A. Rosenhagen, and W. Banzer, "Effects of acupuncture on symptoms and muscle function in delayed-onset muscle soreness," *Journal of Alternative and Complementary Medicine*, vol. 14, no. 8, pp. 1011–1016, 2008.
[27] G. Chevalier and S. Sinatra, "Emotional stress, heart rate variability, grounding, and improved autonomic tone: clinical applications," *Integrative Medicine: A Clinician's Journal*, vol. 10, no. 3, 2011.
[28] J. L. Oschman, "Can electrons act as antioxidants? A review and commentary," *Journal of Alternative and Complementary Medicine*, vol. 13, no. 9, pp. 955–967, 2007.
[29] G. Chevalier, S. T. Sinatra, J. L. Oschman, and R. M. Delany, "Grounding the human body reduces blood viscosity—a major factor in cardiovascular disease," *Journal of Alternative and Complementary Medicine*. In press.
[30] S. Adak, S. Chowdhury, and M. Bhattacharyya, "Dynamic and electrokinetic behavior of erythrocyte membrane in diabetes mellitus and diabetic cardiovascular disease," *Biochimica et Biophysica Acta*, vol. 1780, no. 2, pp. 108–115, 2008.
[31] M. Chahine, A. Chatelier, O. Babich, and J. J. Krupp, "Voltage-gated sodium channels in neurological disorders," *CNS and Neurological Disorders—Drug Targets*, vol. 7, no. 2, pp. 144–158, 2008.
[32] C. Franceschi, M. Bonafè, S. Valensin et al., "Inflamm-aging: an evolutionary perspective on immunosenescence," *Annals of the New York Academy of Sciences*, vol. 908, pp. 244–254, 2000.
[33] S. de Flora, A. Quaglia, C. Bennicelli, and M. Vercelli, "The epidemiological revolution of the 20th century," *FASEB Journal*, vol. 19, no. 8, pp. 892–897, 2005.

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Review Article

A Review of the Diagnosis and Treatment of Ochratoxin A Inhalational Exposure Associated with Human Illness and Kidney Disease including Focal Segmental Glomerulosclerosis

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Ochratoxin A (OTA) exposure via ingestion and inhalation has been described in the literature to cause kidney disease in both animals and humans. This paper reviews Ochratoxin A and its relationship to human health and kidney disease with a focus on a possible association with focal segmental glomerulosclerosis (FSGS) in humans. Prevention and treatment strategies for OTA-induced illness are also discussed, including cholestyramine, a bile-acid-binding resin used as a sequestrant to reduce the enterohepatic recirculation of OTA.

1. Introduction

Ochratoxin A (OTA) is a known nephrotoxic, immunotoxic, and carcinogenic mycotoxin in animals [1, 2] that has long been studied for its role in animal and human disease. Molds associated with the production of OTA include *Aspergillus ochraceus, Aspergillus niger* and *Aspergillus carbonarius, Penicillium verrucosum*, and species of *Penicillium, Petromyces,* and *Neopetromyces* [3]. Concerns regarding exposure to ochratoxin have primarily centered on exposure to food contaminated with OTA such as wine, beer, coffee, dried vine fruit, grape juices, pork, poultry, dairy, spices, and chocolate [1]. Toxicity from ochratoxin is considered serious enough that it is among approximately 20 mycotoxins monitored in food.

Options for diagnosis and treatment of persons diagnosed with chronic kidney disease and FSGS are reviewed. Treatment is then discussed in the context of two patients diagnosed with FSGS who were found to have significantly elevated levels of urine OTA. This exposure is believed to have resulted from inhalational exposure from water damaged indoor environments found to have elevated levels of mold including species of *Aspergillus* and *Penicillium.* This review is intended to highlight the importance of prevention and treatment of human kidney disease and ochratoxin exposure from indoor mold.

2. Mechanisms of Toxicity

Ochratoxins occur in nature as Ochratoxin A, B, and C. OTA is the most prevalent toxin, and our discussion will be limited to OTA. OTA is a proven carcinogen in animals and is classified as a class 2B, possible human carcinogen by the International Agency for Research on Cancer [4]. The National Toxicology Program (NTP) has designated OTA as "reasonably anticipated to be a human carcinogen" based on sufficient evidence of carcinogenicity in experimental animals [1].

After initial exposure from any source, the urinary and fecal excretory routes of OTA are both important with the relative contribution of each dependent upon factors such as route of administration and dose [5]. In the blood, OTA binds to albumin and the bound fraction constitutes a mobile reserve of OTA [6]. The relative contribution of each excretory route is influenced by the degree of serum macromolecular binding as well as differences in the enterohepatic

recirculation of OTA [7]. Elimination of OTA in urine and feces is felt to be relatively slow and has been shown to vary by species and gender as well as specific genotype that may affect the biotransformation of OTA [8, 9].

Intestinal microflora also appear to contribute significantly to the metabolism of OTA via hydrolyzation to the less toxic ochratoxin alpha in rats [10]. Inhibition of microflora in the lower GI tract of rats by neomycin results in decreased hydrolysis of OTA to ochratoxin alpha resulting in elevated levels of OTA [11]. In addition, administration of radiolabeled OTA to rats indicated that effective metabolism of OTA was lacking in most tissues other than the intestines [12]. The importance of digestion in the detoxication of OTA is also supported by the observation that OTA does not readily accumulate in ruminants due to rapid detoxification in the extensive ruminant stomach [5, 13].

Limited information is available on the metabolic disposition of OTA in humans, although it has been suggested that it has a long serum half-life due to strong binding to human serum macromolecules [7, 14].

Individual genetic differences affect the biotransformation and relative toxicity of OTA, with enzymatic hydrolysis and cytochrome p450 induction felt to play a role in toxicity. Studies have indicated that the biotransformation of OTA can be effected by CYP 3A4, CYP 1A1, and CYP 2C9-1 while conflicting results have been found for CYP 1A2 [15, 16].

DNA adducts also occur in animals exposed to OTA in all available studies [17–20]. DNA adducts consist of a chemical covalently bound to DNA. This could interfere with the DNA repair systems and cell cycle controls systems and serve as an initiating point of carcinogenesis.

Oxidative stress is another component of OTA toxicity [21]. Pretreatment of rats with retinol (vitamin A), ascorbic acid (vitamin C), or alpha tocopherol (vitamin E) before OTA administration significantly decreased the number of DNA adducts formed in the kidney by 70 percent, 90 percent, and 80 percent, respectively [22]. In addition, lipid peroxidation and enzymes involved in arachidonic acid metabolism affect the biotransformation of OTA [23].

More recently, it is been shown in rodents that mTOR/AKT pathways are significantly deregulated after exposure to OTA, possibly contributing to carcinogenicity in kidney cells [24].

3. Tissue Distribution

Tissue distribution after exposure of animals to OTA has consistently revealed that the greatest concentration is in the kidneys followed by either liver or muscle and then fat [25–27], but tissues found to contain OTA also include the adrenal medulla and cortex, skin, myocardium, gastric mucosa, and bone marrow [28].

In humans, OTA has been detected in blood, urine, and breast milk [29–31] as well as renal cell carcinomas, breast cancer, astrocytoma, inflamed bladder tissue and transitional cell carcinoma of the bladder, and a skin biopsy sample [32]. Recently, a case has been reported of OTA being found in the umbilical cord and placental tissue of a newborn whose mother had been exposed from a water-damaged home. In addition, the mother had OTA in her breast milk, urine, and nasal secretions [33]. Also, other family members tested positive for OTA in urine and nasal secretion samples, while the pet dog was positive for OTA in its urine and an ear mass [33].

4. Agents Modulating Toxicity

Various substances have been found to either increase or decrease the toxicity of OTA. In mice, pretreatment with phenobarbital decreased the toxicity of OTA with significant increases in LD50 seen [34, 35]. Administration of piperonyl butoxide was also shown to significantly decrease the LD50 of OTA [35] thus increasing toxicity.

A protective effect of melatonin and licorice plant extract was demonstrated in rats exposed to OTA for 28 days and alleviated most of the biochemical abnormalities associated with the exposure [36]. It is significant in this study that the histopathological abnormalities were seen in this relatively short 28 day exposure and showed degenerative symptoms in the proximal tubules, congestion in renal tissue, and a remarkable infiltration of inflammatory cells consistent with OTA nephropathy.

Phenylalanine prevents acute poisoning by OTA in mice [37]. Aspartame, a structural analogue of phenylalanine, is also a powerful inhibitor of OTA toxicity, at least in animals [38].

In male rats, OTA was more toxic in the presence of phenylbutazone (a nonsteroidal anti inflammatory drug/NSAID) and ethyl biscoumacetate (vitamin K antagonist/coumarin) and was less toxic when administered with sulfamethoxypridazine (a sulfonamide antibiotic) [39].

5. Exposure to Ochratoxin A from Food

Dietary exposure to OTA has been extensively documented in the literature and worldwide remains a significant source of OTA exposure in humans [1, 4]. Background studies on average levels of OTA in humans eating a typical diet have, however, only shown modest elevations in urinary OTA levels, well under the 2.0 ppb limit of detection level used by the commercial lab to test these patients [40].

6. Inhalational Exposure to Ochratoxin A

Throughout the literature, much of the study of ochratoxin exposure focuses on exposure through ingestion. However, inhalation exposure in water-damaged buildings with amplified indoor growth of ochratoxin producing species of mold remains a significant risk. OTA has been identified in studies of water-damaged buildings from air [41], dust [42], wallpaper [43], and agricultural dust and conidia [44]. Hooper et al. have reported elevated concentrations of OTA in the urine of individuals exposed to water-damaged buildings versus unexposed controls [40]. The concentrations of mycotoxins for controls not exposed to water damaged buildings were below the detection limit which is 2.0 ppb for ochratoxin [40].

In a study by Skaug et al., dust and aerosol samples were collected from three Norwegian cowsheds [44]. OTA was detected in 6 out of 14 samples with concentrations ranging from 0.2 to 70 μg/kg (ppb) [44]. Collected conidia also contained OTA. The authors concluded that airborne dust and conidia can be sources of OTA and that peak exposures and absorptions from this route can be considerable, especially given the efficiency with which OTA is absorbed through the lung [44, 45]. Testing has also indicated the presence of OTA from samples of air filters, refrigerator filters, dust from air vents, and a towel and sandals from water-damaged buildings [32, 33].

In another study of dust collected from heating ducts in a household where animals were exhibiting signs of ochratoxin poisoning, it was found that all samples in one group yielded ochratoxin, with samples from one duct being over 1500 ppb and another duct showing levels of 306 ppb. In a sample group in the same study, the dust from six samples was measured in a composite showing a level of 58 ppb [42]. In a subsequent study using high-performance liquid chromatography to quantify airborne mycotoxins in a poultry house in Dalian, China, OTA along with aflatoxin and zearalenone was detected [41].

Rapid systemic appearance of OTA after inhalation exposure in rats has been documented with a 98 percent bioavailability [45]. This clearly makes inhalational exposure to OTA a very significant risk.

One of the most striking cases documented in the literature involves the onset of acute renal failure from inhaled OTA after an 8-hour exposure to a granary that had been closed for several years in which an ochratoxin-producing strain of *Aspergillus ochraceus* was isolated [46]. The couple who were exposed to this granary experienced respiratory distress, retrosternal burning, epigastric tension, and asthenia. The husband's condition improved within 24 hours; however, the women's condition worsened and she was admitted 5 days later with nonoliguric renal failure, pulmonary edema, periorbital and lower extremity edema, and proteinuria of 4.6 g/L. A biopsy showed acute tubulonecrosis with interstitial edema with localized infiltration of lymphocytes, granulocytes, and macrophages with thickening of the basement membrane. Fortunately, her kidney function returned to normal 40 days after the exposure.

7. Ochratoxin and Renal Disease

Ochratoxin A has been found to be nephrotoxic in all mammalian species treated [6], although differences in toxicity have been found among species and sex. OTA is excreted in both the stool and urine. Although likely a minor route of excretion, ochratoxin has also been found in human sweat [47].

OTA has been associated with human kidney disease and is the probable causative agent of Balkan Endemic Nephropathy (BEN) [48], a severe, progressive, and ultimately fatal renal disease affecting populations in the Balkan Peninsula. Studies have indicated elevated levels of OTA in persons affected by BEN compared to neighboring persons in unaffected Bulgarian villages [1]. Additionally, several studies in animals have confirmed a causal connection between OTA exposure and cancers of the urinary tract, liver, and mammary glands [49–52]. Striking similarities have been noted between OTA-induced porcine nephropathy in pigs and BEN in humans [21].

The main nephrotoxic effect is in the postproximal nephron and proximal tubule which have been reported as a self-enhancing effect [53, 54].

A recent study in Sri Lanka measuring mycotoxin levels in the urine of patients with kidney disease demonstrated the presence of ochratoxin in 93.5 percent of patients tested although ochratoxin was also found in individuals without kidney disease [55]. Clark's review of ochratoxin in the blood concluded that the highest levels were observed in sampled populations that included persons with kidney disorders with serum level as high as 35–100 ng/mL (ppb) [1]. In contrast, mean serum OTA level in Europe was found to be 0.1–2 ng/mL (ppb) [1].

A correlation of consumption of foods known to contain OTA and the incidence of testicular cancer in 20 countries has suggested the possibility of OTA being related to an increased incidence of testicular carcinoma. The authors also report that there is correlation of pork and coffee intake with testicular carcinoma. In addition, animals exposed to OTA contain OTA in the testes and OTA causes adducts in testicular DNA [56].

Several studies on Tunisians with and without renal disease have shown elevations in serum OTA levels in both populations, with higher levels being found in those with renal disease. In one report, the mean value of OTA for the healthy control population was 3.3 ± 1.5 ng/mL (ppb) compared to a mean value of 18 ± 7 ng/mL (ppb) in those with chronic interstitial nephropathy of unknown origin [57]. Another study of OTA in human blood samples comparing persons with various types of chronic kidney disease to controls showed elevations in serum ochratoxin which were greatest in those diagnosed with chronic interstitial nephropathy at mean values of 25–59 ng/mL (ppb) compared to 0.7–7.8 ng/mL (0.7–7.8 ppb) in the general population and 6–18 ng/mL (ppb) in those with other types of kidney disease [58].

8. Ochratoxin and Reproduction

Ochratoxin can cross the placenta and has been found to be embryotoxic in rats and mice [6]. Studies with radiolabeled OTA in mice showed OTA to cross the placenta [59], preferentially at specific times during gestation. Additionally OTA has been found in breast milk which could represent a significant source of exposure for infants [33, 60].

A study of rats exposed to OTA preconception, during gestation and during lactation showed that the exposed offspring had three to four times higher levels of OTA than the controls. The rat offspring exposed to OTA in both in utero and through breast milk were found to have the highest blood and kidney concentrations of OTA, with the most significant exposure attributed to lactation [60]. In this study, the transfer of OTA to breast milk was found to be very efficient with levels at 60 percent of blood concentrations.

Moreover, a study in rabbits showed an effective transport of OTA from blood to milk and subsequently to the offspring with plasma and kidney concentrations much higher in offspring than adults, possibly due to slower detoxification in the offspring [61]. A study involving 80 Norwegian women found that 21 percent of the breast milk samples showed elevations of OTA ranging from 10 to 182 ng/L (ppt) [62]. The authors believed that these observations were very significant since studies in neonatal rats have shown that neonates are much more susceptible than adult rats with LD50 values for OTA from the oral route being only 3.9 mg/kg in neonates compared with adult LD 50 values of 20–330.3 mg/kg in adults [5]. In addition, testing breast milk samples from 75 women in Ankara, Turkey whose children were patients in the Neonatology Department, showed OTA in all samples tested in the range of 620.87–11311.30 ng/L (ppt) [63]. Additionally, OTA concentration in fetal serum was reported to be twice that of the mother indicating an active transfer of OTA across the placenta [64].

OTA was also shown to decrease testosterone secretion in testicular interstitial cells of gerbils [65].

9. Ochratoxin and the Brain

In vitro and *in vivo* research has demonstrated cerebellar [66, 67], hippocampal [68], and other adverse neurological effects due to OTA [66–69]. A single dose of OTA to Swiss mice was associated with significant oxidative damage in six brain regions—the cerebellum, hippocampus, caudate putamen, pons medulla, substantia nigra, and cerebral cortex. Peak effects were observed in the midbrain, caudate/putamen, and hippocampus [69]. In addition, striatal dopamine was decreased after a single exposure to OTA [69]. *In vitro* experiments have shown decreased proliferation of neural progenitor stem cells in the hippocampal region of mice after exposure to OTA leading the authors to speculate that problems impairing hippocampal neurogenesis *in vivo* could contribute to the memory problems and depression commonly seen in humans exposed to mycotoxins [68]. In another study evaluating the neurotoxicity of ochratoxin A, primary neurons and neuronal cells were incubated with increasing concentrations of OTA [66]. A dose-dependent increase in cytotoxicity was found in both cell types resulting from apoptosis and accompanied by a loss of mitochondrial membrane potential [66]. Based on these data, the authors speculated that OTA may contribute to the development of neurodegenerative diseases such as Alzheimer's and Parkinson's in which apoptotic processes are centrally involved [66].

10. Ochratoxin and Immunity

OTA is known to be immunotoxic in animal studies [1, 2, 70]. The immunosuppressant activity of OTA in animals has been characterized by size reduction of vital immune organs like the thymus, spleen and lymph nodes, depression of antibody responses, alterations in the number and functions of immune cells, and modulation of cytokine production [70].

There are also complex relationships between *Aspergillus*, T regulatory lymphocytes and candidiasis [71], which can be clinically relevant in humans.

11. Focal Segmental Glomerulosclerosis (FSGS)

FSGS is a potentially devastating kidney disease that occurs most frequently in children and young adults but can occur at any age. In early stages of the disease, the kidneys are typically normal or enlarged, while in the late stage of the illness, kidneys are typically shrunken [72].

FSGS accounts for approximately one sixth of the cases of nephrotic syndrome in children and is a common cause of kidney failure in adults. The annual incidence of end-stage renal disease from FSGS has increased 11-fold from 0.2 percent to 2.3 percent between 1980 and 2000, and FSGS is now the most common cause of end-stage renal disease resulting from primary glomerular disease [72].

There has been increasing recognition of causes in primary FSGS including genetic, viral, drug toxicity, and others. Cases are considered primary with no cause identified or secondary due to infections (HIV, Hep B, Parvovirus), toxins (heroin, pamidronate, analgesics), familial, or nephron loss from chronic pyelonephritis, obesity, diabetes, sickle cell disease, or anatomic abnormalities and malignancies [71]. It is not uncommon for the onset of illness to occur after an upper respiratory infection. Ethnic differences are seen in the prevalence of the disease with blacks affected seven times more often as whites with a worse prognosis once the disease is acquired [72].

Treatments for FSGS include salt and protein restriction, diuretics for edema, ACE inhibitors, aldosterone antagonists, steroids, cytotoxics agents (e.g., cyclophosphamide), immunosuppressants (e.g., cyclosporin and tacrolimus), plasmapharesis, and treatment for hyperlipidemia which commonly occurs with the illness [72]. Rarely intravenous albumin or mannitol has been used for intractable edema. Often no treatment is successful and the patient requires dialysis and eventual transplantation. In patients who do not respond to treatment, the average time from onset of the disease to end-stage renal disease is from 6 to 8 years [72].

Recurrence of the disease posttransplantation has long been recognized as a significant risk. In one study of 77, mostly pediatric patients with idiopathic nephrotic syndrome and FSGS who underwent transplantation, 42 had nephrotic range proteinuria posttransplant, and 20 eventually developed pathology consistent with FSGS in the transplanted kidney [73]. Interestingly, the majority of the recurrence occurs in the first 6 months, with the recurrence of illness rare after two years posttransplantation.

12. Human FSGS and Kidney Disease Associated with Ochratoxin A: Patient Histories

Two patients histories of FSGS associated with OTA exposure encountered by the authors are presented.

The first patient was a 48-year-old woman diagnosed with primary idiopathic FSGS who presented with end-stage renal disease 10 days before a scheduled kidney

transplantation. She had been referred by her daughter's pediatrician after she expressed concern about her daughter's serious respiratory symptoms and asthma exacerbations and indicated that her home had recently been found to have significant mold contamination including elevations of *Aspergillus/Penicillium* and *Stachybotrys* on nonviable spore sampling. The most significant elevations were found in the room she had used as a home office. The patient worked as a marriage and family therapist from her home office until she was no longer able to work due to her illness. She moved into the home 12 years prior to presentation and believes the water damage to have been long standing, predating her move. Shortly after moving into the home she developed symptoms of chronic fatigue syndrome which have persisted. Three and a half years before her presentation to the office, she was seen in the emergency room for severe edema and diagnosed with nephrotic syndrome. The diagnosis of FSGS followed several kidney biopsies after it was initially undetected. Attempts to control the disease with long-term high-dose steroids were unsuccessful, and the disease progressed to the point where she was placed on peritoneal dialysis and the search for a donor kidney commenced. At the time of presentation to this office she was taking vitamin D, folic acid, dialyvite, calcitriol, sevelamer carbonate (a phosphate binder), and rosuvastatin for hyperlipidemia. Hyperlipidemia is a common complication of her type of renal disease or nephrotic syndrome. Physical exam was remarkable for a tired, uncomfortable appearing woman who felt nauseated on several occasions during the exam. She displayed 1+ pitting pretibial edema, mucosal nasal swelling with white patches noted, a thick white coating on her tongue, a I/VI systolic murmur which did not radiate, and moderate dysmetria on finger to nose testing. Urine mycotoxin testing was performed, and the patient was found to have significant elevations of ochratoxin A at 11.9 ppb (limit of detection 2.0 ppb). Aflatoxin and trichothecene mycotoxins were tested and were not detected. The testing for ochratoxin A was performed by a CLIA certified lab using immunoaffinity columns and fluorometry [40]. The patient reported a history of recurrent yeast infections necessitating the use of oral fluconazole and was found to be anergic to candida on intradermal skin testing, with a normal response to tetanus noted. A nasal fungal culture showed the presence of *Cladosporium* species, while fungal blood cultures remained negative. Testing for antibodies to *Aspergillus* species of mold was recommended but not performed. Prior to the dietary changes required by the onset of renal failure, the patient's diet was typical of the general population and did not include excessive consumption of foods known to contain OTA.

The patient was encouraged to commence treatment with oral cholestyramine as soon as possible, but met with resistance from her transplant team and ultimately did not initiate therapy. She did, however, follow the recommendation to move from her home to which she has not yet returned and has avoided further exposure to items exposed to the mold contaminated home. Her posttransplantation course was remarkable for the recurrence of proteinuria within days of the transplantation. Her function in the transplanted kidney has continued to deteriorate, and she was diagnosed with FSGS in the previously healthy donor kidney within months of the transplantation. Additionally, she has experienced rejection of the transplanted kidney requiring a course of solumedrol and long-term use of immunosuppressant agents. To date, the patient has not undergone any treatment to reduce the body burden of ochratoxin indicated by her initial elevations in urinary ochratoxin other than avoidance of her water-damaged home.

The second patient was a 5-year-old girl who presented to the office with a history of receiving a diagnosis of FSGS at the age of 3.5 after proteinuria was identified when she presented to her pediatrician experiencing new onset enuresis. The family sought care from the authors due to chronic symptoms and illnesses in all family members and history of exposure to water-damaged environments in past and current homes. Due to the father's work, the family has moved frequently. They recall evidence of water damage and mold in several of their previous homes and confirmed the presence of elevated levels of indoor mold in their current home. The patient's diet was typical for her age and did not include excessive consumption of foods known to contain elevated levels of ochratoxin.

Testing indicated the patient did not have a genetic cause for FSGS. Upon diagnosis she was placed on a 6-week course of high-dose prednisone and has since used tacrolimus and enalapril as well as galactose. Her protein excretion in urine is followed regularly, and her mother reports an increased level of proteinuria upon moving to their most recent home.

She was born by NSVD at 39 weeks gestation. APGAR scores are not available. Her mother reports she required some initial resuscitation, but improved quickly and did not require transfer to neonatal intensive care unit. At 5 days of age, she was found to have severe hyperbilirubinemia, with reported levels of 26 mg/dL. She was breastfed for 14 months.

Her physical exam was remarkable for nasal mucosal swelling with clear discharge and erythematous right tympanic membrane consistent otitis media, slightly enlarged bilateral submandibular lymph nodes, and moderate sway when balancing on toes with eyes open and an inability to balance on toes with eyes closed.

Urine mycotoxin testing was performed, and she was found to have significantly elevated level of ochratoxin A at 9.1 ppb (limit of detection 2.0 ppb). Aflatoxin and trichothecene mycotoxins were not detected in the sample. Of note, elevations in urinary levels of ochratoxin and trichothecene mycotoxins were found in other family members as would be expected with their shared inhalational exposure; however, the patient's level of OTA was significantly higher than that of the other family members.

At the time of presentation to this office, the patient was also reporting symptoms of night sweats, heat intolerance, frequent episodes of otitis media and conjunctivitis, dizziness, hair loss, fungal skin rashes, episodes of excessive thirst, and the recent onset of reversing letters when she writes. Additionally, she has a history of significant dental disease including requiring a root canal at the age of 4.

The patient was started on therapy including avoidance of exposure to water-damaged/moldy environments and property exposed to these environments and the use of nutritional support, liposomal glutathione, and sequestering agents including cholestyramine and charcoal. Within months of starting treatment, the mother reported a significant decrease in the patients urinary protein excretion with the lowest levels found to date on recent testing.

13. Treatment

Given the well-known nephrotoxicity of OTA, as well as reports of human kidney disease associated with elevated levels of OTA, including BEN and other kidney disease, and this report of elevated OTA in two patients diagnosed with FSGS, we believe it is appropriate to obtain a detailed clinical history documenting potential exposures to water-damaged buildings with elevated levels of mold as well as a dietary history of persons diagnosed with FSGS and significant renal disease for which an alternative explanation is not readily available. Since testing for mycotoxins in urine and other tissues is now readily available [40], it is appropriate to obtain this testing on urine and renal biopsy tissue to help elucidate whether this is a potential contributor to illness. In cases where elevated levels of OTA and/or evidence of significant indoor water damage is found, urging avoidance of further exposure would be strongly recommended. In addition, in cases where the disease has progressed to the state where kidney transplantation is indicated, knowing if the existing kidneys have a significant concentration of OTA might affect the decision about whether to leave the diseased kidneys in place at the time of transplantation.

Various other means of lowering the renal burden of OTA have been studied with varying degrees of success. Most promising is the use of cholestyramine as a bile acid resin binding agent to reduce enterohepatic recirculation of OTA, thereby reducing levels that are filtered through the kidneys and shifting excretion to the stool where it is presumably bound to cholestyramine resin. Cholestyramine is not absorbed systemically allowing it to be safe even for those with advanced kidney disease. Studies in rats showed that OTA exposed rats that were fed a diet enriched with cholestyramine experienced decreased OTA concentration in plasma as well as decreased excretion of OTA and its metabolites (ochratoxin alpha and hydroxylated ochratoxin A) in bile and urine [74]. This was associated with an increased excretion of OTA in feces which was felt to reduce the potential nephrotoxicity of OTA.

Studies of sweat have shown the presence of OTA in sweat on at least one occasion [47] supporting treatments such as sauna to increase excretion of OTA.

Several studies have indicated that phenylalanine decreases the absorption and consequent toxicity of OTA, and this is also true of aspartame [34, 37, 38].

14. Discussion

We have reviewed two cases of FSGS associated with significant elevations in urinary OTA excretion following inhalational exposure in water-damaged buildings. The nephrotoxicity of OTA is undisputed in the literature in both extensive animal studies and more limited human studies. The precise role, if any, ochratoxin plays in the onset and progression of FSGS in select individuals has yet to be elucidated.

The etiology of FSGS is generally unknown, while treatments are most often directed towards slowing progression. At least in the case of drug toxicity including heroin and other drugs, toxins have been acknowledged to play an important role in some cases of FSGS. Undoubtedly, like most human illnesses, it will involve a complex interaction of environmental exposures with underlying genetics. Further study is clearly indicated; however, in the interim, it is wise to test for and initiate appropriate, safe measures to lower the body burden of OTA. As has been discussed, a toxin as potent as OTA that is known to be found in the highest concentrations in kidney tissue could certainly play a role in the onset and progression of the disease. A kidney already damaged from any cause would be all the more negatively affected by the strain of excreting a potent nephrotoxin.

It is the recommendation of the authors to obtain a detailed environmental history including exposure to water-damaged indoor environments and a dietary history in the case of primary idiopathic FSGS as well as other kidney disease of unclear etiology. If the history is remarkable for any potential exposures to OTA and perhaps even if it is not, we would urge evaluation for the presence of OTA in urine. If biopsy samples are available, it would also be useful to test these tissues since elevated levels in the kidney, might support the removal of diseased kidneys at the time of transplantation as opposed to the common practice of leaving them in place. In light of the detection of OTA in individuals with chronic fungal sinusitis [33], testing of nasal secretions for OTA and other mycotoxins should also be considered.

Additionally, consideration should be given to the testing of breast milk for OTA and other mycotoxins in mothers exposed to water-damaged environments as the data is strong that OTA can be excreted in breast milk and that the consequences of this exposure can be significant especially given the increased susceptibility of the neonate to toxins. For example, rat neonates exposed to OTA in only lactational milk had a 4 to 5 times higher level of OTA compared with those rats exposed only via the placenta [60]. Results of a recent biomonitoring study in Chile confirmed the presence of OTA in breast milk at levels such that the tolerable daily intake could be exceeded [75]. Of concern, in 50 lactating mothers and their infants in Egypt, the presence of OTA was associated with significantly higher levels of urine microglobulin and microalbuminuria in the infants consistent with early renal injury [76]. Moreover, the level of OTA in the infants sera correlated with the degree of microalbuminuria [76], raising great concern about maternal transfer of OTA and injury to the infants.

If elevated levels of urinary OTA are found, there are a number of safe treatments that should be considered to lower the body burden of this mycotoxin. The most important are the avoidance of further exposure and the use

of the bile acid resin binding agent, cholestyramine, to decrease enterohepatic recirculation of OTA. Studies have shown that animals fed a diet of OTA plus cholestyramine had a significant shift of OTA from the plasma and urine to the stool, where it is presumably excreted bound to cholestyramine [74]. This will safely reduce the burden on the kidneys as cholestyramine is not absorbed systemically and remains in the gastrointestinal tract. Side effects, which are primarily limited to the gastrointestinal tract, must be considered as well as the timing of cholestyramine away from medications and vitamins, primarily fat soluble vitamins. Many patients tolerate the pure resin better than the commercial prescription prepared with sugar, artificial colors, and a number of additives. As a number of patients with kidney disease also have hyperlipidemia, cholestyramine could potentially beneficial for its lipid lowering effect.

Other potential sequestrant treatments include the use of charcoal which is included in the military textbook recommendations for exposure to trichothecene mycotoxins which has been associated with Yellow Rain exposure [77]. Clay and zeolite have been studied for their efficacy of mycotoxin binding in animals [78, 79] and likely have a use in human illness caused by mycotoxins including OTA.

The use of licorice extract and melatonin was mentioned earlier in this paper and require further study, but may offer a safe option for reducing the toxicity of ochratoxin. There is some evidence that sauna shifts the excretion of ochratoxin to sweat; however, use of sauna needs to be very carefully monitored, especially on initiation. Antioxidants, including glutathione, are also likely to be helpful for their antioxidant and detoxification effects [23]. Vitamins A, C, E and selenium are other potentially beneficial antioxidants that may be protective in their role as superoxide anion scavengers [22, 75].

As we learn more about genomic studies, including the evaluation of cytochrome p450 and glutathione (GSTP and GSTM) pathways, it may be possible to identify those who may be most vulnerable to illness after exposure to ochratoxin and other toxic agents and having this information could be invaluable in directing appropriate therapies and medication use in the future.

Given the important metabolism of OTA that occurs in the gut, and evidence of increased toxicity when gut flora is disturbed with an antibiotic, it would pay to direct attention toward achieving and maintaining healthy gastrointestinal functioning. In fact, there is evidence that some beneficial gastrointestinal flora can have positive effects on decreasing toxicity from OTA including specific strains of yeast [80]. Interestingly, aspartame has been found to decrease toxicity of OTA through a phenylalanine-mediated mechanism [38]; however, there may be some concerns about other potential toxicities associated with aspartame.

Most importantly, anyone with chronic illness should carefully evaluate their environment and other sources of potential toxic exposures and make every effort to control these exposures. Unfortunately, in the case of exposures to mycotoxins including ochratoxin, it is imperative to address issues of cross contamination of items exposed to water-damaged/mold contaminated environment. Mycotoxins are very difficult to destroy and travel readily on fine, often submicron-sized particles making simple spore testing inadequate for determining the presence of mycotoxins. Thus, a thorough approach is needed to address contamination of items exposed to water-damaged environments to avoid continued exposure to mycotoxins including ochratoxin through these items even if the building is no longer a source of exposure.

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References

[1] H. A. Clark and S. M. Snedeker, "Ochratoxin A: its cancer risk and potential for exposure," *Journal of Toxicology and Environmental Health B*, vol. 9, no. 3, pp. 265–296, 2006.

[2] J. Varga, E. Kevel, E. Rinyu, J. Teren, and Z. Kozakiewicz, "Ochratoxin production by Aspergillus species," *Applied and Environmental Mircrobiology*, pp. 4461–4464, 1996.

[3] T. O. Larsen, A. Svendsen, and J. Smedsgaard, "Biochemical characterization of ochratoxin A-producing strains of the genus penicillium," *Applied and Environmental Microbiology*, vol. 67, no. 8, pp. 3630–3635, 2001.

[4] L. Reddy and K. Bhoola, "Ochratoxins-food contaminants: impact on human health," *Toxins*, vol. 2, pp. 771–779, 2010.

[5] T. Kuiper-Goodman and P. M. Scott, "Risk assessment of the mycotoxin ochratoxin A," *Biomedical and Environmental Sciences*, vol. 2, no. 3, pp. 179–248, 1989.

[6] D. Benford, C. Boyle, W. Dekant et al., "Ochratoxin A," *JECFA*, vol. 47, 2001.

[7] S. Hagelberg, K. Hult, and R. Fuchs, "Toxicokinetics of ochratoxin A in several species and its plasma-binding properties," *Journal of Applied Toxicology*, vol. 9, no. 2, pp. 91–96, 1989.

[8] P. Galtier, "Pharmacokinetics of ochratoxin A in animals," *IARC Scientific Publications*, no. 115, pp. 187–200, 1991.

[9] H. Zepnik, A. Pahler, U. Schauer, and W. Dekant, "Ochratoxin A induced tumour formation: is there a role of reactive ochratoxin A metabolites?" *Toxicological Sciences*, vol. 59, no. 1, pp. 59–67, 2011.

[10] P. Galtier, "Contribution of pharmacokinetic studies to mycotoxicology-Ochratoxin A," *Veterinary Science Communications*, vol. 1, no. 1, pp. 349–358, 1978.

[11] M. S. Madhyastha, R. R. Marquardt, and A. A. Frohlich, "Hydrolysis of ochratoxin A by the microbial activity of digesta in the gastrointestinal tract of rats," *Archives of Environmental Contamination and Toxicology*, vol. 23, pp. 468–472, 1992.

[12] P. Galtier, J. L. Charpenteau, M. Alvinerie, and C. Labouche, "The pharmacokinetic profile of ochratoxin A in the rat after oral and intravenous administration," *Drug Metabolism and Disposition*, vol. 7, no. 6, pp. 429–434, 1979.

[13] World Health Organization, *Environmental Health Criterai 105: Selected Mycotoxins: Ochratoxin, Trichothecenes, Ergot*, WHO, Geneva, Switzerland, 1990.

[14] J. Bauer and M. Gareis, "Ochratoxin A in the food chain," *Journal of Veterinary Medicine B*, vol. 34, no. 8, pp. 613–627, 1987 (German).

[15] J. C. Gautier, J. Richoz, D. H. Welti et al., "Metabolism of ochratoxin A: absence of formation of genotoxic derivatives

by human and rat enzymes," *Chemical Research in Toxicology*, vol. 14, no. 1, pp. 34–45, 2001.

[16] R. F. Omar, H. V. Gelboin, and A. D. Rahimtula, "Effect of cytochrome P450 induction on the metabolism and toxicity of ochratoxin A," *Biochemical Pharmacology*, vol. 51, no. 3, pp. 207–216, 1996.

[17] A. Pfohl-Leszkowicz, K. Chakor, E. Creppy, and G. Dirheimer, "DNA adduct formation in mice treated with ochratoxin A," in *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*, M. Castegnaro, R. Plestina, G. Dirheimer, I. N. Chernozemsky, and H. Bartsch, Eds., IARC Scientific Publications No. 115, pp. 245–253, IARC Press, Lyon, France, 1991.

[18] A. Pfohl-Leszkowicz, Y. Grosse, M. Castegnaro et al., "Ochratoxin A-related DNA adducts in urinary tract tumours of Bulgarian subjects," in *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*, M. Castegnaro, R. Plestina, G. Dirheimer, I. N. Chernozemsky, and H. Bartsch, Eds., IARC Scientific Publications No. 115, pp. 141–148, IARC Press, Lyon, France, 1993.

[19] A. Pfohl-Leszkowicz, E. Pinelli, H. Bartsch, U. Mohr, and M. Castegnaro, "Sex- and strain-specific expression of cytochrome P450s in ochratoxin A- induced genotoxicity and carcinogenicity in rats," *Molecular Carcinogenesis*, vol. 23, no. 2, pp. 76–85, 1998.

[20] M. Castegnaro, U. Mohr, A. Pfuhl-Leszkowicz et al., "Sex- and strain-specific induction of renal tumors by ochratoxin A in rats correlates with DNA adduction," *International Journal of Cancer*, vol. 77, no. 1, pp. 70–75, 1998.

[21] A. Pfohl-Leszkowicz, M. Tozlovanu, R. Manderville, M. Peraica, M. Castegnaro, and V. Stefanovic, "New molecular and field evidences for the implication of mycotoxins but not aristolochic acid in human nephropathy and urinary tract tumor," *Molecular Nutrition and Food Research*, vol. 51, no. 9, pp. 1131–1146, 2007.

[22] Y. Grosse, L. Chekir-Ghedira, A. Huc et al., "Retinol, ascorbic acid and α-tocopherol prevent DNA adduct formation in mice treated with the mycotoxins ochratoxin A and zearalenone," *Cancer Letters*, vol. 114, no. 1-2, pp. 225–229, 1997.

[23] A. Pfohl-Leszkowicz, H. Bartsh, B. Azemar, U. Mohr, J. Esteve, and M. Castegnaro, "MESNA protects rats against nephrotoxity but not carcinogenicity induced by ochratoxin A implicating two separate pathways," *Medicine and Biology*, vol. 9, no. 1, pp. 57–63, 2002.

[24] K. Stemmer, H. Ellinger-Ziegelbauer, H. J. Ahr, and D. R. Dietrich, "Molecular characterization of preneoplastic lesions provides insight on the development of renal tumors," *American Journal of Pathology*, vol. 175, no. 4, pp. 1686–1698, 2009.

[25] J. Harwig, T. Kuiper-Goodman, and P. M. Scott, "Microbial food toxicants: ochratoxins," in *Handbook of Foodborne Diseases of Biological Origin*, M. Rechcigl, Ed., pp. 193–238, CRC Press, Boca Raton, Fla, USA, 1983.

[26] H. P. Mortensen, B. Hald, and A. Madsen, "Feeding experiments with ochratoxin A contaminated barley for bacon pigs. 5. Ochratoxin A in pig blood," *Acta Agriculturae Scandinavica*, vol. 33, pp. 235–239, 1983.

[27] A. Madsen, H. P. Mortensen, and B. Hald, "Feeding experiments with ochratoxin A contaminated barley for bacon pigs. II. Naturally contaminated barley given for 6 weeks from 20 kg compared with normal barley supplemented with crystalline ochratoxin A and/or citrinin," *Acta Agriculturae Scandinavica A*, vol. 32, no. 4, pp. 369–372, 1982.

[28] A. Breitholtz-Emanuelsson, R. Fuchs, K. Hult, and L. E. Appelgren, "Syntheses of ^{14}C-ochratoxin A and ^{14}C-ochratoxin B and a comparative study of their distribution in rats using whole body autoradiography," *Pharmacology and Toxicology*, vol. 70, no. 4, pp. 255–261, 1992.

[29] C. Micco, A. Ambruzzi, M. Miraglia et al., "Contamination of human milk with ochratoxin A," *Mycotoxins Endemic Nephropathy Urinary Tract Tumors*, vol. 115, pp. 105–108, 1991.

[30] C. Micco, M. Miraglia, C. Brera, S. Corneli, and A. Ambruzzi, "Evaluation of ochratoxin a level in human milk in Italy," *Food Additives and Contaminants*, vol. 12, no. 3, pp. 351–354, 1995.

[31] F. E. Jonsyn, S. M. Maxwell, and R. G. Hendrickse, "Ochratoxin A and aflatoxins in breast milk samples from Sierra Leone," *Mycopathologia*, vol. 131, no. 2, pp. 121–126, 1995.

[32] D. G. Hooper, "Personal communication," June 2011.

[33] J. Thrasher, "A water damaged home and health occupants: a case study," *Journal of Environmental and Public Health*, 2012.

[34] K. Moroi, S. Suzuki, T. Kuga, M. Yamazaki, and M. Kanisawa, "Reduction of ochratoxin A toxicity in mice treated with phenylalanine and phenobarbital," *Toxicology Letters*, vol. 25, pp. 1–5, 1985.

[35] K. Chakor, E. E. Creppy, and G. Dirheimer, "In vivo studies on the relationship between hepatic metabolism and toxicity of ochratoxin A," *Archives of Toxicology*, vol. 62, no. 12, pp. 201–204, 1988.

[36] H. Malekinejad, A. Farshid, and N. Mirzakhani, "Liquorice plant extract reduces ochratoxin A-induced nephrotoxicity in rats," *Experimental and Toxicologic Pathology*, vol. 63, no. 1-2, pp. 125–130, 2011.

[37] E. E. Creppy, M. Schlegel, R. Roschenthaler, and G. Dirheimer, "Phenylalanine prevents acute poisoning by ochratoxin-a in mice," *Toxicology Letters*, vol. 6, no. 2, pp. 77–80, 1980.

[38] E. E. Creppy and A. M. Baudrimontl, "How aspartame prevents the toxicity of ochratoxin A," *The Journal of Toxicological Sciences*, supplement 2, pp. 165–172, 1998.

[39] P. Galtier, R. Camguilhem, and G. Bodin, "Evidence for in vitro and in vivo interaction between ochratoxin A and three acidic drugs," *Food and Cosmetics Toxicology*, vol. 18, no. 5, pp. 493–496, 1980.

[40] D. G. Hooper, V. E. Bolton, F. T. Guilford, and D. C. Straus, "Mycotoxin detection in human samples from patients exposed to environmental molds," *International Journal of Molecular Sciences*, vol. 10, no. 4, pp. 1465–1475, 2009.

[41] Y. Wang, T. Chai, G. Lu et al., "Simultaneous detection of airborne Aflatoxin, Ochratoxin and Zearalenone in a poultry house by immunoaffinity clean-up and high-performance liquid chromatography," *Environmental Research*, vol. 107, no. 2, pp. 139–144, 2008.

[42] J. L. Richard, R. D. Plattner, J. May, and S. L. Liska, "The occurrence of ochratoxin A in dust collected from a problem household," *Mycopathologia*, vol. 146, no. 2, pp. 99–103, 1999.

[43] V. Polizzi, B. Delmulle, A. Adams et al., "JEM Spotlight: fungi, mycotoxins and microbial volatile organic compounds in mouldy interiors from water-damaged buildings," *Journal of Environmental Monitoring*, vol. 11, no. 10, pp. 1849–1858, 2009.

[44] M. A. Skaug, W. Eduard, and F. C. Størmer, "Ochratoxin A in airborne dust and fungal conidia," *Mycopathologia*, vol. 151, no. 2, pp. 93–98, 2001.

[45] A. Breitholtz-Emanuelsson, R. Fuchs, and K. Hult, "Toxicokinetics of ochratoxin A in rat following intratracheal administration," *Natural Toxins*, vol. 3, no. 2, pp. 101–103, 1995.

[46] N. Dipaolo, A. Guarnieri, G. Garosi, G. Sacchi, and A. M. Mangiarotti, "Inhaled mycotoxins lead to acute renal failure,"

Nephrology Dialysis Transplantation, vol. 9, supplement 4, pp. 116–120, 1994.

[47] Genuis and Stephen, "Personal Communication," June 2011.

[48] A. Pfohl-Leszkowicz and R. A. Manderville, "Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans," *Molecular Nutrition and Food Research*, vol. 51, no. 1, pp. 61–99, 2007.

[49] A. M. Bendele, W. W. Carlton, P. Krogh, and E. B. Lillehoj, "Ochratoxin A carcinogenesis in the (C57BL/6J x C3H)F1 mouse," *Journal of the National Cancer Institute*, vol. 75, no. 4, pp. 733–742, 1985.

[50] J. E. Huff, "Carcinogenicity of ochratoxin A in experimental animals," in *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumors*, M. Castegnaro, R. Plestina, G. Dirheimer, I. N. Chemozemsky, and H. Bartsch, Eds., pp. 229–244, France International Agency for Research on Cancer, Lyon, France, 1991.

[51] IARC, "Ochratoxin A," in *IARC Working Group on the Evaluation of Carcinogenic Risks to Humans; Some Naturally Occurring Substances. Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*, pp. 489–521, World Health Organization, International Agency for Research on Cancer, Lyon, France, 1993.

[52] National Toxicology Program, *Toxicology and Carcinogenesis Studies of Ochratoxin A (CAS no. 303-47-9) ub F344/N rats (Gavage Studies)*, National Toxicology Program, Research Triangle Park, NC, USA, 1989.

[53] M. Gekle and S. Silbernagl, "The role of the proximal tubule in ochratoxin A nephrotoxicity in vivo: toxodynamic and toxokinetic aspects," *Renal Physiology and Biochemistry*, vol. 17, no. 1, pp. 40–49, 1994.

[54] M. Gekle, H. Oberleithner, and S. Silbernagl, "Ochratoxin A impairs 'postproximal' nephron function in vivo and blocks plasma membrane anion conductance in Madin-Darby canine kidney cells in vitro," *Pflugers Archiv European Journal of Physiology*, vol. 425, no. 5-6, pp. 401–408, 1993.

[55] B. Desalegn, S. Nanayakkara, K. H. Harada et al., "Mycotoxin detection in urine samples from patients with chronic kidney disease of uncertain etiology in Sri Lanka," *Bulletin of Environmental Contamination and Toxicology*, vol. 87, no. 1, pp. 6–10, 2011.

[56] G. G. Schwartz, "Hypothesis: does ochratoxin A cause testicular cancer?" *Cancer Causes and Control*, vol. 13, no. 1, pp. 91–100, 2002.

[57] C. Zaied, C. Bouaziz, I. Azizi et al., "Presence of ochratoxin A in Tunisian blood nephropathy patients. Exposure level to OTA," *Experimental and Toxicologic Pathology*, vol. 63, no. 7-8, pp. 613–618, 2011.

[58] K. Maaroufi, A. Achour, M. Hammami et al., "Ochratoxin A in human blood in relation to nephropathy in Tunisia," *Human and Experimental Toxicology*, vol. 14, no. 7, pp. 609–614, 1995.

[59] L. E. Appelgren and R. G. Arora, "Distribution of ^{14}C-labelled ochratoxin A in pregnant mice," *Food and Chemical Toxicology*, vol. 21, no. 5, pp. 563–568, 1983.

[60] I. P. Hallen, A. Breitholtz-Emanuelsson, K. Hult, M. Olsen, and A. Oskarsson, "Placental and lactational transfer of ochratoxin A in rats," *Natural Toxins*, vol. 6, no. 1, pp. 43–49, 1998.

[61] E. V. Ferrufino-Guardia, E. K. Tangni, Y. Larondelle, and S. Ponchaut, "Transfer of ochratoxin A during lactation: exposure of suckling via the milli of rabbit does fed a naturally-contaminated feed," *Food Additives and Contaminants*, vol. 17, no. 2, pp. 167–175, 2000.

[62] M. A. Skaug, I. Helland, K. Solvoll, and O. D. Saugstad, "Presence of ochratoxin A in human milk in relation to dietary intake," *Food Additives and Contaminants*, vol. 18, no. 4, pp. 321–327, 2001.

[63] A. Gürbay, G. Girgin, S. A. Sabuncuoğlu et al., "Ochratoxin A: is it present in breast milk samples obtained from mothers from Ankara, Turkey?" *Journal of Applied Toxicology*, vol. 30, no. 4, pp. 329–333, 2010.

[64] B. Zimmerli and R. Dick, "Determination of ochratoxin A at the ppt level in human blood, serum, milk and some foodstuffs by high-performance liquid chromatography with enhanced fluorescence detection and immunoaffinity column cleanup: methodology and Swiss data," *Journal of Chromatography B*, vol. 666, no. 1, pp. 85–99, 1995.

[65] M. Fenske and J. Fink-Gremmels, "Effects of fungal metabolites on testosterone secretion in vitro," *Archives of Toxicology*, vol. 64, no. 1, pp. 72–75, 1990.

[66] X. Zhang, C. Boesch-Saadatmandi, Y. Lou, S. Wolffram, P. Huebbe, and G. Rimbach, "Ochratoxin A induces apoptosis in neuronal cells," *Genes and Nutrition*, vol. 4, pp. 41–48, 2009.

[67] A. Belmadani, P. S. Steyn, G. Tramu, A. M. Betbeder, I. Baudrimont, and E. E. Creppy, "Selective toxicity of ochratoxin a in primary cultures from different brain regions," *Archives of Toxicology*, vol. 73, no. 2, pp. 108–114, 1999.

[68] V. Sava, A. Velasquez, S. Song, and J. Sanchez-Ramos, "Adult hippocampal neural stem/progenitor cells in vitro are vulnerable to the mycotoxin ochratoxin-A," *Toxicological Sciences*, vol. 98, no. 1, pp. 187–197, 2007.

[69] V. Sava, O. Reunova, A. Velasquez, R. Harbison, and J. Sánchez-Ramos, "Acute neurotoxic effects of the fungal metabolite ochratoxin-A," *NeuroToxicology*, vol. 27, no. 1, pp. 82–92, 2006.

[70] L. Al-Anati and E. Petzinger, "Immunotoxic activity of ochratoxin A," *Journal of Veterinary Pharmacology and Therapeutics*, vol. 29, no. 2, pp. 79–90, 2006.

[71] C. Montagnoli, F. Fallarino, R. Gaziano et al., "Immunity and tolerance to Aspergillus involve functionally distinct regulatory T cells and tryptophan catabolism," *Journal of Immunology*, vol. 176, no. 3, pp. 1712–1723, 2006.

[72] T. K. S. Rao, A. Soman, C. M. Nzerue et al., "Focal segmental glomeruloscerosis," *E Medicine*, http://emedicine.medscape.com/article/245915-overview#showall. In press.

[73] G. Canaud, D. Dion, J. Zuber et al., "Recurrence of nephritic syndrome after transplantation in a mixed population of children and adults: course of glomerular lesions and value of the Columbia classification of histological variants of focal ad segmental glomerulosclerosis (FSGS)," *Nephrology Dialysis Transplantation*, vol. 25, no. 4, pp. 1321–1328, 2010.

[74] A. Kerkadi, C. Barriault, B. Tuchweber et al., "Dietary cholestyramine reduces ochratoxin A-induced nephrotoxicity in the rat by decreasing plasma levels and enhancing fecal excretion of the toxin," *Journal of Toxicology and Environmental Health A*, vol. 53, no. 3, pp. 231–250, 1998.

[75] K. Muñoz, V. Campos, M. Blaszkewicz et al., "Exposure of neonates to ochratoxin A: first biomonitoring results in human milk (colostrum) from Chile," *Mycotoxin Research*, vol. 26, no. 2, pp. 59–67, 2010.

[76] A. M. Hassan, H. A. Sheashaa, M. F. A. Fattah, A. Z. Ibrahim, O. A. Gaber, and M. A. Sobh, "Study of ochratoxin A as an environmental risk that causes renal injury in breast-fed Egyptian infants," *Pediatric Nephrology*, vol. 21, no. 1, pp. 102–105, 2006.

[77] R. Wannemacher and S. Weiner, "Chapter 34- trichothecene mycotoxins in the textbook," in *Medical Aspects of Chemical*

and Biological Warfare, pp. 655–676, Office of the Surgeon General at TMM Publications Borden Institute, Walter Reed Army Medical Center, Washington, DC. USA, 1998.

[78] A. Piva and F. Galvano, "Nutritional approaches to reduce the impact of mycotoxins," 2007, http://www.engormix.com/.

[79] L. Whitlow, "Evaluation of mycotoxin binders," in *Proceedings of the 4th Mid-Atlantic Nutrition Conference*, pp. 132–143, Timonium, Maryland, March 2006.

[80] G. Schatzmayr, F. Zehner, M. Täubel et al., "Microbiologicals for deactivating mycotoxins," *Molecular Nutrition and Food Research*, vol. 50, no. 6, pp. 543–551, 2006.

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Clinical Study

Human Excretion of Bisphenol A: Blood, Urine, and Sweat (BUS) Study

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Background. Bisphenol A (BPA) is an ubiquitous chemical contaminant that has recently been associated with adverse effects on human health. There is incomplete understanding of BPA toxicokinetics, and there are no established interventions to eliminate this compound from the human body. Using 20 study participants, this study was designed to assess the relative concentration of BPA in three body fluids—blood, urine, and sweat—and to determine whether induced sweating may be a therapeutic intervention with potential to facilitate elimination of this compound. *Methods*. Blood, urine, and sweat were collected from 20 individuals (10 healthy participants and 10 participants with assorted health problems) and analyzed for various environmental toxicants including BPA. *Results*. BPA was found to differing degrees in each of blood, urine, and sweat. In 16 of 20 participants, BPA was identified in sweat, even in some individuals with no BPA detected in their serum or urine samples. *Conclusions*. Biomonitoring of BPA through blood and/or urine testing may underestimate the total body burden of this potential toxicant. Sweat analysis should be considered as an additional method for monitoring bioaccumulation of BPA in humans. Induced sweating appears to be a potential method for elimination of BPA.

1. Introduction

First synthesized in 1891 and with current production estimated at 4 billion kilograms each year globally [1], bisphenol A (BPA) is a multipurpose compound that is widely used in the modern industrial world. BPA was initially investigated for its potentially therapeutic estrogenic properties in the 1930s; when diethylstilbestrol (DES) was found to be more potent, however, BPA was temporarily cast aside. Its commercial value was reassessed in the 1950s with the introduction of BPA as a fundamental component in the manufacturing of some plastics. As its primary use currently, BPA is a key monomer in the production of the most common form of clear and shatter-proof polycarbonate plastic, but it has also been incorporated into a variety of everyday goods.

Questions regarding the safety and side effects of BPA began to emerge in the late 1990s when BPA was found to leech out of plastics and into experimental animal subjects, resulting in an increased incidence of chromosomal anomalies in offspring [2]. There has since been ongoing discussion in both scientific and political spheres about the potential for harm resulting from human BPA exposure and potential bioaccumulation. An overview of the literature regarding the effects of BPA on human health is provided, followed by a presentation of data from 20 subjects whose blood, urine, and sweat were tested for BPA. Results and discussion regarding BPA bioaccumulation and elimination are presented for consideration.

2. Background

Currently, BPA is most commonly found as a component in polycarbonates (~74% of total BPA produced) and in the production of epoxy resins (~20%). As well as being found in a myriad of products including plastic food and beverage containers (including baby and water bottles), BPA is also

commonly found in various household appliances, electronics, sports safety equipment, adhesives, cash register receipts, medical devices, eyeglass lenses, water supply pipes, and many other products. It is also frequently used as an adjunct in the production of brominated flame retardants and brake fluid [1]. Moreover, BPA derivatives, such as bisphenol A-glycidyl methacrylate and bisphenol A-dimethacrylate, have recently been incorporated into the dental industry and used in dental fillings and sealants. The widespread use of this compound is receiving increasing scrutiny as concerns about BPA effects on human health have recently emerged.

The main mechanism by which the population is exposed to BPA is through leaching from plastic products. This results from either the release of unpolymerized monomers or the slow decay of polymer bonds in polycarbonates leading to monomer release into proximal foods and liquids. Occupational exposures are also present where plastics are burned and manufactured, and thus BPA may be inhaled by workers [3, 4]. An analysis of Chinese employees in factories where BPA and epoxy resins are produced, for example, revealed that over 90% of exposed workers have notable levels of BPA in their serum and urine [5].

A plethora of recent studies affirms that the majority of the population (91–99%) does indeed have detectable levels of BPA, but the level and the toxicological relevance of current exposure levels is a subject of intense academic and public health debate [6–13]. An extensive review conducted in 2007 concluded that BPA levels in human blood and/or urine are within the range shown to be dangerous in animals and are therefore likely to be biologically active in humans [6]. (As will be discussed, however, blood and urine testing may underestimate the full extent of exposure and bioaccumulation.) Conversely, an industry-sponsored literature review from 2008 declared that daily human consumption was far below dangerous levels and is therefore of minimal concern [7].

Sources of BPA ingestion may vary. In infants and children, baby and beverage bottles used by most individuals in the pediatric population provide ongoing daily sources of BPA [14–22]. Le et al. found that at room temperature, leaching of BPA occurred into the contained fluid, which increased 55-fold if boiling water was added [14]. Another study found that exposure levels increased not only with temperature, but also with repeated use of a container [15].

Other common sources of ingestion include foods stored in food cans, which are lined with BPA epoxy resin films to prevent corrosion [23–28], thermal printing paper commonly used in cash register receipts [29–31], and BPA containing dental composites and sealants [32–34]. Medical equipment is also raising concerns about BPA levels as a study of newborns found that those who regularly spent time in a neonatal intensive care unit had significantly higher serum BPA levels than the general population—thought to be due to exposure to plastics in medical devices [35]. Likewise, dialysis patients appear to have higher rates of exposure, which may be attributable to circulating solvents which expedite the leaching of BPA from polycarbonate hemodialysis equipment [36, 37].

When ingested, unconjugated BPA—the biologically active form of BPA—has historically been thought to be rapidly conjugated in the liver and then excreted through bile or urine, with a half life of approximately 5.3 hours [38–40]. This rapid excretion has been the basis of reassuring safety evaluations and declarations given by some public health authorities worldwide. However, within many tissues, particularly the lungs, livers and kidneys in rats, and the placenta of animals and humans, β-glucuronidase enzyme is present at detectable concentration. This enzyme is able to deconjugate BPA and thus release its active form again [41]. This is of great significance, as it is plausible that, in pregnancy, the conjugated form of BPA will circulate through the placenta, undergo deconjugation, and cause subsequent fetal exposure in utero. This may also result in bioaccumulation of some portion of BPA after exposure. In fact, recent evidence suggests that at low concentrations, while most plasma BPA (about 95%) is bound to serum proteins, BPA has lipophilic affinity with a fat: blood coefficient of 3.3 [42]. Furthermore, BPA appears to have a disproportionate affinity to fat in comparison to other tissues such as kidney, muscle, and other sites; "in fat, the accumulation of BPA was about three times higher than in other tissues" [42]. With evidence of potential bioaccumulation, BPA has the prospect of exerting ongoing metabolic effects.

2.1. Potential Implications of BPA Exposure. BPA is thought to wield its effects through endocrine disruption, epigenetic modification, cytokine release, and oxidative stress. When first discovered, BPA was investigated for its estrogenic properties, as it is thought to alter the synthesis of estradiol and testosterone and interfere with receptor binding [43, 44]. Consequently, exposure to BPA has been linked with a number of developmental and reproductive pathologies in both animal models and human subjects. These include abnormalities in reproductive organ function (irregular cycles, multiple ovarian cysts, reduction in primordial follicles [45–49]), placental dysfunction [50], increased incidence of miscarriage and neonatal mortality [50, 51], precocious puberty [52], and sexual dysfunction such as erectile dysfunction, decreased libido, and ejaculation difficulties [53–55]. Moreover, interference with the production and signaling of sex hormones has led to neurological impairment [56–60]. Synapse formation during development is regulated by estrogen and androgens; however with exposure to BPA, a recent study found that levels deemed safe by the US Environment Protection Agency, completely abolish the response of synapses to estrogen in the prefrontal cortex and hippocampus [61].

Epigenetic effects of BPA have been associated with an increased risk of cancer, particularly breast and prostate malignancies [62–69]. The exposure of breast epithelial cells to BPA was found to alter gene expression of 170 genes and increase their vulnerability to other carcinogens [62, 63]. Additionally, there was silencing of lysosomal-associated membrane protein 3, as occurs in ERα-positive breast cancer [62]. Similar effects have been seen with respect to prostatic disease, as exposure to BPA has been repeatedly shown to modify methylation of implicated genes [64–66].

Low dose BPA exposure at weaning during the perinatal period has also been found to increase adipogenesis in female animals [70]. As it is hypothesized that adult body weight may be programmed during early life, these results are noteworthy with regards to the childhood obesity pandemic and the action of endocrine disruptors as determinants of obesity [70]. BPA exposure appears to have widespread impact as it has also been linked by various researchers and studies throughout the world with a whole host of other health problems, including metabolic syndrome, obesity, non-insulin-dependent diabetes mellitus, allergies and asthma, ADHD, autism, cognitive decline, memory impairment, depression, and anxiety [71–92]. With the rise of sensitivity-related illness, there is also concern that BPA may be a determinant of this recently recognized causative mechanism of disease and source etiology of assorted clinical conditions [93] by stimulating the release of proinflammatory adipokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) from human adipose tissue [71].

As a result of all the emerging attention in the scientific literature, various governments have also embarked on research and policy decisions relating to BPA. In 2010, for example, the Minister of Health for the Canadian Government declared the results of a four-year study indicating, "The Government of Canada is a world leader in chemicals management. Our science indicated that Bisphenol A may be harmful to both human health and the environment" [94]. With emerging information of concern about BPA, the Canadian government became the first to prohibit the sale of BPA-containing polycarbonate baby bottles. France, Denmark and several American states have since implemented similar regulations.

2.2. Limitations of Toxicant Biomonitoring. It is often assumed that after exposure, BPA is rapidly metabolized to a hormonally inactive metabolite and efficiently excreted in toto from the body [95–97]. As a result, there has been little concern about bioaccumulation. The question arises, however, that if the compound is rapidly excreted without any accrual, why is there increasing evidence that BPA exposure is anything but innocuous and is able to cause potentially serious problems in animal and human organisms? While some believe that only unremitting ongoing exposure to BPA generates risk, concern has been raised that unrecognized bioaccumulation of some fraction of BPA may occur in some exposed individuals. How does one monitor to determine if toxicant compounds bioaccumulate and thus remain within the human organism?

Throughout the world, blood and urine sampling are the general modalities used to biomonitor levels of most toxicant compounds including toxic elements, synthetic compounds, petrochemical compounds, biologic toxicants such as mycotoxins, and xenobiotics sometimes produced as by-products from processing of parent compounds [98]. There is increasing evidence, however, that relying on blood and urine measurements as indicators of bioaccumulation can be very flawed [99]. Many compounds sequester in tissues and do not remain in blood; testing of whole blood or serum may miss toxicants which have exited the blood compartment and are being stored primarily in tissues such as bone, muscle, or adipose compartments. Levels of toxicant compounds can also rapidly fluctuate with changes in immediate status such as caloric restriction, level of hydration, underlying nutrient status, thermal changes, or exercise [98, 100].

Reliance on blood or urine testing for assessment of the body burden of many toxicants may thus be less than reliable clinically or for public health purposes. As a result, attempts to biomonitor toxicant levels by sampling other tissues and bodily excretions have been explored, including hair sampling, salivary testing, stool sampling, perspiration testing, breath analysis, provocation testing, and biopsies of adipose tissue through needle aspiration into fat pads under the skin. It is evident, however, that there are limitations with each of these approaches. Hair samples, for example may only reflect what has been in the blood stream for the last few weeks, while stool samples only assess what is being eliminated though fecal waste—these do not measure the body burden. As detailed toxicokinetics for many xenobiotic compounds are not fully understood, it is difficult to know which proportion of parent compounds and their metabolites accrue within various bodily compartments.

Some researchers have recently commenced doing fat biopsies as a tool to biomonitor toxicant levels—this technique involves taking a sample of fat, usually from the abdominal or gluteal area and sending the sample for analysis. Recent evidence, however, confirms that toxicants sequester differently even within specific compartments such as adipose tissue; one adipose tissue site may display toxicant concentrations that are totally different than concentrations at another site [101]. So the toxicant concentration in brain adipose tissue, for example, may be very different than that found in breast or abdominal wall adipose tissue.

In review, attempts to biomonitor the levels of toxicant compounds, including BPA, in humans using a single modality such as blood or urine are inadequate at best. This is a challenging realization as most population studies on toxicant compounds reported in the scientific literature as well as most ongoing biomonitoring research is based on blood or urine testing.

2.3. Study Objective. BPA exposure is generally assessed by measuring urine levels of this compound. In this research, we endeavor to determine the relative concentrations of BPA in blood, urine, and sweat. By assessing BPA levels in these three compartments, the possibility of identifying retained BPA will be explored as well as the potential for induced perspiration as a means to eradicate this compound.

3. Methods

3.1. Participant Recruitment. 9 males and 11 females with mean ages 44.5 ± 14.4 years and 45.6 ± 10.3 years, respectively, were recruited to participate in the study after appropriate ethical approval from the Health Research Ethics Board of the University of Alberta. 10 participants were patients with various clinical conditions and 10 were otherwise healthy adults. Participants with health issues were recruited from the first author's clinical practice by invitation. Each

Table 1: Participant results for BPA in three body compartments: serum, urine, and sweat.

Participant	Gender	Age	Clinical diagnosis	Serum conc.	Urine conc.	Sweat conc.	Sweat/urine ratio	Technique used for sweat collection
1	M	61	Diabetes, obesity, hypertension	0	4	82	20.5	Exercise
2	F	40	Rheumatoid arthritis	0	22	24	1.1	Steam sauna
3	M	38	Addiction disorder	0	20	22	1.1	Steam sauna
4	F	25	Bipolar disorder	0	40	22	0.6	Steam sauna
5	F	47	Lymphoma	0	10	24	2.4	Steam sauna
6	F	43	Fibromyalgia	0	32	0	n/a	Steam sauna
7	F	48	Depression	0	0	16	n/a	Steam sauna
8	F	40	Chronic fatigue	0	0	22	n/a	Infrared sauna
9	F	68	Diabetes, fatigue, obesity	0	0	10	n/a	Steam sauna
10	M	49	Chronic pain, cognitive decline	0	8	10	1.3	Exercise
11	M	53	Healthy	10	32	20	0.6	Exercise
12	M	23	Healthy	0	30	46	1.5	Infrared sauna
13	M	21	Healthy	30	4	10	2.5	Infrared sauna
14	F	47	Healthy	0	8	12	1.5	Infrared sauna
15	M	53	Healthy	0	4	35	8.8	Infrared sauna
16	F	43	Healthy	0	0	12	n/a	Infrared sauna
17	F	51	Healthy	0	0	0	n/a	Infrared sauna
18	M	46	Healthy	0	42	0	n/a	Infrared sauna
19	M	57	Healthy	0	0	0	n/a	Infrared sauna
20	F	50	Healthy	0	8	22	2.8	Infrared sauna

participant in the study provided informed consent and volunteered to give one 200 mL random sample of blood, one sample of first morning urine, and one 100 mL sample of sweat. Demographic and clinical characteristics of all research participants are provided in Table 1.

3.2. Samples Collection. All blood samples were collected at one Dynalife laboratory site in Edmonton, Alberta, Canada through vacutainer blood collection equipment (BD Vacutainer, Franklin Lakes, NJ, 07417, USA) using 21 gauge stainless steel needles which were screwed into the "BD Vacutainer One-Use Holder" (REF 364815). The 10 mL glass vacutainer was directly inserted into the holder and into the back end of the needle. This process and the use of glass were used to preclude contamination. Blood was collected directly into plain 10 mL glass vacutainer tubes, allowed to clot, and spun down 30 minutes later. After serum was separated off, samples were picked up by ALS Laboratories (about 3 kilometres from the blood collection site) for storage pending analysis. When received at ALS, serum samples were transferred to 4-mL glass vials and stored in a freezer at −20°C, pending transfer to the analytical laboratory. We chose to analyze BPA in serum rather than in whole blood, based on the fact that matrix effect of serum is much lower than whole blood.

For urine collection, participants were instructed to collect a first morning urine sample directly into a provided 500 mL glass jar container with Teflon-lined lid on the same day that blood samples were collected. Urine samples were delivered by the participants directly to Edmonton ALS Laboratories. Samples were transferred to 4-mL glass vials and stored in a freezer at −20°C, pending transfer.

For sweat collection, participants were instructed to collect perspiration from any site on their body directly into the provided 500 mL glass jar container with Teflon-lined lid—by placing the jar against their prewashed skin when actively sweating or by using a stainless steel spatula against their skin to transfer perspiration directly into the glass jar. (Stainless steel—made up primarily of iron, chromium, and nickel—was chosen as it is the same material as the needles used in standard blood collections and is reported not to off-gas or leach at room or body temperature.) Excess of 100 mL of sweat was provided in all but one case. Each of the glass bottles used for sampling in this study was provided by ALS laboratories and had undergone extensive cleaning and rinsing. The containers were deemed appropriate for sweat collection with negligible risk of contamination: laboratory-grade phosphate-free detergent wash; acid rinse; multiple hot and cold deionized water rinses; oven-dried; capping and packing in quality controlled conditions. Sweat was collected within 1 week before or after doing the blood work. No specifications were given as to how long sweating had commenced before collection. 10 participants collected sweat

Table 2: Comparison of urine BPA levels across published studies.

Urine levels of BPA (ng/mL)-comparison across studies							
Study	Location	Detection method	DL (ng/mL)	Participants	Detection(%)	AM/GM/Median	Range
This study	Canada	LC-MS	0.2	20 adults	70	AM:13 Median 8	0–42
Bushnik et al. [10]	Canada	GC-MS	0.2	5462 (age 6–79)	90.7	GM 1.16 (1.08–1.24)	N/A
Calafat et al. [9]	USA	LC-MS	0.4	950 adults	N/A	GM 2.4	N/A
Calafat et al. [8]	USA	GC-MS	0.1	394 adults	95	GM 1.33 Median 1.28	0.1–5.18 (*95th Centile*)
Moors et al. [108]	Germany	GC-MS	3	15 adults	60		ND-55
Mendiola et al. [107]	USA	LC-MS	0.4	375 males	90	GM 1.50	<0.4–6.5 *(95th Centile)*

DL: detection limit; AM: arithmetic mean; GM: geometric mean; ND: non detect; N/A: not available.
LC-MS: liquid chromatography-mass spectrometry.
GC-MS: gas chromatography-mass spectrometry.

inside an infrared sauna; 7 collected inside a regular steam sauna, and 3 collected during and immediately after exercise—no specific instruction was given regarding the type or location of exercise. Sweat was delivered by the participants directly to ALS laboratories. Samples were transferred to 4 mL glass vials and stored in a freezer at −20°C, pending analysis. No preservatives were used in the jars provided for sweat and urine collection, nor in the serum storage vials.

3.3. Analytical Methods. Human serum was analyzed for levels of bisphenol A (BPA) at ALS Canada following the general procedures presented by the Centres for Disease Control and Prevention [8, 102]. Briefly, samples were fortified with 12.5 nanograms of isotopically labelled phthalate metabolites, 50 nanograms of labeled bisphenol-A, 250 nanograms of 4-methylumbelliferone glucuronide, 300 microliters of ammonium acetate buffer (pH 6.5), and 10 microliters of β-glucuronidase (Escherichia coli K12, Roche Biomedical). The samples were mixed and incubated at 37°C overnight to allow for the deglucuronidation.

Following enzymatic hydrolysis, a 20 uL aliquot of the sample is added to 70 uL of HPLC-grade water and 10 ng of labelled 4-methylumbelliferone to determine deglucuronidation efficiency (done once every 100 samples). The remaining sample is loaded onto a Zymark Rapid Trace Station for automated solid phase extraction (SPE). The 60 milligram/3 mL Oasis-HLB cartridges were conditioned with HPLC-grade methanol (2 mL) and 0.1 M formic acid (2 mL). The samples were diluted with 5 mL of 0.1 M formic acid and loaded onto the SPE cartridge at a rate of 1.0 mL/min. The cartridge was washed with water (1 mL) and 10% methanol in water (2 mL) at a flow rate of 1 mL/min. The samples were eluted with 1.0 mL of acetonitrile at a flow rate of 0.5 mL/min. The eluate was evaporated to dryness under a stream of dry nitrogen and the residue was resuspended in 85% methanol in water (200 microliters) and transferred to glass autosampler vials.

Quality control of the analysis was maintained by analysing a method blank (calf serum) and two spiked calf serum samples (20 ng/mL, all analytes) along with every 17 samples. The detection limit (0.2 ng/mL) was based upon our lower calibration standard (0.5 ng/mL) which gave an instrument signal to noise response of 3 : 1. Analysis was performed using an API 4000 liquid chromatograph/tandem mass spectrometer.

4. Results and Discussion

Demographic characteristics as well as results for each individual participant are summarized in Table 1. All concentrations are in nanograms per milliliter.

The fact that some subjects showed undetectable levels confirms that generalized contamination of these samples is not likely. Furthermore, the levels of BPA were similar to those recently published in studies from Italy and Greece [103] and are comparable with the serum levels found (0.79–7.12 ng/mL) in a recent study by Cobellis et al. in 2009 [104]. The rather low percentage detection among the serum samples in North America is hard to compare as (to our knowledge) there is only one study in the literature that documents BPA levels in blood of North Americans [105]. In that study, using the same extraction method as the methodology used in this study, the authors reported BPA levels in the range of <0.5 (detection limit) to 22.3 ng/mL in the blood plasma of 40 pregnant American women in the state of Michigan. However, the authors did not report in how many of these 40 women that BPA was detected above their current limit of detection of 0.5 ng/mL. In general, there is major variability in the range of concentrations of BPA detected in blood, and this may be explained by the fact that detection methods vary widely and the specific populations studied also vary considerably [106].

For the urine samples, the percentage detection in the current study (70%) is lower than that of the large scale Canadian biomonitoring study (90.7%) also known as the Canadian Health Measures Survey as reported by Bushnik et al. [10]. However, the geometric mean level of urine BPA in this study is generally higher than those in other biomonitoring studies in North America. Comparative levels for urine BPA as found in the literature are presented in Table 2. For example, Bushnik et al. reported a geometric

A	Serum+	Serum−
Urine+	2	12
Urine−	0	6

B	Serum+	Serum−
Sweat+	2	14
Sweat−	0	4

C	Urine+	Urine−
Sweat+	12	4
Sweat−	2	2

Figure 1: 2×2 tables indicating the presence of BPA in specific body compartments. Each cell represents number of study participants.

mean of 1.15 ng/mL urine BPA in Canada for a sample size of 5462 [10], Calafat et al. found a value of 2.4 ng/mL for urine BPA among 950 American adults aged between 20 and 59 [9] and more recently Mendolia et al. reported on 375 American males with a geometric mean value of 1.50 ng/mL of urine BPA [107]. As far as sweat data is concerned, comparison across studies is impossible as to our knowledge this is the first study which attempts to quantify BPA in sweat.

One obvious qualitative interpretation of the data from the cohort of 20 study participants is that BPA is rarely detected in blood, and this is probably why most large scale biomonitoring studies, such as the NHANES (National Health and Nutrition Examination Survey) and CHMS (Canadian Health Measures Survey), use urine as the human sample of choice to determine exposure levels in populations. In an attempt to summarize the findings on the distribution of BPA in the 3 different body fluids, we give three 2 × 2 tables in Figure 1. As discussed earlier, the 2 discordant pairs urine+/serum− and sweat+/serum−, with 12 and 14 in their respective grids show clearly that serum is not the appropriate body fluid to test if BPA biomonitoring in humans is to be characterised. Although there seems to be high correlation between urine and sweat in terms of the presence/absence of BPA in these media, with 12 individuals in the urine+/sweat+ concordant pair, what is more surprising is that there are 4 individuals for which BPA was detected in sweat but undetectable in urine (Figure 1C).

In an attempt to compare the excretion efficiencies of urine and sweat for BPA, we calculated the ratio of BPA concentration in sweat versus urine for those 12 individuals who are in the urine+/sweat+ concordant pair. As shown in Table 1, with the exception of 2 individuals (participants 4 and 11) where urine concentration of BPA is slightly higher than in sweat, in general the ratio is higher than 1, suggesting that induced sweating may be an efficient method for eliminating BPA from the body. This is not surprising in the light of the findings of Csanády et al. whereby they found a preferential partitioning of BPA in adipose tissue compared to blood with a ratio of 3.3 [42]. This suggests that the BPA in blood which is then conjugated and excreted in the urine may only represent about one-third of the body burden of BPA. It is not surprising therefore that induced sweating in saunas can mobilise BPA in adipose tissue thus leading to enhanced excretion in sweat. Given that BPA in body fat is mostly unconjugated, further studies looking at the ratio of free BPA to conjugated BPA in sweat will help to confirm whether the BPA excreted in sweat comes from adipose tissue. Given that in normal circumstances the daily volume of urine is much higher than sweat, urine remains an important mode of elimination of BPA from the human body.

An important consideration in response to the results is why there are two participants with evidence of BPA in their urine with no positive level in their sweat. Presumably, the sweat level may be reflective of accrued toxicants in tissue, whereas urine may reflect in part at least recent exposure which the body is endeavoring to eliminate. It may be that BPA begins to bioaccumulate only after a threshold of exposure is reached or once the detoxification mechanisms of the organism are unable to completely eliminate the BPA load that presents after exposure. Further research is required in order to clarify, but this result may indicate that the two individuals have had some recent exposure, but no significant level of stockpiled toxicant. Similarly, the marked variation in the range of the sweat/urine ratio may once again be reflective of different phenomenon: the sweat results may represent transcutaneous excretion of accured BPA toxicant, while the urine results are perhaps reflecting recent BPA exposure in addition to some release of accrued BPA that the body is able to eliminate through renal mechanisms. It is also noteworthy that there was no statistically significant difference (P-value >0.05) in sweat BPA levels depending on the method of sweat collection whether through exercise, infrared sauna, or regular sauna.

Although this study sheds light on the importance of sweat as a pathway of elimination for BPA from the body, it has some limitations. First, given the small study size (n = 20) it is not possible to extrapolate the findings to the general population. Secondly, samples were analysed for total BPA, instead of unconjugated BPA and conjugated BPA separately. Thirdly, other rare forms of BPA such as chlorinated and sulfated BPA were not analysed for.

5. Conclusion

As a result of increased scrutiny of health sequelae associated with human BPA exposure, it is apparent that this endocrine-disrupting compound has potentially negative consequences for the human organism. With new evidence for the possibility of BPA accrual within the body, interventions to facilitate elimination of this toxic compound have clinical relevance with regards to the prevention and treatment of adverse outcomes associated with BPA bioaccumulation. The results of this study suggest that: (i) Sweat testing may be an additional tool for BPA bio-monitoring; and (ii) Induced sweating appears to be a clinically useful tool to facilitate the release of BPA through the skin in order to eliminate this toxicant from the human body.

Notable findings and implications of data from this study
(i) BPA is excreted in sweat
(ii) Sweat BPA concentrations are consistently much higher than urine
(iii) Only 2/20 participants had BPA in serum, while 16/20 had BPA in sweat
(iv) The data suggests that BPA likely bioaccumulates to some degree in humans
(v) The data suggests that BPA retained in tissues (likely adipose) excretes via sweat
(vi) The finding in some individuals that little or no BPA is excreted in urine while considerable levels are found in sweat suggests that current biomonitoring via serum (as done in Europe) or urine (as done in North America) may not provide a reliable indication of the BPA toxicant burden
(vii) With the recognition that BPA has the potential for hormonal dysregulation, the significance of accrued BPA remains to be conclusively elucidated

Conflict of Interests

There is no conflict of interests. No funding has been received for any part of this work.

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References

[1] Environment Canada, Consultation Document Bisphenol A—Phenol, 4,4′-(1-Methylethylidene) bis-(Bisphenol A), 2009, http://www.ec.gc.ca/lcpe-cepa/79A93C66-1EE0-4F72-89C1-114DFD7B7292/pba-eng.pdf.

[2] P. A. Hunt, K. E. Koehler, M. Susiarjo et al., "Bisphenol a exposure causes meiotic aneuploidy in the female mouse," *Current Biology*, vol. 13, no. 7, pp. 546–553, 2003.

[3] N. K. Wilson, J. C. Chuang, M. K. Morgan, R. A. Lordo, and L. S. Sheldon, "An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare," *Environmental Research*, vol. 103, no. 1, pp. 9–20, 2007.

[4] L. N. Vandenberg, M. V. Maffini, C. Sonnenschein, B. S. Rubin, and A. M. Soto, "Bisphenol-a and the great divide: a review of controversies in the field of endocrine disruption," *Endocrine Reviews*, vol. 30, no. 1, pp. 75–95, 2009.

[5] Y. He, M. Miao, C. Wu et al., "Occupational exposure levels of Bisphenol a among Chinese workers," *Journal of Occupational Health*, vol. 51, no. 5, pp. 432–436, 2009.

[6] L. N. Vandenberg, R. Hauser, M. Marcus, N. Olea, and W. V. Welshons, "Human exposure to bisphenol A (BPA)," *Reproductive Toxicology*, vol. 24, no. 2, pp. 139–177, 2007.

[7] W. Dekant and W. Völkel, "Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures," *Toxicology and Applied Pharmacology*, vol. 228, no. 1, pp. 114–134, 2008.

[8] A. M. Calafat, Z. Kuklenyik, J. A. Reidy, S. P. Caudill, J. Ekong, and L. L. Needham, "Urinary concentrations of bisphenol A and 4-Nonylphenol in a human reference population," *Environmental Health Perspectives*, vol. 113, no. 4, pp. 391–395, 2005.

[9] A. M. Calafat, X. Ye, L. Y. Wong, J. A. Reidy, and L. L. Needham, "Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004," *Environmental Health Perspectives*, vol. 116, no. 1, pp. 39–44, 2008.

[10] T. Bushnik, D. Haines, P. Levallois, J. Levesque, J. Van Oostdam, and C. Viau, "Lead and bisphenol A concentrations in the Canadian population," *Health Reports*, vol. 21, no. 3, pp. 7–18, 2010.

[11] H. Mielke and U. Gundert-Remy, "Bisphenol A levels in blood depend on age and exposure," *Toxicology Letters*, vol. 190, no. 1, pp. 32–40, 2009.

[12] K. Becker, T. Güen, M. Seiwert et al., "GerES IV: phthalate metabolites and bisphenol A in urine of German children," *International Journal of Hygiene and Environmental Health*, vol. 212, no. 6, pp. 685–692, 2009.

[13] J. H. Kang, F. Kondo, and Y. Katayama, "Human exposure to bisphenol A," *Toxicology*, vol. 226, no. 2-3, pp. 79–89, 2006.

[14] H. H. Le, E. M. Carlson, J. P. Chua, and S. M. Belcher, "Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons," *Toxicology Letters*, vol. 176, no. 2, pp. 149–156, 2008.

[15] S. H. Nam, Y. M. Seo, and M. G. Kim, "Bisphenol A migration from polycarbonate baby bottle with repeated use," *Chemosphere*, vol. 79, no. 9, pp. 949–952, 2010.

[16] J. L. Carwile, H. T. Luu, L. S. Bassett et al., "Polycarbonate bottle use and urinary bisphenol A concentrations," *Environmental Health Perspectives*, vol. 117, no. 9, pp. 1368–1372, 2009.

[17] Health C, "Survey of bisphenol A in bottled water products," 2009, http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/securit/bpa_survey-enquete-bot-bou-eng.pdf.

[18] K. A. Ehlert, C. W. E. Beumer, and M. C. E. Groot, "Migration of bisphenol A into water from polycarbonate baby bottles during microwave heating," *Food Additives and Contaminants—Part A*, vol. 25, no. 7, pp. 904–910, 2008.

[19] N. De Coensel, F. David, and P. Sandra, "Study on the migration of bisphenol-A from baby bottles by stir bar sorptive extractionthermal desorption-capillary GC-MS," *Journal of Separation Science*, vol. 32, no. 21, pp. 3829–3836, 2009.

[20] C. Kubwabo, I. Kosarac, B. Stewart, B. R. Gauthier, K. Lalonde, and P. J. Lalonde, "Migration of bisphenol A from plastic baby bottles, baby bottle liners and reusable polycarbonate drinking bottles," *Food Additives and Contaminants—Part A*, vol. 26, no. 6, pp. 928–937, 2009.

[21] X. L. Cao and J. Corriveau, "Migration of bisphenol A from polycarbonate baby and water bottles into water under severe conditions," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 15, pp. 6378–6381, 2008.

[22] N. C. Maragou, A. A. Makri, E. Lampi, N. S. Thomaidis, and M. A. Koupparis, "Migration of bisphenol A from polycarbonate baby bottles under real use conditions," *Food Additives and Contaminants—Part A*, vol. 25, no. 3, pp. 373–383, 2008.

[23] J. Sajiki, F. Miyamoto, H. Fukata, C. Mori, J. Yonekubo, and K. Hayakawa, "Bisphenol A (BPA) and its source in foods in Japanese markets," *Food Additives and Contaminants*, vol. 24, no. 1, pp. 103–112, 2007.

[24] R. Braunrath, D. Podlipna, S. Padlesak, and M. Cichna-Markl, "Determination of bisphenol A in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 23, pp. 8911–8917, 2005.

[25] L. Grumetto, D. Montesano, S. Seccia, S. Albrizio, and F. Barbato, "Determination of bisphenol A and bisphenol B residues in canned peeled tomatoes by reversed-phase liquid chromatography," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 22, pp. 10633–10637, 2008.

[26] X. L. Cao, J. Corriveau, S. Popovic, G. Clement, F. Beraldin, and G. Dufresne, "Bisphenol A in baby food products in glass jars with metal lids from canadian markets," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 12, pp. 5345–5351, 2009.

[27] X. L. Cao, J. Corriveau, and S. Popovic, "Bisphenol a in canned food products from canadian markets," *Journal of Food Protection*, vol. 73, no. 6, pp. 1085–1089, 2010.

[28] X. L. Cao, J. Corriveau, and S. Popovic, "Levels of bisphenol a in canned soft drink products in Canadian markets," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 4, pp. 1307–1311, 2009.

[29] S. Biedermann, P. Tschudin, and K. Grob, "Transfer of bisphenol A from thermal printer paper to the skin," *Analytical and Bioanalytical Chemistry*, vol. 398, no. 1, pp. 571–576, 2010.

[30] S. Lunder, D. Andrews, and J. Houlihan, "Synthetic estrogen BPA coats cash register receipts," 2010, http://www.ewg.org/bpa-in-store-receipts.

[31] T. Mendum, E. V. Stoler, and J. Warner, "Concentration of bisphenol A in thermal paper," 2010, http://advancinggreenchemistry.org/?p=1176.

[32] N. Olea, R. Pulgar, P. Pérez et al., "Estrogenicity of resin-based composites and sealants used in dentistry," *Environmental Health Perspectives*, vol. 104, no. 3, pp. 298–305, 1996.

[33] D. Arenholt-Bindslev, V. Breinholt, A. Preiss, and G. Schmalz, "Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants," *Clinical Oral Investigations*, vol. 3, no. 3, pp. 120–125, 1999.

[34] J. M. Zimmerman-Downs, D. Shuman, S. C. Stull, and R. E. Ratzlaff, "Bisphenol A blood and saliva levels prior to and after dental sealant placement in adults," *Journal of Dental Hygiene*, vol. 84, no. 3, pp. 145–150, 2010.

[35] A. M. Calafat, J. Weuve, X. Ye et al., "Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants," *Environmental Health Perspectives*, vol. 117, no. 4, pp. 639–644, 2009.

[36] Y. Kanno, H. Okada, T. Kobayashi, T. Takenaka, and H. Suzuki, "Effects of endocrine disrupting substance on estrogen receptor gene transcription in dialysis patients," *Therapeutic Apheresis and Dialysis*, vol. 11, no. 4, pp. 262–265, 2007.

[37] K. Murakami, A. Ohashi, H. Hori et al., "Accumulation of bisphenol A in hemodialysis patients," *Blood Purification*, vol. 25, no. 3, pp. 290–294, 2007.

[38] W. Völkel, T. Colnot, G. A. Csanády, J. G. Filser, and W. Dekant, "Metabolism and kinetics of bisphenol a in humans at low doses following oral administration," *Chemical Research in Toxicology*, vol. 15, no. 10, pp. 1281–1287, 2002.

[39] H. Inoue, G. Yuki, H. Yokota, and S. Kato, "Bisphenol A glucuronidation and absorption in rat intestine," *Drug Metabolism and Disposition*, vol. 31, no. 1, pp. 140–144, 2003.

[40] H. Yokota, H. Iwano, M. Endo et al., "Glucuronidation of the environmental oestrogen bisphenol A by an isoform of UDP-glucuronosyltransferase, UGT2B1, in the rat liver," *Biochemical Journal*, vol. 340, no. 2, pp. 405–409, 1999.

[41] G. Ginsberg and D. C. Rice, "Does rapid metabolism ensure negligible risk from bisphenol A?" *Environmental Health Perspectives*, vol. 117, no. 11, pp. 1639–1643, 2009.

[42] G. Csanády, H. Oberste-Frielinghaus, B. Semder, C. Baur, K. Schneider, and J. Filser, "Distribution and unspecific protein binding of the xenoestrogens bisphenol A and daidzein," *Archives of Toxicology*, vol. 76, no. 5-6, pp. 299–305, 2002.

[43] T. Galloway, R. Cipelli, J. Guralnik et al., "Daily bisphenol a excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study," *Environmental Health Perspectives*, vol. 118, no. 11, pp. 1603–1608, 2010.

[44] S. Arase, K. Ishii, K. Igarashi et al., "Endocrine disrupter bisphenol a increases in situ estrogen production in the mouse urogenital sinus," *Biology of Reproduction*, vol. 84, no. 4, pp. 734–742, 2011.

[45] H. Kato, T. Ota, T. Furuhashi, Y. Ohta, and T. Iguchi, "Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period," *Reproductive Toxicology*, vol. 17, no. 3, pp. 283–288, 2003.

[46] S. Honma, A. Suzuki, D. L. Buchanan, Y. Katsu, H. Watanabe, and T. Iguchi, "Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction," *Reproductive Toxicology*, vol. 16, no. 2, pp. 117–122, 2002.

[47] R. R. Newbold, W. N. Jefferson, and E. Padilla-Banks, "Prenatal Exposure to Bisphenol A at environmentally relevant doses adversely affects the murine female reproductive tract later in life," *Environmental Health Perspectives*, vol. 117, no. 6, pp. 879–885, 2009.

[48] C. Lawson, M. Gieske, B. Murdoch et al., "Gene expression in the fetal mouse ovary is altered by exposure to low doses of bisphenol A," *Biology of Reproduction*, vol. 84, no. 1, pp. 79–86, 2011.

[49] H. A. Rodríguez, N. Santambrosio, C. G. Santamaría, M. Muñoz-de-Toro, and E. H. Luque, "Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary," *Reproductive Toxicology*, vol. 30, no. 4, pp. 550–557, 2010.

[50] T. Tachibana, Y. Wakimoto, N. Nakamuta et al., "Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice," *Journal of Reproduction and Development*, vol. 53, no. 3, pp. 509–514, 2007.

[51] M. Sugiura-Ogasawara, Y. Ozaki, S. I. Sonta, T. Makino, and K. Suzumori, "Exposure to bisphenol A is associated with recurrent miscarriage," *Human Reproduction*, vol. 20, no. 8, pp. 2325–2329, 2005.

[52] K. L. Howdeshell, A. K. Hotchkiss, K. A. Thayer, J. G. Vandenbergh, and F. S. Vom Saal, "Environmental toxins: exposure to bisphenol A advances puberty," *Nature*, vol. 401, no. 6755, pp. 763–764, 1999.

[53] D. Li, Z. Zhou, D. Qing et al., "Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction," *Human Reproduction*, vol. 25, no. 2, pp. 519–527, 2010.

[54] D. K. Li, Z. Zhou, M. Miao et al., "Relationship between urine bisphenol-A Level and declining male sexual function," *Journal of Andrology*, vol. 31, no. 5, pp. 500–506, 2010.

[55] R. A. Keri, S. M. Ho, P. A. Hunt, K. E. Knudsen, A. M. Soto, and G. S. Prins, "An evaluation of evidence for the carcinogenic activity of bisphenol A," *Reproductive Toxicology*, vol. 24, no. 2, pp. 240–252, 2007.

[56] C. Leranth, T. Hajszan, K. Szigeti-Buck, J. Bober, and N. J. MacLusky, "Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 37, pp. 14187–14191, 2008.

[57] N. J. MacLusky, T. Hajszan, and C. Leranth, "The environmental estrogen bisphenol A inhibits estradiol-induced hippocampal synaptogenesis," *Environmental Health Perspectives*, vol. 113, no. 6, pp. 675–679, 2005.

[58] J. H. Morrison, "Environmental estrogens impact primate brain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 37, pp. 13705–13706, 2008.

[59] M. Yokosuka, R. Ohtani-Kaneko, K. Yamashita, D. Muraoka, Y. Kuroda, and C. Watanabe, "Estrogen and environmental estrogenic chemicals exert developmental effects on rat hypothalamic neurons and glias," *Toxicology in Vitro*, vol. 22, no. 1, pp. 1–9, 2008.

[60] R. Zhou, Z. Zhang, Y. Zhu, L. Chen, M. Sokabe, and L. Chen, "Deficits in development of synaptic plasticity in rat dorsal striatum following prenatal and neonatal exposure to low-dose bisphenol A," *Neuroscience*, vol. 159, no. 1, pp. 161–171, 2009.

[61] C. Leranth, K. Szigeti-Buck, N. J. MacLusky, and T. Hajszan, "Bisphenol A prevents the synaptogenic response to testosterone in the brain of adult male rats," *Endocrinology*, vol. 149, no. 3, pp. 988–994, 2008.

[62] Y. I. Weng, P. Y. Hsu, S. Liyanarachchi et al., "Epigenetic influences of low-dose bisphenol A in primary human breast epithelial cells," *Toxicology and Applied Pharmacology*, vol. 248, no. 2, pp. 111–121, 2010.

[63] S. Jenkins, N. Raghuraman, I. Eltoum, M. Carpenter, J. Russo, and C. A. Lamartiniere, "Oral exposure to Bisphenol A increases dimethylbenzanthraceneo-induced mammary cancer in rats," *Environmental Health Perspectives*, vol. 117, no. 6, pp. 910–915, 2009.

[64] G. S. Prins, W. Y. Tang, J. Belmonte, and S. M. Ho, "Developmental exposure to bisphenol A increases prostate cancer susceptibility in adult rats: epigenetic mode of action is implicated," *Fertility and Sterility*, vol. 89, no. 2, article e41, 2008.

[65] G. S. Prins, W. Y. Tang, J. Belmonte, and S. M. Ho, "Perinatal exposure to oestradiol and bisphenol A alters the prostate epigenome and increases susceptibility to carcinogenesis," *Basic and Clinical Pharmacology and Toxicology*, vol. 102, no. 2, pp. 134–138, 2008.

[66] G. S. Prins, "Endocrine disruptors and prostate cancer risk," *Endocrine-Related Cancer*, vol. 15, no. 3, pp. 649–656, 2008.

[67] M. D. Anway and M. K. Skinner, "Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease," *Reproductive BioMedicine Online*, vol. 16, no. 1, pp. 23–25, 2008.

[68] S. M. Ho, W. Y. Tang, J. Belmonte De Frausto, and G. S. Prins, "Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4," *Cancer Research*, vol. 66, no. 11, pp. 5624–5632, 2006.

[69] T. J. Murray, M. V. Maffini, A. A. Ucci, C. Sonnenschein, and A. M. Soto, "Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure," *Reproductive Toxicology*, vol. 23, no. 3, pp. 383–390, 2007.

[70] E. Somm, V. M. Schwitzgebel, A. Toulotte et al., "Perinatal exposure to bisphenol A alters early adipogenesis in the rat," *Environmental Health Perspectives*, vol. 117, no. 10, pp. 1549–1555, 2009.

[71] N. Ben-Jonathan, E. R. Hugo, and T. D. Brandebourg, "Effects of bisphenol A on adipokine release from human adipose tissue: implications for the metabolic syndrome," *Molecular and Cellular Endocrinology*, vol. 304, no. 1-2, pp. 49–54, 2009.

[72] F. Grün and B. Blumberg, "Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling," *Endocrinology*, vol. 147, no. 6, pp. S50–S55, 2006.

[73] F. Grun, "Obesogens," *Current Opinion in Endocrinology, Diabetes and Obesity*, vol. 17, no. 5, pp. 453–459, 2010.

[74] R. R. Newbold, E. Padilla-Banks, W. N. Jefferson, and J. J. Heindel, "Effects of endocrine disruptors on obesity," *International Journal of Andrology*, vol. 31, no. 2, pp. 201–207, 2008.

[75] K. Wada, H. Sakamoto, K. Nishikawa et al., "Life style-related diseases of the digestive system: endocrine disruptors stimulate lipid accumulation in target cells related to metabolic syndrome," *Journal of Pharmacological Sciences*, vol. 105, no. 2, pp. 133–137, 2007.

[76] R. R. Newbold, E. Padilla-Banks, R. J. Snyder, and W. N. Jefferson, "Perinatal exposure to environmental estrogens and the development of obesity," *Molecular Nutrition and Food Research*, vol. 51, no. 7, pp. 912–917, 2007.

[77] J. Miyawaki, K. Sakayama, H. Kato, H. Yamamoto, and H. Masuno, "Perinatal and postnatal exposure to bisphenol A increase adipose tissue mass and serum cholesterol level in mice," *Journal of Atherosclerosis and Thrombosis*, vol. 14, no. 5, pp. 245–252, 2007.

[78] F. S. Vom Saal and J. P. Myers, "Bisphenol A and risk of metabolic disorders," *JAMA—Journal of the American Medical Association*, vol. 300, no. 11, pp. 1353–1355, 2008.

[79] R. M. Sharpe and A. J. Drake, "Bisphenol A and metabolic syndrome," *Endocrinology*, vol. 151, no. 6, pp. 2404–2407, 2010.

[80] P. Alonso-Magdalena, S. Morimoto, C. Ripoll, E. Fuentes, and A. Nadal, "The estrogenic effect of bisphenol a disrupts pancreatic β-cell function in vivo and induces insulin resistance," *Environmental Health Perspectives*, vol. 114, no. 1, pp. 106–112, 2006.

[81] R. R. Newbold, E. Padilla-Banks, and W. N. Jefferson, "Environmental estrogens and obesity," *Molecular and Cellular Endocrinology*, vol. 304, no. 1-2, pp. 84–89, 2009.

[82] D. Sharp, "Environmental toxins, a potential risk factor for diabetes among Canadian Aboriginals," *International Journal of Circumpolar Health*, vol. 68, no. 4, pp. 316–326, 2009.

[83] X. Tian, M. Takamoto, and K. Sugane, "Bisphenol A promotes IL-4 production by Th2 cells," *International Archives of Allergy and Immunology*, vol. 132, no. 3, pp. 240–247, 2003.

[84] H. Yan, M. Takamoto, and K. Sugane, "Exposure to bisphenol A prenatally or in adulthood promotes T_H2 cytokine production associated with reduction of $CD4^+CD25^+$ regulatory T cells," *Environmental Health Perspectives*, vol. 116, no. 4, pp. 514–519, 2008.

[85] Y. Masuo, M. Ishido, M. Morita, and S. Oka, "Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats," *Neural Plasticity*, vol. 11, no. 1-2, pp. 59–76, 2004.

[86] Y. Masuo, M. Morita, S. Oka, and M. Ishido, "Motor hyperactivity caused by a deficit in dopaminergic neurons and the effects of endocrine disruptors: a study inspired by the physiological roles of PACAP in the brain," *Regulatory Peptides*, vol. 123, no. 1–3, pp. 225–234, 2004.

[87] J. Kimura-Kuroda, I. Nagata, and Y. Kuroda, "Disrupting effects of hydroxy-polychlorinated biphenyl (PCB) congeners on neuronal development of cerebellar Purkinje cells: a possible causal factor for developmental brain disorders?" *Chemosphere*, vol. 67, no. 9, pp. S412–S420, 2007.

[88] T. Fujimoto, K. Kubo, and S. Aou, "Prenatal exposure to bisphenol a impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats," *Brain Research*, vol. 1068, no. 1, pp. 49–55, 2006.

[89] H. B. Patisaul and H. L. Bateman, "Neonatal exposure to endocrine active compounds or an ERβ agonist increases adult anxiety and aggression in gonadally intact male rats," *Hormones and Behavior*, vol. 53, no. 4, pp. 580–588, 2008.

[90] B. C. Ryan and J. G. Vandenbergh, "Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice," *Hormones and Behavior*, vol. 50, no. 1, pp. 85–93, 2006.

[91] Y. H. Tian, J. H. Baek, S. Y. Lee, and C. G. Jang, "Prenatal and postnatal exposure to bisphenol A induces anxiolytic behaviors and cognitive deficits in mice," *Synapse*, vol. 64, no. 6, pp. 432–439, 2010.

[92] K. H. Cox, J. D. Gatewood, C. Howeth, and E. F. Rissman, "Gestational exposure to bisphenol A and cross-fostering affect behaviors in juvenile mice," *Hormones and Behavior*, vol. 58, no. 5, pp. 754–761, 2010.

[93] S. J. Genuis, "Sensitivity-related illness: the escalating pandemic of allergy, food intolerance and chemical sensitivity," *Science of the Total Environment*, vol. 408, no. 24, pp. 6047–6061, 2010.

[94] S. Dearing, "Canada sets the bar high by declaring Bisphenol A toxic," October 2010, http://www.digitaljournal.com/article/298935#ixzz1R5Q3MJqzhttp://www.digitaljournal.com/article/298935#ixzz12YD1MxcT.

[95] R. W. Snyder, S. C. Maness, K. W. Gaido, F. Welsch, S. C. J. Sumner, and T. R. Fennell, "Metabolism and disposition of bisphenol A in female rats," *Toxicology and Applied Pharmacology*, vol. 168, no. 3, pp. 225–234, 2000.

[96] J. B. Matthews, K. Twomey, and T. R. Zacharewski, "In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors α and β," *Chemical Research in Toxicology*, vol. 14, no. 2, pp. 149–157, 2001.

[97] L. H. Pottenger, J. Y. Domoradzki, D. A. Markham, S. C. Hansen, S. Z. Cagen, and J. M. Waechter, "The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration," *Toxicological Sciences*, vol. 54, no. 1, pp. 3–18, 2000.

[98] S. J. Genuis, "Elimination of persistent toxicants from the human body," *Human and Experimental Toxicology*, vol. 30, no. 1, pp. 3–18, 2011.

[99] S. J. Genuis, D. Birkholz, I. Rodushkin, and S. Beesoon, "Blood, Urine, and Sweat (BUS) study: monitoring and elimination of bioaccumulated toxic elements," *Archives of Environmental Contamination and Toxicology*, vol. 61, no. 2, pp. 344–357, 2010.

[100] R. J. Jandacek, N. Anderson, M. Liu, S. Zheng, Q. Yang, and P. Tso, "Effects of yo-yo diet, caloric restriction, and olestra on tissue distribution of hexachlorobenzene," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 288, no. 2, pp. G292–G299, 2005.

[101] G. W. Yu, J. Laseter, and C. Mylander, "Persistent organic pollutants in serum and several different fat compartments in humans," *Journal of Environmental and Public Health*, vol. 2011, Article ID 417980, 2011.

[102] X. Ye, Z. Kuklenyik, L. L. Needham, and A. M. Calafat, "Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry," *Analytical and Bioanalytical Chemistry*, vol. 383, no. 4, pp. 638–644, 2005.

[103] E. Kandaraki, A. Chatzigeorgiou, S. Livadas et al., "Endocrine disruptors and Polycystic Ovary Syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 3, pp. E480–E484, 2011.

[104] L. Cobellis, N. Colacurci, E. Trabucco, C. Carpentiero, and L. Grumetto, "Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women," *Biomedical Chromatography*, vol. 23, no. 11, pp. 1186–1190, 2009.

[105] V. Padmanabhan, K. Siefert, S. Ransom et al., "Maternal bisphenol-A levels at delivery: a looming problem?" *Journal of Perinatology*, vol. 28, no. 4, pp. 258–263, 2008.

[106] L. N. Vandenberg, I. Chahoud, J. J. Heindel, V. Padmanabhan, F. J. R. Paumgartten, and G. Schoenfelder, "Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A," *Environmental Health Perspectives*, vol. 118, no. 8, pp. 1055–1070, 2010.

[107] J. Mendiola, N. Jørgensen, A. M. Andersson et al., "Are environmental levels of bisphenol A associated with reproductive function in fertile men?" *Environmental Health Perspectives*, vol. 118, no. 9, pp. 1286–1291, 2010.

[108] S. Moors, M. Blaszkewicz, H. M. Bolt, and G. H. Degen, "Simultaneous determination of daidzein, equol, genistein and bisphenol A in human urine by a fast and simple method using SPE and GC-MS," *Molecular Nutrition and Food Research*, vol. 51, no. 7, pp. 787–798, 2007.

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Review Article

Mercury Toxicity and Treatment: A Review of the Literature

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Mercury is a toxic heavy metal which is widely dispersed in nature. Most human exposure results from fish consumption or dental amalgam. Mercury occurs in several chemical forms, with complex pharmacokinetics. Mercury is capable of inducing a wide range of clinical presentations. Diagnosis of mercury toxicity can be challenging but can be obtained with reasonable reliability. Effective therapies for clinical toxicity have been described.

1. Introduction

Mercury is a heavy metal of known toxicity, noted for inducing public health disasters in Minamata Bay, Japan [1] and in Iraq [2–4]. The clinical impact of smaller mercury exposures remains controversial. It exists in several forms: inorganic mercury, which includes metallic mercury and mercury vapor (Hg^0) and mercurous (Hg_2^{++}) or mercuric (Hg^{++}) salts; and organic mercury, which includes compounds in which mercury is bonded to a structure containing carbon atoms (methyl, ethyl, phenyl, or similar groups). The biological behavior, pharmacokinetics, and clinical significance of the various forms of mercury vary with chemical structure. There is some interconversion in vivo between the various forms of mercury. Inhaled elemental mercury vapor, for example, is easily absorbed through mucus membranes and the lung and rapidly oxidized to other forms (but not so quickly as to prevent considerable deposition of elemental mercury in the brain). Methyl mercury is easily absorbed through the gut and deposits in many tissues, but does not cross the blood-brain barrier as efficiently as elemental mercury; however, on entering the brain it is progressively demethylated to elemental mercury [5]. Mercury salts, in contrast, tend to be insoluble, relatively stable, and poorly absorbed.

Human toxicity varies with the form of mercury, the dose and the rate of exposure. The target organ for inhaled mercury vapor is primarily the brain [5]. Mercurous and mercuric salts chiefly damage the gut lining and kidney [5], while methyl mercury is widely distributed throughout the body [5]. Toxicity varies with dosage: large acute exposures to elemental mercury vapor induce severe pneumonitis, which in extreme cases can be fatal [5]. Low-grade chronic exposure to elemental or other forms of mercury induces subtler symptoms and clinical findings, as discussed hereinafter.

There is considerable controversy about the clinical significance of exposure to the various forms of mercury and some disagreement regarding techniques for clinical assessment of mercury burden. This paper is intended to review published data on these issues and to assess published clinical experience using DMPS to remove mercury from the human body. Most of the authors cited hereinafter consider DMPS to be a stronger chelator than DMSA, with one ex-

ception citing evidence that DMSA is more effective at removing organic mercury [6]. This is a complicated issue. The absorption of DMPS and DMSA by ingestion is highly variable from one patient to the next; DMPS can be given intravenously, while DMSA cannot. DMPS is considerably safer than penicillamine or British anti-Lewisite, as discussed hereinafter. It is available for compounding in the United States and is available over the counter in Germany.

2. Sources of Mercury Exposure

Most human exposure to mercury is caused by outgassing of mercury from dental amalgam, ingestion of contaminated fish, or occupational exposure, according to the World Health Organization [7, 8].

Mercury exists in nature primarily as elemental mercury or as a sulfide and is found in the earth's crust at approximately 0.5 parts per million. Atmospheric exposures occur from outgassing from rock or through volcanic activity. Human sources of atmospheric mercury include coal burning [9] and mining (mercury and gold in particular). Atmospheric elemental mercury settles in water, where it is converted by microorganisms into organic (methyl or ethyl) mercury, which is ingested by smaller creatures which are eventually consumed by larger fish. Fish at the top of the food chain (e.g., tuna, swordfish, or shark) may concentrate considerable mercury in their tissues.

Human mercury exposures occur chiefly [7, 8] through inhalation of elemental mercury vapor via occupational or dental amalgam exposure or through ingestion of mercury bonded to organic moieties (methyl, dimethyl, or ethyl mercury), primarily from seafood. Most human metallic mercury exposure comes from mercury vapor outgassing from amalgam fillings, at a rate of 2 to 28 micrograms per facet surface per day, of which about 80% is absorbed, according to the World Health Organization [7, 8] and Berglund et al. [10]. A less common source of mercury vapor is spilled mercury [11], and there is a report in the literature of Idiopathic Thrombocytopenic Purpura [12] caused by vacuuming spilled mercury (thereby producing a major acute exposure to mercury vapor).

Methyl and dimethyl mercury (organic mercury) usually originate from biological sources, chiefly fresh or salt water fish. Over three thousand lakes in the United States have been closed to fishing due to mercury contamination [5] and many species of ocean fish are also tainted with considerable concentrations of mercury [13].

3. Pharmacokinetics of Mercury Exposure

3.1. Inorganic Mercury

3.1.1. Elemental or Metallic (Hg^0) Mercury. Approximately 80% of metallic mercury vapor outgassed from amalgams is absorbed through inhalation [10, 14, 15], compared with about 7 to 10% absorption of ingested metallic mercury [5], and about 1% absorption of metallic mercury through skin contact [5]. On entry to the body, mercury vapor has great affinity for sulfhydryl groups and bonds to sulfur-containing amino acids throughout the body. Mercury vapor is transported to the brain [16], either dissolved in serum or adherent to red cell membranes. Metallic mercury passes easily through the blood brain barrier [17] and through the placenta, where it lodges in the fetal brain [18]. Metallic mercury is, however, rapidly oxidized to mercuric mercury on entry to the blood stream [5], although not so quickly as to prevent considerable uptake by the central nervous system while still in the metallic form.

In addition to the brain [16, 19–26], metallic mercury is also deposited in the thyroid [5, 19, 21], breast [27], myocardium [28, 29], muscles [5, 21], adrenals [5], liver [5, 30–32], kidneys [5, 7, 8, 19, 20, 23, 30–32], skin [5, 7, 8], sweat glands [5], pancreas [5], enterocytes [5, 30], lungs [5, 23, 30], salivary glands [5], testes, and prostate [5] and may be associated with dysfunction of those organs. Mercury also has affinity for binding sites on the surface of T cells and for sulfhydryl groups influencing T cell function [33, 34]. Mercury deposits readily in placenta and fetal tissues and is found in breast milk. [5, 18, 31, 35]

Metallic mercury is largely excreted as mercuric mercury [5]. The excretory half lives of metallic and mercuric mercury vary widely, depending on the organ of deposition and redox state, with values ranging from a few days to several months [5], with some pools (e.g., CNS) having a half life exceeding several years [5]. Hair mercury does not correlate with brain content of metallic mercury [5]. These complexities make accurate assessment of body burden challenging (see Section 9 hereinafter).

3.1.2. Mercurous (Hg_2^{++}) Mercury. Mercurous mercury salt in the form of Hg_2Cl_2 (calomel) is poorly soluble in water and poorly absorbed by the intestine, although some portion is thought to undergo oxidation to more readily absorbable forms [36]. It is doubtful that mercurous mercury survives in the body, other than as a transitional form between metallic and mercuric mercury [5].

Some absorption evidently occurs, however, as calomel is occasionally associated with pink disease, or acrodynia.

3.1.3. Mercuric (Hg^{++}) Mercury. Historically, mercuric chloride ($HgCl_2$) was used as a preservative and for development of photographic film and was ingested accidentally or as a suicide measure. It is a component of some skin-lightening creams. Only about 2% of ingested mercuric chloride is absorbed initially [37], although it is believed that its corrosive effect on the intestine may increase permeability and, hence, absorption, with prolonged exposure [38]. Available data on skin penetration of mercuric mercury are insufficient to make quantitative comparison with ingestion or with metallic mercury.

Like metallic mercury, mercuric mercury in the bloodstream adheres to sulfhydryl groups on erythrocytes, metallothionein, or glutathione or is suspended in plasma [26]. Mercuric mercury does not cross the blood-brain barrier efficiently, but it does accumulate in quantity in the placenta, fetal tissues, and amniotic fluid [35]. Evidence exists showing transport of mercuric mercury via one or more amino acid transporters [39], particularly that for cysteine, which may

account for accumulation in the brain [5]. Much of the body burden of mercuric mercury resides in the proximal convoluted renal tubule [40] bonded to metallothionein [41]. Significant deposition also occurs periportally in the liver [42] and lesser amounts in epithelial tissues, choroidal plexus, and testes.

Excretion of mercuric mercury is largely through urine and stool, although significant amounts are shed through sweat, tears, breast milk, and saliva [5, 43]. Half lives appear to be multiphasic, as with metallic mercury, with human studies suggesting an effective half life of 42 days for 80% of an oral tracer dose; the other 20% did not appear to have a measureable rate of excretion [44]. This may reflect demethylation to metallic mercury in the brain and other organs or mechanisms yet to be determined.

3.2. Organic Mercury Compounds. Most available data on organic mercury compounds refer to methyl mercury, which is a major source of human mercury exposure, is found naturally in fish, and is relatively stable. Ethyl mercury behaves in a similar fashion to methyl mercury at the cellular level, but with an excretory half life about one third as long [5].

Methyl mercury vapor is absorbed with similar (80%) efficiency as metallic mercury vapor [5]. Intestinal absorption of methyl mercury from fish is also fairly efficient, as is absorption through the skin [5]. On entry to the bloodstream, methyl mercury adheres to sulfhydryl groups, particularly to those in cysteine. Methyl mercury is deposited throughout the body, with equilibrium between blood and body occurring approximately four days after exposure [45]. Distribution to peripheral tissues seems to occur through one or more transporters, especially the cysteine transporter, probably adherent to the sulfhydryl group in cysteine [5].

Concentration of methyl mercury occurs in the brain, liver, kidneys, placenta, and fetus, especially in the fetal brain, as well as in peripheral nerves and bone marrow [5]. Deposited methyl mercury slowly undergoes demethylation to inorganic mercury [46].

The excretory half life of methyl mercury in man is about 70 days, with approximately 90% being excreted in stool. Some degree of enterohepatic circulation apparently occurs. Perhaps 20% of methyl mercury is excreted in breast milk, with the actual amount varying with severity of exposure [5]. Hair mercury reflects blood methyl mercury at the time of incorporation, but not elemental mercury [47], and hence is not a good index of total body burden [5], given the short half life of methyl mercury in blood.

Dimethyl mercury is also efficiently absorbed through the skin, and there is a reported death of a scientist caused by minimal skin contact [48].

4. Toxicity

4.1. Inorganic Mercury

4.1.1. Metallic Mercury Vapor. Mercury in all forms poisons cellular function by altering the tertiary and quaternary structure of proteins and by binding with sulfhydryl and selenohydryl groups. Consequently, mercury can potentially impair function of any organ, or any subcellular structure. The chief target organ of mercury vapor is the brain, but peripheral nerve function, renal function, immune function, endocrine and muscle function, and several types of dermatitis have been described [49].

With massive acute exposure to mercury vapor, erosive bronchitis and bronchiolitis potentially leading to respiratory failure may be accompanied by CNS symptoms such as tremor or erethism [50].

Chronic exposure to clinically significant doses of mercury vapor usually produces neurological dysfunction. At low-level exposures, nonspecific symptoms like weakness, fatigue, anorexia, weight loss, and gastrointestinal disturbance have been described [51]. Higher exposure levels are associated with mercurial tremor: fine muscle fasciculations punctuated every few minutes by coarse shaking. Erethism may also be observed: severe behavior and personality changes, emotional excitability, loss of memory, insomnia, depression, fatigue, and in severe cases delirium and hallucination [10]. Gingivitis and copious salivation have been described [5].

These symptoms may regress with cessation of exposure, but in many cases do not. Persistent neurological symptoms are common [52].

4.1.2. Mercurous Mercury. Calomel (Hg_2Cl_2) is still used in some regions of the world as a laxative. Although poorly absorbed, some is converted to mercuric mercury, which is absorbed, and induces toxicity as expected with mercuric mercury.

4.1.3. Mercuric Mercury. Acute poisoning with mercuric salts (typically $HgCl_2$) generally targets the gastrointestinal tract and the kidneys. Extensive precipitation of enterocyte proteins occurs, with abdominal pain, vomiting, and bloody diarrhea with potential necrosis of the gut mucosa. This may produce death either from peritonitis or from septic or hypovolemic shock. Surviving patients commonly develop renal tubular necrosis with anuria [53].

Chronic poisoning with mercury salts is rare, usually also involving concomitant occupational exposure to mercury vapor. Kidney toxicity involves either renal tubular necrosis or autoimmune glomerulonephritis, or both [53]. Immune dysfunctions include hypersensitivity reactions to mercury exposure, including asthma and dermatitis, various types of autoimmunity [54], and suppression of natural killer cells [55] and disruption of various other lymphocyte subpopulations [5].

Brain dysfunction is less evident than with other forms of mercury. Thyroid dysfunction seems associated with inhibition of the 5′ deiodonases, with decreased free T3 and increased reverse T3 [56]. Accumulation in the testicles appears to inhibit spermatogenesis [57]. Atrophy and capillary damage have been described in thigh muscle [58].

4.2. Organic Mercury. Methyl mercury reacts with sulfhydryl groups throughout the body, therefore potentially interfering with the function of any cellular or subcellular structure.

Mercury is believed to interfere with DNA transcription and protein synthesis [59], including protein synthesis in the developing brain, with destruction of endoplasmic reticulum and disappearance of ribosomes [60]. Evidence suggests disruption of numerous subcellular elements in the central nervous system and other organs and in mitochondria; adverse effects have also been described on heme synthesis [61], cell membrane integrity in many locations [5], free radical generation [27, 62, 63], neurotransmitter disruption, and stimulation of neural excitoxins [5], resulting in damage to many parts of the brain and peripheral nervous system [5].

Methyl mercury has been associated with reduction in Natural Killer cell activity [64–67], as well as an imbalance in Th2 : Th1 ratios favoring autoimmunity [34, 68, 69]. Mercury is also possibly associated with disruption of DNA repair [5, 27]. The affinity of mercury for sulfhydryl groups of the mitochondrial oxidative phosphorylation complex [70] associated with destruction of mitochondrial membranes may contribute to chronic fatigue syndrome.

5. Clinical Presentation

5.1. Inorganic

5.1.1. Elemental (Metallic) Mercury. Acute exposure to a large quantity of mercury vapor induces pneumonitis, as discussed previously. Symptoms of low-grade chronic exposure are more subtle and nonspecific: weakness, fatigue, anorexia, weight loss, and gastrointestinal distress [5], sometimes referred to as micromercurialism [71]. At higher exposures, the mercurial fine tremor punctuated by coarse shaking occurs; erethism, gingivitis, and excessive salivation have also been described [5], as has immune dysfunction [34].

Objective findings include altered evoked potentials and decreased peripheral nerve conduction velocity [72]. Objective measures of short-term memory may be inversely correlated with urinary mercury in chloralkali workers [73]. Reduced color vision and visual acuity have also been observed [74]. Changes in coordination, tremor, mental concentration capacity, facial expression, and emotional state are also described [75], as are polyarthritis, various forms of dermatitis, and a syndrome mimicking pheochromocytoma [76].

Subtler clinical findings among dentists have been documented, including delayed reaction time, poor fine motor control, and deficits in mental concentration, vocabulary, task switching, and the One Hole test, as well as mood lability, all correlating with urinary mercury excretion [75]. Evidence also links elemental mercury to depression, excessive anger, and anxiety [77], as well as acute myocardial infarction, lipid peroxidation, and carotid atherosclerosis, in Finland [78]; the Finnish experience may possibly be explained by dietary selenium deficiency, since selenium antagonizes mercury toxicity. Other investigators, however, have described associations between mercury and hypertension, lipid peroxidation, ischemic heart disease, and stroke [79].

5.1.2. Mercuric Salts. Ingestion of mercuric chloride produces extensive precipitation of intestinal mucosal proteins, mucosal necrosis, generalized abdominal pain, bloody diarrhea, and shock. If the patient survives, acute renal failure may follow [5].

5.2. Organic Mercury. Methyl mercury and ethyl mercury produce similar signs and symptoms. Most published data refer to methyl mercury. Symptoms relate more to magnitude of methyl mercury retention than to the rate of deposition. Acute exposures tend to have a latency period of one or more weeks; once acquired, toxic doses are cleared slowly, if at all [5].

Massive prenatal poisoning may induce a form of cerebral palsy [5]. Lesser prenatal doses have been associated with neurodevelopmental delays and cognitive deficits [80–82].

Postnatal exposures generate a range of symptoms ranging from paresthesias, with lesser exposures, to ataxia, visual, auditory, and extrapyramidal impairments with moderate exposures and clonic seizures in more severe exposures, as in Minamata [1] and Iraq [2–4].

Objective physical findings are similar to those seen with elemental mercury exposure.

6. Laboratory Assessment of Mercury Exposure

Given the wide range of excretory half lives of the various mercury pools, discussion of laboratory assessment will combine the various forms into one discussion. It is important to recall that blood, hair, and urine mercury levels reflect recent exposure and do not correlate with total body burden [83–86]. Blood and urine levels correlate fairly well to each other, but not to total body burden [87]. With half life of all mercury pools in the blood estimated to be in the range of three to five days [88], during which either excretion or deposition in solid organs occurs, more accurate means of estimating body burden have been required.

That being said, the US federal biological exposure index (BEI) is currently set at 50 mcg/L urine. Aside from the obvious problems associated with basing a monitoring index on a measurement which only reflects current or recent exposure, and not overall body burden, several clinical studies show objective symptoms well below 50 mcg/L, with many proband values extending down into the low end of the reference range for urinary mercury excretion [75, 89–94], effectively rendering the US federal BEI useless for clinical or investigational purposes. Similar criticisms have been made of the EPA Reference Dose for methylmercury [95]. As summarized by Kazantzis, "it has not been possible to set a level for mercury in blood or urine below which mercury related symptoms will not occur" [96].

Because of these difficulties, provocation with a chelator has been proposed as providing a more reliable estimate of body burden, and DMPS (2,3 Dimercapto-1-Propanesulfonate) has been found by a number of investigators to provide a reliable estimate of body burden, safer than British Anti-Lewisite and more potent than DMSA [75, 97–101].

7. DMPS: Safety

DMPS is an analog of British Anti-Lewisite (BAL) with high affinity for mercury. Due to its superior safety, it has been widely used in Germany for the past fifty years and is available over the counter in that country. Protocols determining the pharmacokinetics of DMPS and evaluating its use for diagnostic purposes have been published in Germany [101], Sweden [102, 103], New Zealand [100], and Mexico [104] and in the United States [105–109].

Maiorino et al. [106] gave his volunteers DMPS 300 mg orally; over 90% of the absorbed DMPS was converted rapidly to disulfide forms. Published absorption of ingested DMPS varies from 39% [107] to 60% [110]. The excretory half life of unaltered DMPS was 4.4 ± 1.1 hours. The excretory half life of the disulfide forms of DMPS was 9.9 ± 1.6 hours.

Hurlbut et al.'s [107] volunteers were given an unusually large dose of DMPS (3 mg/kg intravenously over 5 minutes). Two subjects had a transient 20 mmHg drop in systolic blood pressure during infusion, without other changes in vital signs. Excretory half life of unaltered DMPS ranged from 1.3 to 4.0 hours. Half life of the altered DMPS was from 19.8 to 37.5 hours.

In each of the cited studies, mercury output following provocation with DMPS correlated significantly with amalgam number and/or occupational or dietary exposure. There were no significant complications in any of the trials. Consequently, all the investigators but one [111] concluded that urine output provoked by DMPS represented a fair estimate of body burden.

8. DMPS: Efficacy

Each of the test trials cited in the previous section and others [112] showed statistically significant increases in urinary mercury output with administration of DMPS. With prolonged treatment, evidence of decreased body burden has been inferred [113].

Several controlled clinical trials support this conclusion. The largest was undertaken in the Phillippines in a gold mining area [114]. Workers in gold mining who sustained ongoing exposure to elemental mercury were compared to people living downstream who ate fish, which contained considerable methyl mercury, and to controls without significant known mercury exposure. Probands from the two exposed areas were chosen with elevated blood, urine and hair mercury levels, and appropriate symptoms (tremor, sleeplessness, memory loss, etc.)[115]; controls had normal levels and were asymptomatic.

One hundred six probands completed the fourteen-day trial with oral DMPS 400 mg per day. The only complication was an allergic rash in one patient, who was excluded from the trial. Blood mercury did not decrease during the trial, despite increases in urine mercury up to 85-fold.

Despite the short (fourteen-day) duration of the trial, significant improvements were observed in objective measures like hypomimia, Romberg test, tests for tremor and ataxia, pencil tapping, and Frostig visual perception. Most of the patients reported subjective improvement in memory, sleeplessness, metallic taste, fatigue, anxiety, and paresthesias. Treatment efficacy was similar in the metallic mercury group (miners) and in the methyl mercury group (downstream fish eaters). Similar results were presented in a parallel study by Drasch et al. [115].

A university case report from the United States of treatment of occupational exposures to mercury vapor [116] showed relief of muscle twitching, arthralgias, paresthesias, night sweats, weight loss, and excessive salivation following two weeks of oral DMPS 100 mg TID followed by DMPS 100 mg QID for an additional six weeks. Reduction of symptoms closely paralleled urine mercury output, which tapered over time.

9. Discussion

Mercury toxicity is not often included in the differential diagnosis of common subjective complaints such as fatigue, anxiety, depression, odd paresthesias, weight loss, memory loss, and difficulty concentrating, but these are the symptoms of low-grade chronic mercury exposure described by the investigators cited previously. Given the ability of the various forms of mercury to deposit in most parts of the human body, the range of symptoms potentially caused by mercury is quite large.

Animal studies linking mercury toxicity to neurodegenerative diseases [117, 118] raise clinical concern, as do a series of associations between mercury and neurodegenerative diseases in humans [119–123].

Mercury exposure is not insignificant according to WHO, as cited previously, and the NHANES reports suggest widespread exposure in the United States, especially among women [124, 125].

Diagnosis of mercury overload is difficult. The commonly used modalities (blood, urine, and/or hair levels) do not correlate with total body burden and offer little diagnostically useful information. Provocation with DMPS appears to offer a more accurate assessment of body burden.

Since provocation is safe and inexpensive, indications for provocation must rest on clinical grounds: does the patient have multiple, vague symptoms similar to those described in the mercury literature, without other plausible, and potentially reversible, explanation? Is there a significant history of mercury exposure: multiple amalgam fillings, high seafood intake, and history of multiple thimerosal-containing vaccinations or significant occupational exposures? Is there a family history of Alzheimer's, Parkinson's, or other diseases with postulated links to mercury exposure? Is there a history of known glutathione transferase (GST) polymorphisms, which decrease the body's ability to clear heavy metals like mercury?

If so, then provocation with a chelator may be indicated. Published protocols [126–130] exist which call for provocation with DMPS with or without EDTA, in sequence. These are designed for safety, and for diagnostic breadth. DMPS has far better affinity for mercury than EDTA, but EDTA is more effective in removing lead, cadmium, nickel, and other toxic metals. Provocation with both gives a fuller

picture of overall metal burden. Patients with GST enzyme abnormalities may also receive glutathione to expedite excretion of chelated metal. For unknown reasons, patients with GST polymorphisms tend to excrete mercury later in their course of treatment than other heavy metals [131]; this can sometimes produce early false negatives for mercury, due to preferential excretion of lead and other metals. All effective chelation protocols call for replacement of beneficial minerals, which are also removed by EDTA and DMPS.

There are currently no consensus criteria for the diagnosis of mercury overload, nor for overload of other toxic metals. Clinicians who specialize in this area generally consider a provoked urine metal output more than 2 standard deviations above the NHANES reference range a positive result.

Further research is required to clarify the relation between provoked urine results and clinical disease and to document clinical outcomes.

References

[1] Environmental Health Department, Ministry of the Environment, *Minimata Disease: The History and Measures*, Ministry of the Environment, Government of Japan, Tokyo, Japan, 2002.

[2] F. Bakir, S. F. Damluji, and I. Amin Zaki, "Methylmercury poisoning in Iraq: an interuniversity report," *Science*, vol. 181, no. 4096, pp. 230–241, 1973.

[3] S. B. Skerfving and J. F. Copplestone, "Poisoning caused by the consumption of organomercury dressed seed in Iraq," *Bulletin of the World Health Organization*, vol. 54, no. 1, pp. 101–112, 1976.

[4] T. W. Clarkson, L. Magos, and C. Cox, "Tests of efficacy of antidotes for removal of methylmercury in human poisoning during the Iraq outbreak," *Journal of Pharmacology and Experimental Therapeutics*, vol. 218, no. 1, pp. 74–83, 1981.

[5] M. Berlin, R. K. Zalups, and B. A. Fowler, "Mercury," in *Handbook on the Toxicology of Metals*, G. F. Nordberg, B. A. Fowler, M. Nordberg, and L. T. Friberg, Eds., chapter 33, Elsevier, New York, NY, USA, 3rd edition, 2007.

[6] H. V. Aposhian, "DMSA and DMPS—water soluble antidotes for heavy metal poisoning," *Annual Review of Pharmacology and Toxicology*, vol. 23, pp. 193–215, 1983.

[7] World Health Organization, "Inorganic mercury: environmental health criteria 118," in *International Programme on Chemical Safety*, World Health Organization, Geneva, Switzerland, 1991.

[8] M. Richardson, *The Safety of Dental Amalgam*, ISBN 0-662-24873-2, Minister of Health, Canada, 1996.

[9] H. M. Boylan, R. D. Cain, and H. M. Kingston, "A new method to assess mercury emissions: a study of three coal-fired electric-generating power station configurations," *Journal of the Air and Waste Management Association*, vol. 53, no. 11, pp. 1318–1325, 2003.

[10] A. Berglund, L. Pohl, S. Olsson, and M. Bergman, "Determination of the rate of release of intra-oral mercury vapor from amalgam," *Journal of Dental Research*, vol. 67, no. 9, pp. 1235–1242, 1988.

[11] P. Zeitz, M. F. Orr, and W. E. Kaye, "Public health consequences to mercury spills: hazardous substances emergency events surveillance system, 1993–1998," *Environmental Health Perspectives*, vol. 110, no. 2, pp. 129–132, 2002.

[12] J. G. Schwartz, T. E. Snider, and M. M. Montiel, "Toxicity of a family from vacuumed mercury," *American Journal of Emergency Medicine*, vol. 10, no. 3, pp. 258–261, 1992.

[13] J. Burger, C. Jeitner, and M. Gochfeld, "Locational differences in mercury and selenium levels in 19 species of saltwater fish from New Jersey," *Journal of Toxicology and Environmental Health*, vol. 74, no. 13, pp. 863–874, 2011.

[14] F. N. Kudsk, "The influence of ethyl alcohol on the absorption of mercury vapour from the lungs in man," *Acta Pharmacologica et Toxicologica*, vol. 23, no. 2, pp. 263–274, 1965.

[15] J. B. Hursh, T. W. Clarkson, and M. G. Cherian, "Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subjects," *Archives of Environmental Health*, vol. 31, no. 6, pp. 302–309, 1976.

[16] D. W. Eggleston and M. Nylander, "Correlation of dental amalgam with mercury in brain tissue," *The Journal of Prosthetic Dentistry*, vol. 58, no. 6, pp. 704–707, 1987.

[17] G. F. Nordberg and F. Serenius, "Distribution of inorganic mercury in the guinea pig brain," *Acta Pharmacologica et Toxicologica*, vol. 27, no. 4, pp. 269–283, 1969.

[18] T. W. Clarkson, L. Magos, and M. R. Greenwood, "The transport of elemental mercury into fetal tissues," *Biology of the Neonate*, vol. 21, no. 3, pp. 239–244, 1972.

[19] G. Guzzi, M. Grandi, C. Cattaneo et al., "Dental amalgam and mercury levels in autopsy tissues: food for thought," *American Journal of Forensic Medicine and Pathology*, vol. 27, no. 1, pp. 42–45, 2006.

[20] J. W. Reinhardt, "Side-effects: mercury contribution to body burden from dental amalgam," *Advances in Dental Research*, vol. 6, pp. 110–113, 1992.

[21] L. Björkman, B. F. Lundekvam, T. Lægreid et al., "Mercury in human brain, blood, muscle and toenails in relation to exposure: an autopsy study," *Environmental Health*, vol. 6, article 30, 2007.

[22] D. Wenstrup, W. D. Ehmann, and W. R. Markesbery, "Trace element imbalances in isolated subcellular fractions of Alzheimer's disease brains," *Brain Research*, vol. 533, no. 1, pp. 125–131, 1990.

[23] M. Nylander, L. Friberg, and B. Lind, "Mercury concentrations in the human brain and kidneys in relation to exposure from dental amalgam fillings," *Swedish Dental Journal*, vol. 11, no. 5, pp. 179–187, 1987.

[24] J. Mutter, A. Curth, J. Naumann, R. Deth, and H. Walach, "Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrated molecular mechanism," *Journal of Alzheimer's Disease*, vol. 22, no. 2, pp. 357–374, 2010.

[25] R. L. Siblerud, "The relationship between mercury from dental amalgam and mental health," *American Journal of Psychotherapy*, vol. 43, no. 4, pp. 575–587, 1989.

[26] T. W. Clarkson, J. Gatzy, and E. Dalton, *UR-582*, Division of Radiation Chemistry and Toxicology, University of Rochester Atomic Energy Project, Rochester, NY, USA, 1961.

[27] M. E. Crespo-López, G. L. Macêdo, S. I. D. Pereira et al., "Mercury and human genotoxicity: critical considerations and possible molecular mechanisms," *Pharmacological Research*, vol. 60, no. 4, pp. 212–220, 2009.

[28] A. Frustaci, N. Magnavita, C. Chimenti et al., "Marked elevation of myocardial trace elements in idiopathic dilated cardiomyopathy compared with secondary cardiac dysfunction," *Journal of the American College of Cardiology*, vol. 33, no. 6, pp. 1578–1583, 1999.

[29] N. Matsuo, T. Suzuki, and H. Akagi, "Mercury concentration

in organs of contemporary Japanese," *Archives of Environmental Health*, vol. 44, no. 5, pp. 298–303, 1989.

[30] L. J. Hahn, R. Kloiber, M. J. Vimy, Y. Takahashi, and F. L. Lorscheider, "Dental 'silver' tooth fillings: a source of mercury exposure revealed by whole-body image scan and tissue analysis," *FASEB Journal*, vol. 3, no. 14, pp. 2641–2646, 1989.

[31] M. J. Vimy, Y. Takahashi, and F. L. Lorscheider, "Maternal-fetal distribution of mercury released from dental amalgam fillings," *American Journal of Physiology*, vol. 258, no. 4, pp. R939–R945, 1990.

[32] G. Danscher, P. Horsted-Bindslev, and J. Rungby, "Traces of mercury in organs from primates with amalgam fillings," *Experimental and Molecular Pathology*, vol. 52, no. 3, pp. 291–299, 1990.

[33] P. Hultman, "Immunotoxicology of metals," in *Handbook on the Toxicology of Metals*, G. F. Nordberg, B. A. Fowler, M. Nordberg, and L. T. Friberg, Eds., pp. 205–206, Elsevier, Amsterdam, The Netherlands, 3rd edition, 2007.

[34] S. H. Park, S. Araki, A. Nakata et al., "Effects of occupational metallic mercury vapour exposure on suppressor-inducer (CD4+CD45RA+) T lymphocytes and CD57+CD16+ natural killer cells," *International Archives of Occupational and Environmental Health*, vol. 73, no. 8, pp. 537–542, 2000.

[35] T. Suzuki, T. I. Takemoto, S. Shishido, and K. Kani, "Mercury in human amniotic fluid," *Scandinavian Journal of Work, Environment and Health*, vol. 3, no. 1, pp. 32–35, 1977.

[36] Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, 8th edition, 1990.

[37] T. Norseth and T. W. Clarkson, "Intestinal transport of ^{203}Hg-labeled methyl mercury chloride. Role of biotransformation in rats," *Archives of Environmental Health*, vol. 22, no. 5, pp. 568–577, 1971.

[38] K. Kostial, D. Kello, and S. Jugo, "Influence of age on metal metabolism and toxicity," *Environmental Health Perspectives*, vol. 25, pp. 81–86, 1978.

[39] T. Jansson, "Amino acid transporters in the human placenta," *Pediatric Research*, vol. 49, no. 2, pp. 141–147, 2001.

[40] R. Taugner, K. zum Winkel, and J. Iravani, "The localization of mercuric chloride concentration in the rat kidney," *Virchows Archiv*, vol. 340, no. 4, pp. 369–383, 1966.

[41] M. G. Cherian and T. W. Clarkson, "Radioactive mercury distribution in biological fluids and excretion in human subjects after inhalation of mercury vapor," *Archives of Environmental Health*, vol. 33, no. 3, pp. 109–114, 1978.

[42] M. Berlin and S. Ullberg, "Accumulation and retention of mercury in the mouse. I. An autoradiographic study after a single intravenous injection of mercuric chloride," *Archives of Environmental Health*, vol. 6, pp. 589–601, 1963.

[43] K. A. Björnberg, M. Vahter, B. Berglund, B. Niklasson, M. Blennow, and G. Sandborgh-Englund, "Transport of methylmercury and inorganic mercury to the fetus and breast-fed infant," *Environmental Health Perspectives*, vol. 113, no. 10, pp. 1381–1385, 2005.

[44] T. Rahola, T. Hattula, and A. Lorolainen, "The Elimination of ^{203}Hg-methylmercury in man," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 27, supplement 116, p. 77, 1971.

[45] T. G. Kershaw, T. W. Clarkson, and P. H. Dhahir, "The relationship between blood levels and dose of methylmercury in man," *Archives of Environmental Health*, vol. 35, no. 1, pp. 28–36, 1980.

[46] I. Suda and H. Takahashi, "Degradation of methyl and ethyl mercury into inorganic mercury by other reactive oxygen species besides hydroxyl radical," *Archives of Toxicology*, vol. 66, no. 1, pp. 34–39, 1992.

[47] M. Berglund, B. Lind, K. A. Björnberg, B. Palm, O. Einarsson, and M. Vahter, "Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment," *Environmental Health*, vol. 4, article 20, 2005.

[48] D. W. Nierenberg, R. E. Nordgren, M. B. Chang et al., "Delayed cerebellar disease and death after accidental exposure to dimethylmercury," *New England Journal of Medicine*, vol. 338, no. 23, pp. 1672–1676, 1998.

[49] M. Berlin, *Dental Materials and Health*, Statens Offentliga Utredningar, Stockholm, 2003.

[50] R. Garnier, J. M. Fuster, and F. Conso, "Acute mercury vapour poisoning," *Toxicological European Research*, vol. 3, no. 2, pp. 77–86, 1981.

[51] L. Friberg and G. F. Nordberg, Eds., *Mercury in the Environment*, CRC Press, Boca Raton, Fla, USA, 1972.

[52] G. Baldi, E. C. Vigliani, and N. Zurlo, "Mercury poisoning in hat industry," *La Medicina del Lavoro*, vol. 44, no. 4, pp. 160–199, 1953.

[53] J. L. Barnes, E. M. McDowell, and J. S. McNeil, "Studies on the pathophysiology of acute renal failure. V. Effect of chronic saline loading on the progression of proximal tubular injury and functional impairment following administration of mercuric chloride in the rat," *Virchows Archiv Abteilung B Cell Pathology*, vol. 32, no. 3, pp. 233–260, 1980.

[54] G. de Vos, S. Abotaga, Z. Liao, E. Jerschow, and D. Rosenstreich, "Selective effect of mercury on Th2-type cytokine production in humans," *Immunopharmacology and Immunotoxicology*, vol. 29, no. 3-4, pp. 537–548, 2007.

[55] N. G. Ilback, J. Sundberg, and A. Oskarsson, "Methyl mercury exposure via placenta and milk impairs natural killer (NK) cell function in newborn rats," *Toxicology Letters*, vol. 58, no. 2, pp. 149–158, 1991.

[56] D. G. Ellingsen, J. Efskind, E. Haug, Y. Thomassen, I. Martinsen, and P. I. Gaarder, "Effects of low mercury vapour exposure on the thyroid function in chloralkali workers," *Journal of Applied Toxicology*, vol. 20, no. 6, pp. 483–489, 2000.

[57] M. V. Rao and P. S. N. Sharma, "Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice," *Reproductive Toxicology*, vol. 15, no. 6, pp. 705–712, 2001.

[58] E. Nadorfy-Lopez, S. H. Torres, H. Finol, M. Mendez, and B. Bello, "Skeletal muscle abnormalities associated with occupational exposure to mercury vapours," *Histology and Histopathology*, vol. 15, no. 3, pp. 673–682, 2000.

[59] D. W. Gruenwedel and D. S. Lu, "Changes in the sedimentation characteristics of DNA due to methylmercuration," *Biochemical and Biophysical Research Communications*, vol. 40, no. 3, pp. 542–548, 1970.

[60] S. P. Herman, R. Klein, F. A. Talley, and M. R. Krigman, "An ultrastructural study of methylmercury-induced primary sensory neuropathy in the rat," *Laboratory Investigation*, vol. 28, no. 1, pp. 104–118, 1973.

[61] B. A. Fowler and J. S. Woods, "Ultrastructural and biochemical changes in renal mitochondria during chronic oral methyl mercury exposure. The relationship to renal function," *Experimental and Molecular Pathology*, vol. 27, no. 3, pp. 403–412, 1977.

[62] G. Olivieri, C. Brack, F. Müller-Spahn et al., "Mercury induces cell cytotoxicity and oxidative stress and increases β-amyloid secretion and tau phosphorylation in SHSY5Y

neuroblastoma cells," *Journal of Neurochemistry*, vol. 74, no. 1, pp. 231–236, 2000.

[63] S. Yee and B. H. Choi, "Oxidative stress in neurotoxic effects of methylmercury poisoning," *NeuroToxicology*, vol. 17, no. 1, pp. 17–26, 1996.

[64] N. G. Ilback, J. Sundberg, and A. Oskarsson, "Methyl mercury exposure via placenta and milk impairs natural killer (NK) cell function in newborn rats," *Toxicology Letters*, vol. 58, no. 2, pp. 149–158, 1991.

[65] S. H. Park, S. Araki, A. Nakata et al., "Effects of occupational metallic mercury vapour exposure on suppressor-inducer (CD4+CD45RA+) T lymphocytes and CD57+CD16+ natural killer cells," *International Archives of Occupational and Environmental Health*, vol. 73, no. 8, pp. 537–542, 2000.

[66] L. Santarelli, M. Bracci, and E. Mocchegiani, "In vitro and in vivo effects of mercuric chloride on thymic endocrine activity, NK and NKT cell cytotoxicity, cytokine profiles (IL-2, IFN-γ, IL-6): role of the nitric oxide-l-arginine pathway," *International Immunopharmacology*, vol. 6, no. 3, pp. 376–389, 2006.

[67] L. Vimercati, L. Santarelli, G. Pesola et al., "Monocyte-macrophage system and polymorphonuclear leukocytes in workers exposed to low levels of metallic mercury," *Science of the Total Environment*, vol. 270, no. 1–3, pp. 157–163, 2001.

[68] G. de Vos, S. Abotaga, Z. Liao, E. Jerschow, and D. Rosenstreich, "Selective effect of mercury on Th2-type cytokine production in humans," *Immunopharmacology and Immunotoxicology*, vol. 29, no. 3-4, pp. 537–548, 2007.

[69] T. Tanigawa, H. Takehashi, and A. Nakata, "Naïve (CD4+CD45RA+) T cell subpopulation is susceptible to various types of hazardous substances in the workplace," *International journal of immunopathology and pharmacology*, vol. 17, supplement 2, pp. 109–114, 2004.

[70] D. W. Gruenwedel and M. K. Cruickshank, "Effect of methylmercury(II) on the synthesis of deoxyribonucleic acid, ribonucleic acid and protein in HeLa S3 cells," *Biochemical Pharmacology*, vol. 28, no. 5, pp. 651–655, 1979.

[71] L. Friberg and G. F. Nordberg, Eds., *Mercury in the Environment*, CRC Press, Boca Raton, Fla, USA, 1972.

[72] A. Andersen, D. G. Ellingsen, T. Morland, and H. Kjuus, "A neurological and neurophysiological study of chloralkali workers previously exposed to mercury vapour," *Acta Neurologica Scandinavica*, vol. 88, no. 6, pp. 427–433, 1993.

[73] P. J. Smith, G. D. Langolf, and J. Goldberg, "Effects of occupational exposure to elemental mercury on short term memory," *British Journal of Industrial Medicine*, vol. 40, no. 4, pp. 413–419, 1983.

[74] A. Cavalleri, L. Belotti, F. Gobba, G. Luzzana, P. Rosa, and P. Seghizzi, "Colour vision loss in workers exposed to elemental mercury vapour," *Toxicology Letters*, vol. 77, no. 1–3, pp. 351–356, 1995.

[75] D. Echeverria, N. J. Heyer, M. D. Martin, C. A. Naleway, J. S. Woods, and A. C. Bittner Jr., "Behavioral effects of low-level exposure to Hg$^{\circ}$ among dentists," *Neurotoxicology and Teratology*, vol. 17, no. 2, pp. 161–168, 1995.

[76] C. Kosan, A. K. Topaloglu, and B. Ozkan, "Chronic mercury intoxication simulating pheochromocytoma: effect of captopril on urinary mercury excretion," *Pediatrics International*, vol. 43, no. 4, pp. 429–430, 2001.

[77] R. L. Siblerud, J. Motl, and E. Kienholz, "Psychometric evidence that mercury from silver dental fillings may be an etiological factor in depression, excessive anger, and anxiety," *Psychological Reports*, vol. 74, no. 1, pp. 67–80, 1994.

[78] J. T. Salonen, K. Seppanen, K. Nyyssonen et al., "Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in Eastern Finnish men," *Circulation*, vol. 91, no. 3, pp. 645–655, 1995.

[79] H. M. Chan and G. M. Egeland, "Fish consumption, mercury exposure, and heart diseases," *Nutrition Reviews*, vol. 62, no. 2, pp. 68–72, 2004.

[80] S. Booth and D. Zeller, "Mercury, food webs, and marine mammals: implications of diet and climate change for human health," *Environmental Health Perspectives*, vol. 113, no. 5, pp. 521–526, 2005.

[81] P. Grandjean, P. Weihe, R. F. White et al., "Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury," *Neurotoxicology and Teratology*, vol. 19, no. 6, pp. 417–428, 1997.

[82] G. B. Ramirez, O. Pagulayan, H. Akagi et al., "Tagum study II: follow-up study at two years of age after prenatal exposure to mercury," *Pediatrics*, vol. 111, no. 3, pp. 289–295, 2003.

[83] A. Aitio, S. Valkonen, H. Kivisto, and E. Yrjanheikki, "Effect of occupational mercury exposure on plasma lysosomal hydrolases," *International Archives of Occupational and Environmental Health*, vol. 53, no. 2, pp. 139–147, 1983.

[84] M. Berlin, J. Fazackerley, and G. Nordberg, "The uptake of mercury in the brains of mammals exposed to mercury vapor and to mercuric salts," *Archives of Environmental Health*, vol. 18, no. 5, pp. 719–729, 1969.

[85] M. Berlin and S. Gibson, "Renal uptake, excretion, and retention of mercury. I. A study in the rabbit during infusion of mercuric chloride," *Archives of Environmental Health*, vol. 6, pp. 617–625, 1963.

[86] G. Drasch, E. Wanghofer, and G. Roider, "Are blood, urine, hair, and muscle valid biomonitors for the internal burden of men with the heavy metals mercury, lead and cadmium? An investigation on 150 deceased," *Trace Elements in Medicine*, vol. 14, no. 3, pp. 116–123, 1997.

[87] R. G. Smith, A. J. Vorwald, L. S. Patil, and T. F. Mooney, "Effects of exposure to mercury in the manufacture of chlorine," *American Industrial Hygiene Association journal*, vol. 31, no. 6, pp. 687–700, 1970.

[88] "3rd National report on human exposure to environmental chemicals," Tech. Rep., Centers for Disease Control and Prevention, 2005.

[89] M. W. Haut, L. A. Morrow, D. Pool, T. S. Callahan, J. S. Haut, and M. D. Franzen, "Neurobehavioral effects of acute exposure to inorganic mercury vapor," *Applied Neuropsychology*, vol. 6, no. 4, pp. 193–200, 1999.

[90] D. B. Chaffin, D. B. Dinman, J. M. Miller et al., "Final report NIOSH," Tech. Rep. contract no HSM-099-71-62, University of Michigan, Ann Arbor, Mich, USA, 1973.

[91] M. Meyer-Baron, M. Schaeper, and A. Seeber, "A meta-analysis for neurobehavioural results due to occupational mercury exposure," *Archives of Toxicology*, vol. 76, no. 3, pp. 127–136, 2002.

[92] R. Lucchini, I. Cortesi, P. Facco et al., "Neurotoxic effect of exposure to low doses of mercury," *Medicina del Lavoro*, vol. 93, no. 3, pp. 202–214, 2002.

[93] D. Echeverria, H. Vasken Aposhian, J. S. Woods et al., "Neurobehavioral effects from exposure to dental amalgam Hg^0: new distinctions between recent exposure and Hg body burden," *FASEB Journal*, vol. 12, no. 11, pp. 971–980, 1998.

[94] H. A. Roels, P. Hoet, and D. Lison, "Usefulness of biomarkers of exposure to inorganic mercury, lead, or cadmium in

controlling occupational and environmental risks of nephrotoxicity," *Renal Failure*, vol. 21, no. 3-4, pp. 251–262, 1999.

[95] D. C. Rice, "The US EPA reference dose for methylmercury: sources of uncertainty," *Environmental Research*, vol. 95, no. 3, pp. 406–413, 2004.

[96] G. Kazantzis, "Mercury exposure and early effects: an overview," *Medicina del Lavoro*, vol. 93, no. 3, pp. 139–147, 2002.

[97] H. V. Aposhian, A. Arroyo, M. E. Cebrian et al., "DMPS-arsenic challenge test. I: increased urinary excretion of monomethylarsonic acid in humans given dimercaptopropane sulfonate," *Journal of Pharmacology and Experimental Therapeutics*, vol. 282, no. 1, pp. 192–200, 1997.

[98] G. Kazantzis, "Diagnosis and treatment of metal poisoning—general aspects," in *Handbook on The Toxicology of Metals*, G. F. Nordberg, Ed., chapter 15, pp. 313–314, Elsevier, New York, NY, USA, 3rd edition, 2007.

[99] V. R. Schiele, K. H. Schaller, and D. Weltle, "Aus dem Institut fur Arbeits-und Sozialmedizin und der Poliklinik fur Berufskrankheiten de Universitat Erlangen-Nurnberg," pp. 249–251, 1989.

[100] M. Godfrey and N. Campbell, "Confirmation of mercury retention and toxicity using 2,3 dimercapto-1-propane sulphonic acid sodium salt (DMPS)," *Journal of Advancement in Medicine*, vol. 7, no. 1, pp. 19–30, 1994.

[101] M. Daunderer, "Mobilization test for environmental metal poisonings," *Forum des Praktischen und Allgemein-Arztes*, vol. 28, no. 3, pp. 88–94, 1989.

[102] M. Molin, A. Schutz, S. Skerfving, and G. Sallsten, "Mobilized mercury in subjects with varying exposure to elemental mercury vapour," *International Archives of Occupational and Environmental Health*, vol. 63, no. 3, pp. 187–192, 1991.

[103] J. Aaseth, D. Jacobsen, O. Andersen, and E. Wickstrom, "Treatment of mercury and lead poisonings with dimercaptosuccinic acid and sodium dimercaptopropanesulfonate. A review," *Analyst*, vol. 120, no. 3, pp. 853–854, 1995.

[104] O. Torres-Alanis, L. Garza-Ocanas, and A. Pineyro-Lopez, "Evaluation of urinary mercury excretion after administration of 2,3-dimercaptol-propane sulfonic acid to occupationally exposed men," *Journal of Toxicology—Clinical Toxicology*, vol. 33, no. 6, pp. 717–720, 1995.

[105] H. V. Aposhian, R. M. Maiorino, M. Rivera et al., "Human studies with the chelating agents, DMPS and DMSA," *Journal of Toxicology—Clinical Toxicology*, vol. 30, no. 4, pp. 505–528, 1992.

[106] R. M. Maiorino, R. C. Dart, D. E. Carter, and H. V. Aposhian, "Determination and metabolism of dithiol chelating agents. XII. Metabolism and pharmacokinetics of sodium 2,3-dimercaptopropane-1-sulfonate in humans," *Journal of Pharmacology and Experimental Therapeutics*, vol. 259, no. 2, pp. 808–814, 1991.

[107] K. M. Hurlbut, R. M. Maiorino, M. Mayersohn, R. C. Dart, D. C. Bruce, and H. V. Aposhian, "Determination and metabolism of dithiol chelating agents XVI: pharmacokinetics of 2,3-dimercapto-1-propanesulfonate after intravenous administration to human volunteers," *Journal of Pharmacology and Experimental Therapeutics*, vol. 268, no. 2, pp. 662–668, 1994.

[108] H. V. Aposhian, "Mobilization of mercury and arsenic in humans by sodium 2,3-dimercapto-1-propane sulfonate (DMPS)," *Environmental Health Perspectives*, vol. 106, no. 4, pp. 1017–1025, 1998.

[109] H. V. Aposhian, R. M. Maiorino, D. Gonzalez-Ramirez et al., "Mobilization of heavy metals by newer, therapeutically useful chelating agents," *Toxicology*, vol. 97, no. 1–3, pp. 23–38, 1995.

[110] "2,3 Dimercapto-1-propane sulfonic acid (DMPS) in the treatment of heavy metal poisoning," FDA Docket 98-n-0182 entry for DMPS and Nguyen H. T., 1999, http://www.fda.gov/ohrms/dockets/DOCKETS/98n0182/nom005b.pdf.

[111] G. Sallsten, L. Barregard, and A. Schutz, "Clearance half life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on excretion of mercury in urine," *Occupational and Environmental Medicine*, vol. 51, no. 5, pp. 337–342, 1994.

[112] O. Torres-Alanis, L. Garza-Ocanas, and A. Pineyro-Lopez, "Evaluation of urinary mercury excretion after administration of 2,3-dimercaptol-propane sulfonic acid to occupationally exposed men," *Journal of Toxicology—Clinical Toxicology*, vol. 33, no. 6, pp. 717–720, 1995.

[113] D. Gonzalez-Ramirez, M. Zuniga-Charles, A. Narro-Juarez et al., "DMPS (2,3-dimercaptopropane-1-sulfonate, dimaval) decreases the body burden of mercury in humans exposed to mercurous chloride," *Journal of Pharmacology and Experimental Therapeutics*, vol. 287, no. 1, pp. 8–12, 1998.

[114] S. Böse-O'Reilly, G. Drasch, C. Beinhoff et al., "The Mt. Diwata study on the Philippines 2000—Treatment of mercury intoxicated inhabitants of a gold mining area with DMPS (2,3-Dimercapto-1-propane-sulfonic acid, Dimaval®)," *Science of the Total Environment*, vol. 307, no. 1–3, pp. 71–82, 2003.

[115] G. Drasch, S. Böse-O'Reilly, C. Beinhoff, G. Roider, and S. Maydl, "The Mt. Diwata study on the Philippines 1999—Assessing mercury intoxication of the population by small scale gold mining," *Science of the Total Environment*, vol. 267, no. 1–3, pp. 151–168, 2001.

[116] J. R. Campbell, T. W. Clarkson, and M. D. Omar, "The therapeutic use of 2,3-dimercaptopropane-1-sulfonate in two cases of inorganic mercury poisoning," *Journal of the American Medical Association*, vol. 256, no. 22, pp. 3127–3130, 1986.

[117] J. C. Pendergrass, B. E. Haley, M. J. Vimy, S. A. Winfield, and F. L. Lorscheider, "Mercury vapor inhalation inhibits binding of GTP to tubulin in rat brain: similarity to a molecular lesion in Alzheimer diseased brain," *NeuroToxicology*, vol. 18, no. 2, pp. 315–324, 1997.

[118] M. Fujimura, F. Usuki, M. Sawada, and A. Takashima, "Methylmercury induces neuropathological changes with tau hyperphosphorylation mainly through the activation of the c-jun-N-terminal kinase pathway in the cerebral cortex, but not in the hippocampus of the mouse brain," *NeuroToxicology*, vol. 30, no. 6, pp. 1000–1007, 2009.

[119] F. Monnet-Tschudi, M. G. Zurich, C. Boschat, A. Corbaz, and P. Honegger, "Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases," *Reviews on Environmental Health*, vol. 21, no. 2, pp. 105–117, 2006.

[120] M. Aschner, N. Onishchenko, and S. Ceccatelli, "Toxicology of alkylmercury compounds," *Metal Ions in Life Sciences*, vol. 7, pp. 403–423, 2010.

[121] J. Mutter, A. Curth, J. Naumann, R. Deth, and H. Walach, "Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrated molecular mechanism," *Journal of Alzheimer's Disease*, vol. 22, no. 2, pp. 357–374, 2010.

[122] F. O. Johnson and W. D. Atchison, "The role of environmental mercury, lead and pesticide exposure in development of amyotrophic lateral sclerosis," *NeuroToxicology*, vol. 30, no. 5, pp. 761–765, 2009.

[123] D. J. Yang, S. Shi, L. F. Zheng, T. M. Yao, and L. N. Ji, "Mercury(II) promotes the in vitro aggregation of tau fragment corresponding to the second repeat of microtubule-binding domain: coordination and conformational transition," *Biopolymers*, vol. 93, no. 12, pp. 1100–1107, 2010.
[124] "(NHANES III) 3rd National report on human exposure to environmental chemicals," Tech. Rep., Centers for Disease Control and Prevention, 2005.
[125] "(NHANES IV) 4th National Report on Human Exposure to Environmental Chemicals," Tech. Rep., Centers for Disease Control and Prevention, 2009.
[126] "Toxicological profile for mercury," U.S. Department of Health and Human Services, Public Health Service, 1999, http://www.atsdr.cdc.gov/toxprofiles/tp46.pdf.
[127] "Diagnostic and Treatment Protocols for safer, effective mercury human biohazard management," Consensus Development Working Group of the International College of Integrative Medicine, 2003.
[128] *Chelation Module*, American College for Advancement in Medicine, Irvine, Calif, USA, 2010.
[129] Advanced Medical Education and Services Physician Association, *Introduction to Clinical Metal Toxicology*, Advanced Medical Education and Services Physician Association, San Antonio, Tex, USA, 2007.
[130] *Clinician Seminar Level 1*, Autism Research Institute, San Diego, Calif, USA, 2010.
[131] R. A. Bernhoft and R. A. Buttar, "Autism: a multi-system oxidative and inflammatory disorder," *Townsend Letter*, pp. 86–90, 2008.

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Research Article

Psychophysical Evaluation of Achromatic and Chromatic Vision of Workers Chronically Exposed to Organic Solvents

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The purpose of this paper was to evaluate achromatic and chromatic vision of workers chronically exposed to organic solvents through psychophysical methods. Thirty-one gas station workers (31.5 ± 8.4 years old) were evaluated. Psychophysical tests were achromatic tests (Snellen chart, spatial and temporal contrast sensitivity, and visual perimetry) and chromatic tests (Ishihara's test, color discrimination ellipses, and Farnsworth-Munsell 100 hue test—FM100). Spatial contrast sensitivities of exposed workers were lower than the control at spatial frequencies of 20 and 30 cpd whilst the temporal contrast sensitivity was preserved. Visual field losses were found in 10–30 degrees of eccentricity in the solvent exposed workers. The exposed workers group had higher error values of FM100 and wider color discrimination ellipses area compared to the controls. Workers occupationally exposed to organic solvents had abnormal visual functions, mainly color vision losses and visual field constriction.

1. Introduction

Studies about the effect of organic solvents in biological systems are more frequent in occupational medicine, and most commonly the intoxication is occupational and caused by solvent mixtures [1–7]. In addition, inhalation is the major pathway of intoxication in occupational environment [8–10].

Occupational exposure to organic solvents can cause damage in both central and peripheral nervous system [11–14], and the visual system is one of the main targets of organic solvent intoxication [15]. As a result, acquired dyschromatopsias usually have been found in chronically exposed subjects to organic solvent mixtures [3, 16–19], as well as to specific solvents as n-hexane, styrene, and toluene [20–25]. Most color vision deficiencies due to exposure to solvents have subclinical symptoms, and a loss of the blue-yellow discrimination has been the most frequently reported impairment [3, 16, 18, 20–22, 24, 26–30], although some studies described altered red-green discrimination [3, 31].

It has been described that chronic exposure to n-hexane may cause color discrimination losses, associated with maculopathy [32] and visual perimetry losses at the periphery, with optic nerve atrophy and retrobulbar neuritis [33]. Optic neuropathy is a finding associated with polyneuropathy in cases of alcohol, methanol, styrene, toluene, trichloroethylene, and solvent misture intoxication [34]. Decreased spatial contrast sensitivity in the middle range (6–12 cpd) of spatial frequencies associated to normal visual acuity seems to be an indicator of visual impairment induced by chronic exposure to styrene, acute exposure to tetrachloroethylene or triethylamine [21, 25, 35] and organic solvent mixtures [6, 36–38]. Losses of spatial vision can be dependent of the intoxication level [38–40].

Painters, factory workers, and cleaners are subject to continuous exposure to organic solvents. Investigation of their visual system to look for functional deficits has been performed by several authors, showing the impact of this exposure [6, 7, 25, 30, 41]. In some countries, Brazil included, automobile tanks are filled by gas station workers. Therefore, in this job the person is subject to a long period of organic solvent exposure. Automobile fuel is composed of a mixture of organic solvent including gasoline, alcohol, and diesel oil. They are composed by several hydrocarbons such as methane, ethane, propane, pentane, methanol, ethanol, propanol, methyl tertiary butyl ester, benzene, toluene, and xylene.

2. Methods

Thirty-one gas station workers agreed to participate in the study. Two subjects were excluded due to congenital red-green dyschromatopsia. Twenty-nine (27 males, 31.5 ± 8.4 years old) workers were evaluated. All procedures were evaluated by Ethical Committee in Research in Humans of the Tropical Medicine Nucleus of Federal University of Pará (Protocol no. 075/2006-CEP/NMT). These subjects had normal visual acuity or corrected to 20/20 (Snellen test).

Gas station workers participated in the current work according to their availability and in some cases they were unable to do all the tests due to their heavy work schedule. Control group for each psychophysical test was composed by the same number of subjects as the exposed group, matched in age and gender (32.8 ± 9.5 years old). The control subjects worked in environments free of solvent exposure.

2.1. Psychophysical Tests. Achromatic (spatial and temporal contrast sensitivity and visual perimetry) and chromatic (Farnsworth-Munsell 100 hue test, color discrimination ellipses) psychophysical tests were performed. Stimuli were displayed in a CRT high spatial and temporal resolution (Monitor Trinitron en Color Sony model CPG-G420). Spatial contrast sensitivity was measured using static vertical sinusoidal luminance gratings, of 6.5°× 5° of visual angle, and 43.5 cd/m^2 mean luminance. Eleven spatial frequencies were used ranging between 0.2–30 cpd. Contrast thresholds were estimated using a staircase (10 reversals) protocol which started from subthreshold to suprathreshold contrasts. Contrast sensitivity was expressed as the inverse of contrast threshold values. Twenty-five workers were tested in spatial contrast sensitivity and the control group was composed by 25 subjects.

Temporal contrast sensitivity was measured using a square field (2.5°× 2.5° of visual angle) that flickered at seven temporal frequencies ranging between 0.5–32 Hz. The background luminance was equal to the mean stimulus luminance (43.5 cd/m^2). A staircase procedure, analogous to that described for the spatial contrast sensitivity measurements, was used. Twenty-five workers were tested in temporal contrast sensitivity and the control group was composed by 25 subjects.

Visual perimetry assessment was performed using the Humphrey field analyzer (model 745, Humphrey System, CA). Central 10-2 (SITA-fast strategy, Central 30-2 (SITA-standard strategy) and Peripheral 60-4 (SITA-standard strategy) protocols were used. At each point in the visual field, thresholds were estimated using a staircase procedure, in which, correct responses were followed by a 4 dB luminance decrease, and mistakes by a 2 dB luminance increase. Results of visual perimetry were analyzed in eight eccentricity rings (0°–3.3°, 3.3°–6.6°, 6.6°–10°, 10°–20°, 20°–30°). Twenty-one workers were tested in visual perimetry and the control group was composed by 21 subjects.

Color discrimination was estimated by two different procedures: the Farnsworth-Munsell 100 hue (FM100) arrangement test and the Mollon-Reffin color test.

The FM 100 test consisted of 85 stimuli (each stimuli was a disk of 1° of visual angle, mean luminance of 41.75 cd/m^2) of different hues and same saturation (30%), distributed in a chromatic axis in Munsell color space. At the beginning of the test, the subject was shown the correct sequence of the stimuli, arranged in a gradually changing order in the hue dimension in the Munsell color space. The stimuli were then disarranged and the subject was instructed to order the stimuli in a hue sequence as shown at the beginning of the test. Errors in the positioning of the different color disks were measured as indicator of the test performance [42]. Twenty-six workers were tested in the FM 100 test and the control group was composed by 26 subjects.

Color discrimination ellipses were estimated using the Mollon-Reffin test for color discrimination evaluation [43]. The test had a pseudoisochromatic design, in which the target, a Landolt C, differed from the background only in chromaticity. Mean luminance of the target and background were the same. The target had 4.3° of outer diameter and 2.2° of inner diameter. The gap of the Landolt C was 1° of visual angle. The task of the subject was to identify the gap position. After each hit, the chromaticity of the target approached the chromaticity of the background. A staircase was used to estimate the minimum distance in chromaticity in the CIE1976 color space. Five background chromaticities were used (CIE1976 color space coordinates: E1. u′: 0.215, v′: 0.531; E2. u′: 0.219, v′: 0.481; E3. u′: 0.225, v′: 0.415; E4. u′: 0.175, v′: 0.485; E5. u′: 0.278, v′: 0.472), and each background chromaticity was discriminated from 8 chromaticity lines of different orientations. An ellipse fitted the threshold results. The area of a circle with equivalent area of the ellipses was chosen as indicator of color discrimination performance. Seventeen workers were tested in color discrimination ellipses and the control group was composed by 17 subjects.

2.2. Evaluation of the Exposure to Organic Solvents Mixture. Six out of 32 gas station workers reported use of individual safety instruments (masks and gloves). Mean duration of occupational exposure was 47.4 ± 61.7 months, with an exposure of 45.23 ± 4.4 hours/week.

2.3. Data Analysis. The normal range in each of the tests was defined by tolerance limits corresponding to 90% of

the population with a 95% confidence [44]. The confidence interval was used to compare the exposed group with the control group. The t-test was used to compare data with one variable between gas station workers group and control group. Two-way ANOVA was used to compare the exposed group with the control group on data with more than one variable. Linear correlation was used to estimate the dependence of the psychophysical performance upon exposure time.

3. Results

3.1. Spatial Luminance Contrast Sensitivity. Eight out of 25 gas station workers showed spatial luminance contrast sensitivity below the lower tolerance limit for at least one spatial frequency. Mean contrast sensitivity at 20 and 30 cpd of the gas station workers group was out of the interval of confidence of the mean of the control group (two-way ANOVA, $P < 0.01$; Figure 1). Correlations between the spatial luminance contrast sensitivity at different spatial frequencies and exposure time were very low (highest correlation (r^2) was lesser than 0.2).

3.2. Temporal Luminance Contrast Sensitivity. All gas station workers showed temporal luminance contrast sensitivity within the control group tolerance limits. Mean contrast sensitivity was inside of the interval of confidence of the control (two-way ANOVA, $P > 0.05$; Figure 2). Correlations between the temporal luminance contrast sensitivity at different spatial frequencies and exposure time were very low (highest r^2 lesser than 0.1).

3.3. Visual Perimetry. Six out of 21 gas station workers had detection threshold below of the control tolerance limits for at least one eccentricity ring (Figure 3). Mean detection threshold of the exposed group was below the lower limit of confidence of control group in the rings of eccentricity between 10°–60°. Two-way ANOVA showed statistical difference of the detection threshold between both groups ($P < 0.05$). There was low linear correlation between the detection thresholds and exposure time ($P < 0.45$). Mean deviation (MD) and pattern standard deviation (PSD) of one worker was out of the control tolerance limits for the eccentricities below 10°. MD of four subjects and PSD of six subjects were out of the control tolerance limits for eccentricities between 10° and 30°. Two-way ANOVA showed statistical differences of MD values between both groups ($P < 0.01$) for eccentricities between 10°–30°, but no differences for MD values at eccentricities below 10° or PSD values for any eccentricity. Low linear correlations were found between MD or PSD and exposure time ($r < 0.2$).

3.4. Farnsworth-Munsell Hue 100 Test. Fifteen out of 26 gas station workers had errors above the upper tolerance limit of the control group. Mean error value of the exposed group was higher than upper limit of confidence (t-test $P < 0.01$; Figure 4), low linear correlation between the exposure time and errors of FM100 test ($r < 0.2$).

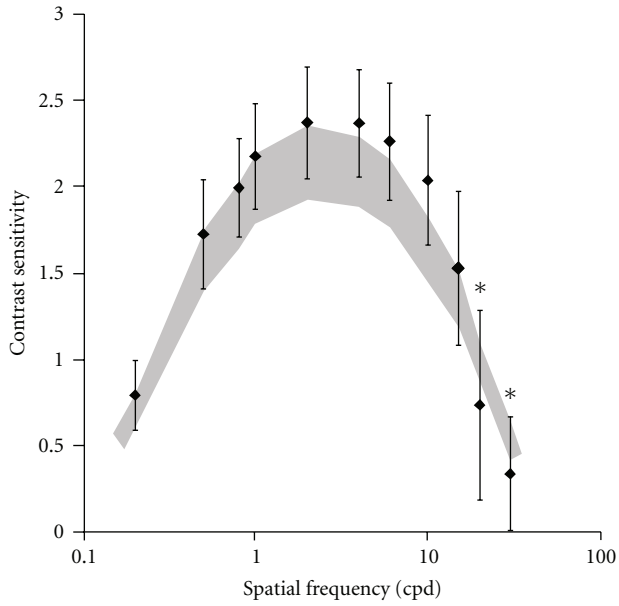

Figure 1: Mean spatial luminance contrast sensitivity at 11 spatial frequencies. Black diamonds represent the gas station workers contrast sensitivity and dark area represents the interval of confidence of control group.

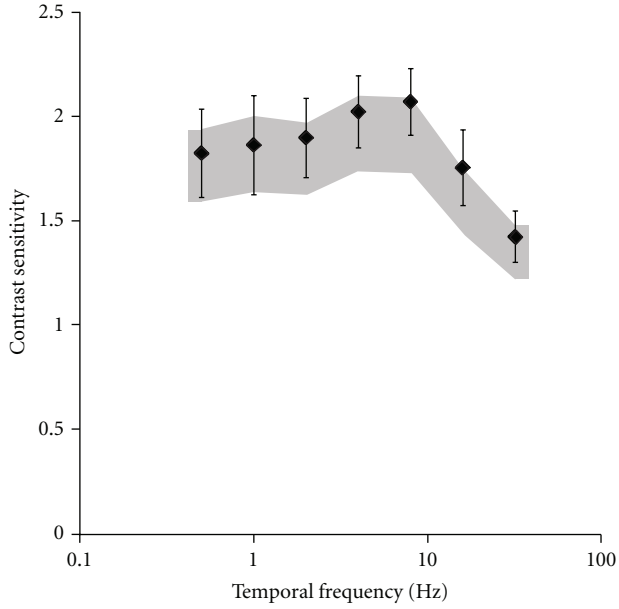

Figure 2: Mean temporal luminance contrast sensitivity at 7 temporal frequencies. Black diamonds represent the gas station workers contrast sensitivity and dark area represents the interval of confidence of control group.

3.5. Color Discrimination Ellipses. Six out of 17 workers showed increased equivalent circle diameter (D) to the area of the ellipse for at least one of five center references, when compared with the control tolerance limits. Mean D values of exposed group were higher than the upper limit of confidence of control group for all the color discrimination

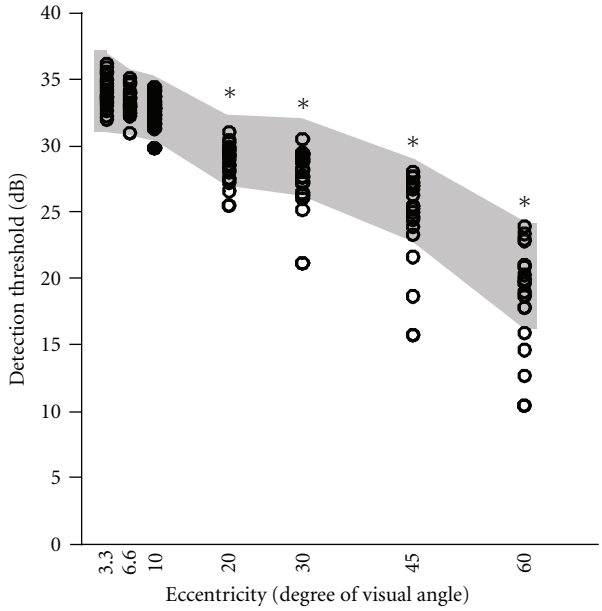

Figure 3: Detection threshold estimated by visual perimetry. Detection threshold as function of visual field eccentricity. Dark area represents the tolerance interval of detection thresholds of the control group.

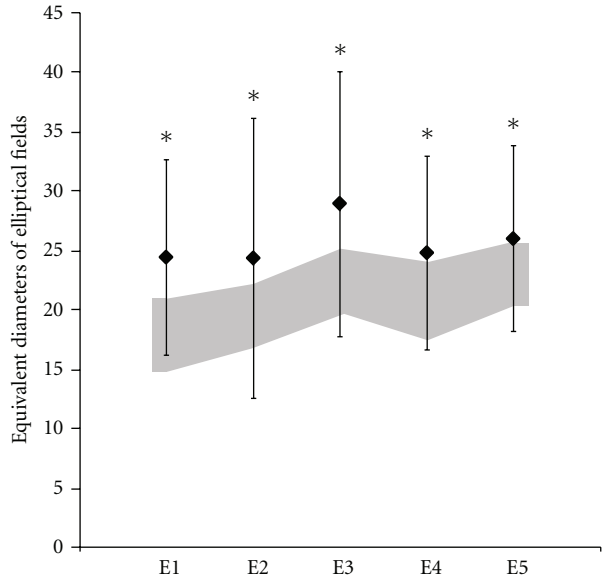

Figure 5: Equivalent circle diameter of color discrimination ellipses centered in five chromaticities in CIE1976. Black diamonds represent the equivalent circle diameter of elliptical area for different ellipses. Dark area represents the interval of confidence of the control group.

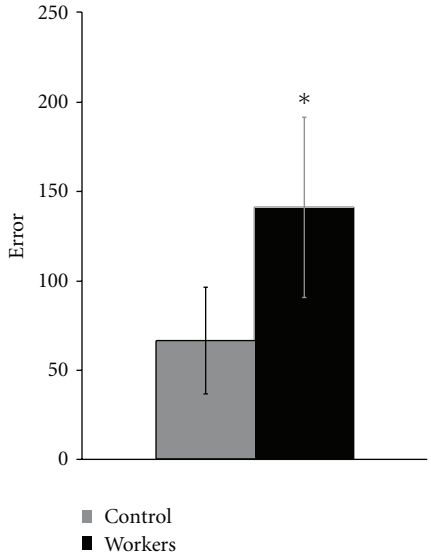

Figure 4: Mean FM100 errors of exposed (black bar) and control (gray bar) groups.

ellipses (Two-way ANOVA, $P < 0.05$; Figure 5). Low linear correlations were found between D from five ellipses and exposure time ($r < 0.46$).

4. Discussion

In the present study we assessed visual functions of gas station workers. In this profession, common in some countries, the job of the worker is to fill the automobile gas tanks. The worker is thus continuously exposed to a mixture of organic solvents throughout his work shift. We observed that twenty-five out of twenty-nine gas station workers had some kind of visual loss evaluated by psychophysical methods.

Many studies have demonstrated that workers exposed to organic solvent have visual impairments, mainly in color vision [3, 16–25, 45]. The mechanisms of neuronal dysfunction elicited by exposure to organic solvents are still unclear, but the affinity of organic solvent to lipid enriched tissues is well known. The nervous system is therefore a potential target of the solvent intoxication [46].

Most color vision studies have reported mainly blue-yellow color vision losses, and a secondary red-green color dyschromatopsia as shown in the present study [3, 16–25, 27–31]. Previous studies have investigated the color vision of solvent exposed workers using color arrangement test as FM100 test or Lanthony D15. As far we know, the present study is the first time that color discrimination ellipses test was applied in the solvent exposed subjects [16, 23–25, 27, 28, 30, 31, 38, 47–51]. As the tasks of color discrimination and FM100 test are quite different it is difficult to assert which test would be best to evaluate the color vision of the workers. This acquired dyschromatopsia might be the result of optics and neural causes [52, 53]. Aging can also lead to macular degeneration [52, 53]. The present study did not find worker diagnosed with any change in the ophthalmic clinical evaluation, suggesting that the color vision losses have neural predominant origin [31, 54].

Study in rats and nonhuman primates demonstrated an accumulation of metabolites from methanol in the vitreous and retina [55, 56], which could cause degeneration of

outer nuclear layer and ganglion cell layer suggested for histopathologic studies by Potts and colleges [57]. For Köllner [58] blue-yellow color vision loss reflects changes in outer retina whilst losses in the red-green axes reflect abnormalities in the inner retina or optic nerve. This became known as Köllner's rule. Muttray et al. [22] argued that Köllner rule [58] could be combined with more recent findings [59], considering that outer retinal damage in the central retina would lead the subject to fixate at more eccentric retinal points. We agreed with Muttray's argument, because the pathologic eccentric fixation could result in impairment of red-green discrimination.

Dyschromatopsia associated to organic solvents intoxication has been attributed to maculopathies caused by damage in cone photoreceptors, ganglion cells and optic nerve demielinization [20, 32, 60]. Blain and Mergler [61] suggested that the fact solvent intoxication led to blue-yellow color vision losses and later may develop to red-green color vision loss, reflects progressive degeneration from outer retina to optic nerve [61]. Cortical changes in the visual processing can occur after organic solvent intoxication [16, 20, 32, 60, 62–64]. We described diffuse color vision losses, with no preferences for blue-yellow or red-green chromatic axes. This kind of color vision loss is associated to high exposure to organic solvents [16, 54].

Eight out of 25 gas station workers had luminance spatial contrast sensitivity lower than the tolerance limits defined by the control group. There was statistical difference between the organic solvent exposed workers and the control group at 20 and 30 cpd, but there was no change in their Snellen visual acuity. Boeckelmann and Pfister [6] and Järvinen and Hyvärinen [35] suggested measuring contrast sensitivity at low and intermediate spatial frequencies which reflect changes in the neural processing whereas loss of contrast sensitivity at high spatial frequencies reflects impairment of the optics of the eye. In the present work, all subjects had normal or corrected visual acuity to 20/20. Other studies on intoxication by organic solvents intoxication showed spatial vision impairments without changes of visual acuity [6, 21, 25, 35–40]. We found no impairments in the temporal contrast sensitivities in the organic solvent exposed workers.

Our results of visual field losses are similar to the findings of Yamamura [33]. Six out of 21 gas station workers had impairment of contrast sensitivity in eccentricities above 10°. Even the workers who were in the normal range of contrast sensitivity in eccentricities that ranged between 10°–60°, there was significant decreasing between the values of the exposed group and control group. This impairment is detected by MD (low values) and PSD (high values) analysis, reflecting in altered visual field with constriction of the visual field towards the central field. Grant and Schuman [34] suggest that this type of visual loss indicates a beginning process of optical neuropathy after exposure to methanol, styrene, toluene, trichloroethylene, and organic solvents mixtures.

In the present study, the exposed subjects have worked at the gas station from one month to twenty-one years (47.4 ± 61.7 months) and the period of exposure varied from 36 to 48 hours a week (45.23 ± 4.4 hours a week). Three subjects reported that they used protective safety equipment, but they lack specific training for use of this kind of equipment. Some studies found weak correlation between psychophysical performance of exposed subjects and their exposure to organic solvent mixtures, styrene, perchloroethylene, or benzene [22, 24, 65]. Although we also expected to find some correlation between total time of exposure and/or amount of daily exposure and the performance of exposed subjects in the psychophysical tests that we used, that was not the case. We did not find any significant correlation between the exposed subject performances and the duration or amount of exposure to organic solvents.

Concentration of organic solvents or their metabolites in tissues are not directly related to time of exposure. There are genes that code enzymes that work in the metabolism of organic solvents in the organisms, and gene polymorphism modifies the absorption and the neurotoxicity effects of the organic solvents [25, 66]. We suggest that visual system damage probably occurred at early moment of solvent exposure, and the intersubject variability in the psychophysical tests could be explained by individual genetic predisposition.

The current study investigated psychophysically the achromatic and chromatic vision of gas station workers, and correlated the psychophysical results with time of exposure. These results have previously been published as abstracts [67].

Conflict of Interests

The authors declare no conflict of interests.

Ethical Approval

All procedures were evaluated by Ethical Committee in Research in Humans of the Tropical Medicine Nucleus of Federal University of Pará (protocol no. 075/2006-CEP/NMT).

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References

[1] H. Hänninen, J. Eskelinen, K. Husman, and M. Nurminen, "Behavioral effects of long term exposure to a mixture of organic solvents," *Scandinavian Journal of Work, Environment and Health*, vol. 2, no. 4, pp. 240–255, 1976.

[2] J. Olsen and S. Sabroe, "A case-reference study of neuropsychiatric disorders among workers exposed to solvents in the Danish wood and furniture industry," *Scandinavian Journal of Social Medicine*, vol. 8, no. 16, pp. 44–49, 1980.

[3] D. Mergler, R. Bowler, and J. Cone, "Colour vision loss among disabled workers with neuropsychological impairment," *Neurotoxicology and Teratology*, vol. 12, no. 6, pp. 669–672, 1990.

[4] E. Pfister, I. Böckelmann, S. Darius, and C. Wurthmann, "Diagnosis of early toxic effects of lead or mixed organic solvents - Inclusion of psychopathological methods," *Fortschritte der Neurologie Psychiatrie*, vol. 67, no. 10, pp. 435–440, 1999.

[5] A. Ahmadi, P. Jönsson, U. Flodin, and P. Söderkvist, "Interaction between smoking and glutathione S-transferase polymorphisms in solvent-induced chronic toxic encephalopathy," *Toxicology and Industrial Health*, vol. 18, no. 6, pp. 289–296, 2002.

[6] I. Boeckelmann and E. A. Pfister, "Influence of occupational exposure to organic solvent mixtures on contrast sensitivity in printers," *Journal of Occupational and Environmental Medicine*, vol. 45, no. 1, pp. 25–33, 2003.

[7] S. Semple, F. Dick, A. Osborne et al., "Impairment of colour vision in workers exposed to organic solvents," *Occupational and Environmental Medicine*, vol. 57, no. 9, pp. 582–587, 2000.

[8] L. Amaducci, C. Arfaioli, D. Inzitari, and M. Marchi, "Multiple sclerosis among shoe and leather workers: an epidemiological survey in Florence," *Acta Neurologica Scandinavica*, vol. 65, no. 2, pp. 94–103, 1982.

[9] A. M. Chancellor, J. M. Slattery, H. Fraser, and C. P. Warlow, "Risk factors for motor neuron disease: a case-control study based on patients from the Scottish motor neuron disease register," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 56, no. 11, pp. 1200–1206, 1993.

[10] A. Smargiassi, A. Mutti, A. De Rosa, G. De Palma, A. Negrotti, and S. Calzetti, "A case-control study of occupational and environmental risk factors for Parkinson's disease in the Emilia-Romagna region of Italy," *NeuroToxicology*, vol. 19, no. 4-5, pp. 709–712, 1998.

[11] J. Baelum, I. Andersen, and L. Molhave, "Acute and subacute symptoms among workers in the printing industry," *British Journal of Industrial Medicine*, vol. 39, no. 1, pp. 70–75, 1982.

[12] R. Chen, S. Semple, F. Dick, and A. Seaton, "Nasal, eye, and skin irritation in dockyard painters," *Occupational and Environmental Medicine*, vol. 58, no. 8, pp. 542–543, 2001.

[13] F. D. Dick, "Solvent neurotoxicity," *Journal of Occupational and Environmental Medicine*, vol. 63, pp. 221–226, 2007.

[14] I. Böckelmann, S. Darius, N. McGauran, B. P. Robra, B. Peter, and E. A. Pfister, "The psychological effects of exposure to mixed organic solvents on car painters," *Disability and Rehabilitation*, vol. 24, no. 9, pp. 455–461, 2002.

[15] C. Till, C. A. Westall, J. F. Rovet, and G. Koren, "Effects of maternal occupational exposure to organic solvents on offspring visual functioning: a prospective controlled study," *Teratology*, vol. 64, no. 3, pp. 134–141, 2001.

[16] D. Mergler, S. Belanger, S. De Grosbois, and N. Vachon, "Chromal focus of acquired chromatic discrimination loss and solvent exposure among printshop workers," *Toxicology*, vol. 49, no. 2-3, pp. 341–348, 1988.

[17] F. Gobba, C. Galassi, M. Imbriani, S. Ghittori, S. Candela, and A. Cavalleri, "Acquired dyschromatopsia among styrene-exposed workers," *Journal of Occupational Medicine*, vol. 33, no. 7, pp. 761–765, 1991.

[18] M. Zavalic, Z. Mandic, R. Turk et al., "Assessment of colour vision impairment in male workers exposed to toluene generally above occupational exposure limits," *Occupational Medicine*, vol. 48, no. 3, pp. 175–180, 1998.

[19] F. Dick, S. Semple, R. Chen, and A. Seaton, "Neurological deficits in solvent-exposed painters: a syndrome including impaired colour vision, cognitive defects, tremors and loss of vibration sensation," *QJM: Monthly Journal of the Association of Physicians*, vol. 93, no. 10, pp. 655–661, 2000.

[20] C. Raitta, H. Teir, M. Tolonen, M. Nurminen, E. Helpiö, and S. Malmström, "Impaired color discrimination among viscose rayon workers exposed to carbon disulfide," *Journal of Occupational Medicine*, vol. 23, no. 3, pp. 189–192, 1981.

[21] D. Campagna, D. Mergler, G. Huel et al., "Visual dysfunction among styrene-exposed workers," *Scandinavian Journal of Work, Environment and Health*, vol. 21, no. 5, pp. 382–390, 1995.

[22] A. Muttray, U. Wolff, D. Jung, and J. Konietzko, "Blue-yellow deficiency in workers exposed to low concentrations of organic solvents," *International Archives of Occupational and Environmental Health*, vol. 70, no. 6, pp. 407–412, 1997.

[23] D. Campagna, B. Stengel, D. Mergler et al., "Color vision and occupational toluene exposure," *Neurotoxicology and Teratology*, vol. 23, no. 5, pp. 473–480, 2001.

[24] H. Issever, G. Malat, H. H. Sabuncu, and N. Yuksel, "Impairment of colour vision in patients with n-hexane exposure-dependent toxic polyneuropathy," *Occupational Medicine*, vol. 52, no. 4, pp. 183–186, 2002.

[25] J. S. Schreiber, H. Kenneth Hudnell, A. M. Geller et al., "Apartment residents' and day care workers' exposures to tetrachloroethylene and deficits in visual contrast sensitivity," *Environmental Health Perspectives*, vol. 110, no. 7, pp. 655–664, 2002.

[26] F. Gobba, C. Galassi, M. Imbriani, S. Ghittori, S. Candela, and A. Cavalleri, "Acquired dyschromatopsia among styrene-exposed workers," *Journal of Occupational Medicine*, vol. 33, no. 7, pp. 761–765, 1991.

[27] C. Fallas, J. Fallas, P. Maslard, and S. Dally, "Subclinical impairment of colour vision among workers exposed to styrene," *British Journal of Industrial Medicine*, vol. 49, no. 10, pp. 679–682, 1992.

[28] T. Eguchi, R. Kishi, I. Harabuchi et al., "Impaired colour discrimination among workers exposed to styrene: relevance of a urinary metabolite," *Occupational and Environmental Medicine*, vol. 52, no. 8, pp. 534–538, 1995.

[29] M. Schäper, P. Demes, E. Kiesswetter, M. Zupanic, and A. Seeber, "Colour vision and occupational toluene exposure: results of repeated examinations," *Toxicology Letters*, vol. 151, no. 1, pp. 193–202, 2004.

[30] E. H. Lee, K. D. Eum, S. I. Cho, H. K. Cheong, and D. M. Paek, "Acquired dyschromatopsia among petrochemical industry workers exposed to benzene," *NeuroToxicology*, vol. 28, no. 2, pp. 356–363, 2007.

[31] J. Päällysaho, R. Näsänen, M. Mäntyjärvi, A. Kaukiainen, and M. Sainio, "Colour vision defects in occupational chronic solvent encephalopathy," *Human and Experimental Toxicology*, vol. 26, no. 4, pp. 375–383, 2007.

[32] C. Raitta, A. M. Seppalainen, and M. S. Huuskonen, "N-hexane maculopathy in industrial workers," *Graefe's Archives of Clinical and Experimental Ophthalmology*, vol. 209, no. 2, pp. 99–110, 1978.

[33] Y. Yamamura, "N-hexane polyneuropathy," *Folia Psychiatrica et Neurologica Japonica*, vol. 23, no. 1, pp. 45–57, 1969.

[34] W. M. Grant and J. S. Schuman, *Toxicology of the Eye*, edited by C. Charles, Thomas Publisher, Springfield, Ill, USA, 1993.

[35] P. Järvinen and L. Hyvärinen, "Contrast sensitivity measurement in evaluations of visual symptoms caused by exposure to triethylamine," *Occupational and Environmental Medicine*, vol. 54, no. 7, pp. 483–486, 1997.

[36] B. Frenette, D. Mergler, and R. Bowler, "Contrast-sensitivity loss in a group of former microelectronics workers with normal visual acuity," *Optometry and Vision Science*, vol. 68, no. 7, pp. 556–560, 1991.

[37] D. K. Broadwell, D. J. Darcey, H. K. Hudnell, D. A. Otto, and W. K. Boyes, "Work-site clinical and neurobehavioral assessment of solvent-exposed microelectronics workers," *American Journal of Industrial Medicine*, vol. 27, no. 5, pp. 677–698, 1995.

[38] Y. Gong, R. Kishi, S. Kasai et al., "Visual dysfunction in workers exposed to a mixture of organic solvents," *NeuroToxicology*, vol. 24, no. 4-5, pp. 703–710, 2003.

[39] D. Mergler, G. Huel, R. Bowler, B. Frenette, and J. Cone, "Visual dysfunction among former microelectronics assembly workers," *Archives of Environmental Health*, vol. 46, no. 6, pp. 326–334, 1991.

[40] A. Iregren, M. Andersson, and P. Nylén, "Color vision and occupational chemical exposures: I. An overview of tests and effects," *NeuroToxicology*, vol. 23, no. 6, pp. 719–733, 2002.

[41] S. Mikkelsen, "A cohort study of disability pension and death among painters with special regard to disabling presenile dementia as an occupational disease," *Scandinavian Journal of Social Medicine*, vol. 8, no. 16, pp. 34–43, 1980.

[42] D. Farnsworth, *The Farnsworth-Munsell 100-Hue Test for the Examination of Color Discrimination (Manual)*, Munsell Color, MacBeth, A Division of Kollmorgen Corporation, Baltimore, Md, USA, 1957.

[43] B. C. Regan, J. P. Reffin, and J. D. Mollon, "Luminance noise and the rapid determination of discrimination ellipses in colour deficiency," *Vision Research*, vol. 34, no. 10, pp. 1279–1299, 1994.

[44] W. J. Dixon and F. J. Massey, *Introduction to Statistical Analysis*, McGraw-Hill, New York, NY, USA, 1969.

[45] J. A. Rosen, "Pseudoisochromatic visual testing in the diagnosis of disseminated sclerosis," *Transactions of the American Neurological Association*, vol. 90, pp. 283–284, 1965.

[46] G. N. Rosenberg, "Nervous system effects of toluene and other organic solvents," *Western Journal of Medicine*, vol. 5, pp. 571–572, 1989.

[47] D. Mergler and L. Blain, "Assessing color vision loss among solvent-exposed workers," *American Journal of Industrial Medicine*, vol. 12, no. 2, pp. 195–203, 1987.

[48] S. Semple, F. Dick, A. Osborne et al., "Impairment of colour vision in workers exposed to organic solvents," *Occupational and Environmental Medicine*, vol. 57, no. 9, pp. 582–587, 2000.

[49] F. Dick, S. Semple, A. Osborne et al., "Organic solvent exposure, genes, and risk of neuropsychological impairment," *QJM: Monthly Journal of the Association of Physicians*, vol. 95, no. 6, pp. 379–387, 2002.

[50] A. Ihrig, M. Nasterlack, M. C. Dietz, J. Hoffmann, and G. Triebig, "Pilot study on prevalence of color vision dysfunction in long-term solvent-exposed painters," *Industrial Health*, vol. 41, no. 1, pp. 39–42, 2003.

[51] F. Dick, S. Semple, A. Soutar, A. Osborne, J. W. Cherrie, and A. Seaton, "Is colour vision impairment associated with cognitive impairment in solvent exposed workers?" *Occupational and Environmental Medicine*, vol. 61, no. 1, pp. 76–78, 2004.

[52] J. Pokorny, V. C. Smith, G. Verriest, and A. J. L. G. Pinkers, *Congenital and Acquired Colour Defects*, Grune and Stratton, New York, NY, USA, 1979.

[53] K. J. Bowman, "A method for quantitative scoring of the Farnsworth Panel D-15," *Acta Ophthalmologica*, vol. 60, no. 6, pp. 907–916, 1982.

[54] D. Mergler, L. Blain, and J. P. Lagace, "Solvent related colour vision impairment: an indicator of neural changes?" *International and Archives of Occupational and Environmental Health*, vol. 59, p. 313, 1987.

[55] M. S. Hayreh, S. S. Hayreh, G. L. Baumbach, P. Cancilla, G. Martin-Amat, and T. R. Tephly, "Ocular toxicity of methanol: an experimental study," in *Neurotoxicity of the Visual System*, W. Merigan and B. Weiss, Eds., pp. 35——53, Raven Press, New York, NY, USA, 1980.

[56] J. T. Eells, "Methanol-induced visual toxicity in the rat," *Journal of Pharmacology and Experimental Therapeutics*, vol. 257, no. 1, pp. 56–63, 1991.

[57] A. M. Potts, J. Praglin, I. Farkas, L. Orbison, and D. Chickering, "Studies on the visual toxicity of methanol. VIII. Additional observations on methanol poisoning in the primate test object," *American Journal of Ophthalmology*, vol. 40, no. 5, pp. 76–83, 1955.

[58] H. Köllner, *Die Störungen des Farbensinnes, ihre klinische Bedeutung und ihre Diagnose*, Karger, Berlin, Germany, 1912.

[59] A. Pinckers and M. Marre, "Basic phenomena in acquired colour vision deficiency," *Documenta Ophthalmologica*, vol. 55, no. 3, pp. 251–271, 1983.

[60] H. H. Schaumburg and P. S. Spencer, "Environmental hydrocarbons produce degeneration in cat hypo-thalamus and optic tract," *Science*, vol. 199, pp. 199–200, 1978.

[61] L. Blain and D. Mergler, "Dyschromatopsia in workers exposed to organic solvents," *Journal Francais d'Ophtalmologie*, vol. 9, no. 2, pp. 127–133, 1986.

[62] W. H. Merigan, "Chromatic and achromatic vision of macaques: role of the P pathway," *Journal of Neuroscience*, vol. 9, no. 3, pp. 776–783, 1989.

[63] P. H. Schiller, N. K. Logothetis, and E. R. Charles, "Role of the color-opponent and broad-band channels in vision," *Visual Neuroscience*, vol. 5, no. 4, pp. 321–346, 1990.

[64] W. H. Merigan, L. M. Katz, and J. H. R. Maunsell, "The effects of parvocellular lateral geniculate lesions on the acuity and contrast sensitivity of macaque monkeys," *Journal of Neuroscience*, vol. 11, no. 4, pp. 994–1001, 1991.

[65] F. Gobba, "Color vision: a sensitive indicator of exposure to neurotoxins," *NeuroToxicology*, vol. 21, no. 5, pp. 857–862, 2000.

[66] P. Söderkvist, A. Ahmadi, A. Åkerbäck, O. Axelson, and U. Flodin, "Glutathione S-transferase M1 null genotype as a risk modifier for solvent-induced chronic toxic encephalopathy," *Scandinavian Journal of Work, Environment and Health*, vol. 22, no. 5, pp. 360–363, 1996.

[67] E. M. C. B. Lacerda, M. G. Lima, G. S. Souza et al., "Chromatic and achromatic psychophysical evaluations from chronically exposed workers to mixture of organic solvents," in *Proceedings of the Annual Meeting of ARVO*, p. 368, Winter Park, Fla, USA, 2010.

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Research Article

A Water-Damaged Home and Health of Occupants: A Case Study

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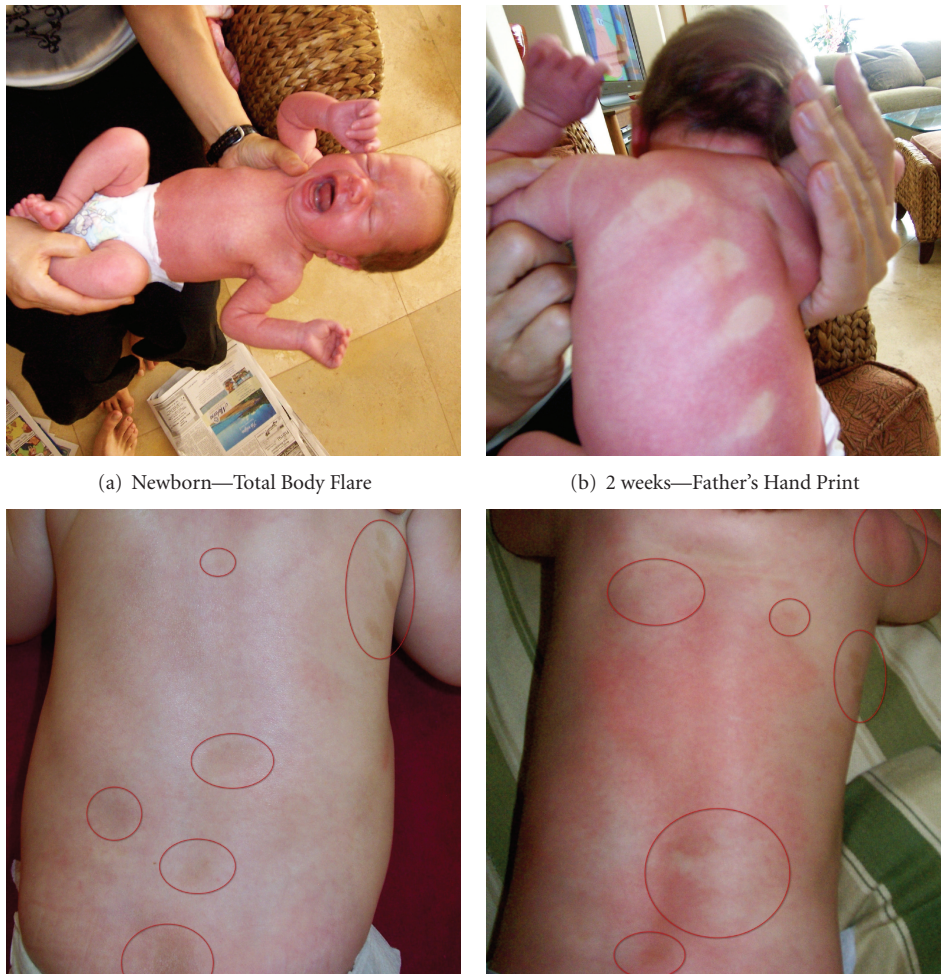
A family of five and pet dog who rented a water-damaged home and developed multiple health problems. The home was analyzed for species of mold and bacteria. The diagnostics included MRI for chronic sinusitis with ENT and sinus surgery, and neurological testing for neurocognitive deficits. Bulk samples from the home, tissue from the sinuses, urine, nasal secretions, placenta, umbilical cord, and breast milk were tested for the presence of trichothecenes, aflatoxins, and Ochratoxin A. The family had the following diagnosed conditions: chronic sinusitis, neurological deficits, coughing with wheeze, nose bleeds, and fatigue among other symptoms. An infant was born with a total body flare, developed multiple Cafe-au-Lait pigmented skin spots and diagnoses with NF1 at age 2. The mycotoxins were detected in bulk samples, urine and nasal secretions, breast milk, placenta, and umbilical cord. *Pseudomonas aueroginosa, Acinetobacter, Penicillium,* and *Aspergillus fumigatus* were cultured from nasal secretions (father and daughter). RT-PCR revealed *A. fumigatus* DNA in sinus tissues of the daughter. The dog had 72 skin lesions (sebaceous glands and lipomas) from which trichothecenes and ochratoxin A. were detected. The health of the family is discussed in relation to the most recent published literature regarding microbial contamination and toxic by-products present in water-damaged buildings.

1. Introduction

Indoor dampness and fungal contamination have been shown in qualitative reviews to be associated with a variety of respiratory health effects, including infections, sinusitis, and otitis media [1–4]. In addition, case studies with and without controls have demonstrated the existence of severe sinusitis as well as neurological deficits in occupants in water-damaged homes and buildings [5–12]. Currently, it is recognized that the indoor water-damaged environment resulting from microbial growth is a complex mixture of mold and bacteria along with their by-products [13–15]. Thus, the illnesses resulting from exposure cannot be defined by any specific component of the affected environment [2, 13–17]. In this paper we present a family of five exposed to fungi and bacteria in a water-damaged home located in Maui, Hawaii. Members of the family developed multiple health problems, including sinusitis and neurological deficits. In addition, the mother was pregnant during occupation of the contaminated home giving birth to a girl who had a total body flare with development of Cafe-au-Lait spots. Her condition has been diagnosed with Neurofibromastosis type (NF1).

2. The Family

The family of five moved from Canada to Maui, Hawaii, in February 2008, where they rented a home. All were healthy prior to the move and began experiencing symptoms shortly after the move in. Chief health complaints were as follows. Father (age 40) had persistent cough with phlegm, throat irritation, headaches, sinusitis, severe fatigue, somnolence, decreased concentration, long-term and recent memory loss, nose bleeds, decreased libido, hair loss, and shortness of



(a) Newborn—Total Body Flare

(b) 2 weeks—Father's Hand Print

(c) 23 Weeks—Café-au-Lait Pigment Spots

(d) 28 Weeks—Pigment Spots on the Back

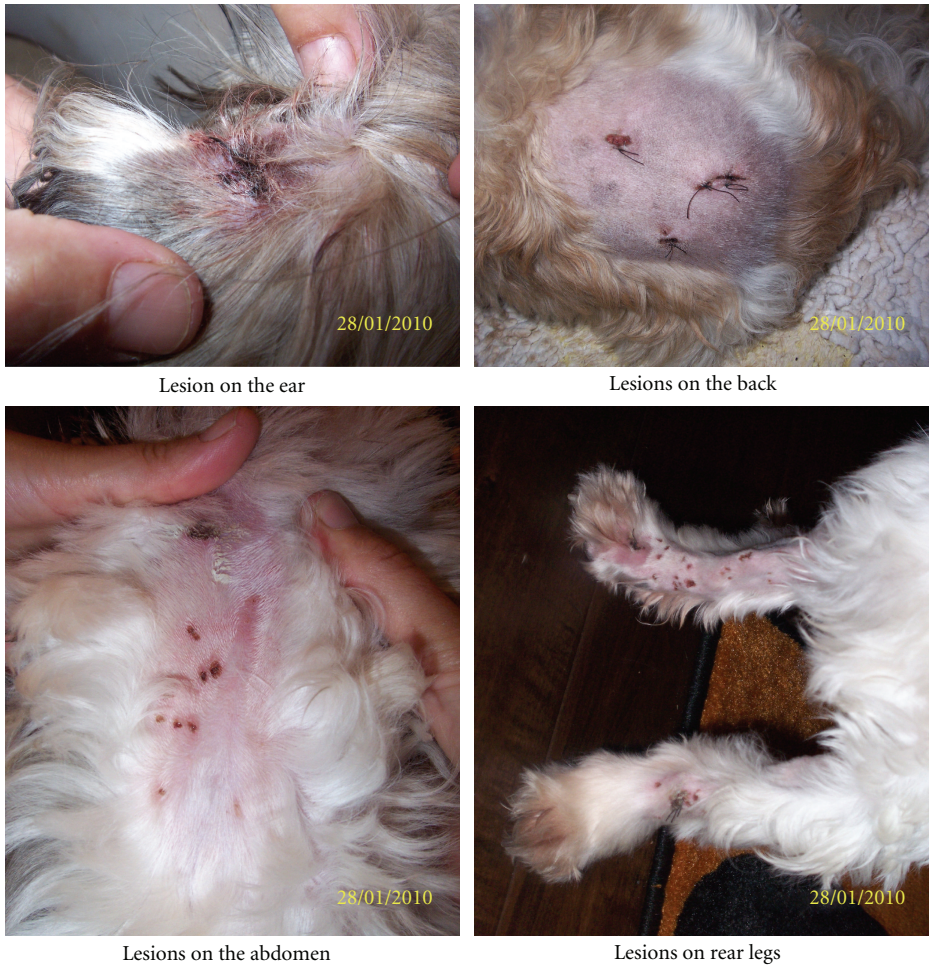
Figure 1: The upper two photos are of the newborn girl demonstrating the total body flare and the impression of the Father's hand on her back. The bottom two photos show the pigmented sports that appear to be Cafe-au-Lait skin pigmentation that were apparent at birth and are still present. The flare reaction was present at birth, began to subside at 10–12 weeks, and occurred periodically through 55 weeks of age. The multiple pigmented spots has been diagnosed as NF1 at U.S. San Francisco, Department of Dermatology.

breath with wheezing. The mother (age 39) complained of cough with phlegm, throat irritation, headaches, sinusitis, extreme fatigue, somnolence, recent and long-term memory loss, decreased libido, and shortness of breath with wheezing. She became pregnant while living in the home and gave birth to a girl 3 months after moving out of the home. The eldest daughter (age 8) had the same symptoms as the parents, except she had decreased concentration, nausea, and loss of appetite. The son (age 5) had frequent headaches, fatigue and tiredness, nasal congestion, nose bleeds, throat irritation, shortness of breath with mild wheezing, and decreased attention in classroom activities. The newborn had a total body flare (pinkish red) that continued to age 10–12 weeks, after which the flare would appear periodically. She had multiple pigmented skin spots on her back, chest, and abdomen at birth that appeared to be Cafe-au-Lait spots. The pigmented areas are still present at 2 years of that are scheduled for additional diagnostics for neurofibromatosis (Figure 1). Finally, the pet dog developed approximately 72 skin lesions diagnosed as sebaceous and lipoma tumors (Figure 2).

3. Neurological Evaluation

The family sought neurological consultation from one of the authors as previously published [8, 9]. The results of the evaluations are briefly summarized as follows.

The father had 17 neurological deficits as follows: simple and choice reaction time, sway-balance with eyes open and closed, decreased right and left grip strength, abnormal right and left color vision, abnormal visual field performance (right and left), abnormal digit symbol, abnormal perceptual motor speed (dominant pegboard, Trails A and B, right and left finger writing errors), abnormal smell score, abnormal picture completion and elevated Profile of Mood States (POMS), Beck's depression inventory, and Limbic System Check List score. The increased POMS score was consistent



Lesion on the ear

Lesions on the back

Lesions on the abdomen

Lesions on rear legs

FIGURE 2: This figure demonstrates the sites of the subcutaneous and lipoma tumors that were removed from the pet dog. The Veterinarian stated that the presence of 72 such lesions on an animal is a very rare observation.

with elevated confusion, fatigue, and tension. The mother also had 17 abnormalities, identical to those of the husband (data not repeated). The neurological scores for the daughter were within normal ranges. However, the physical exam revealed abnormal past pointing without dysmetria (finger to nose) and fine resting tremors at 3-4 per second increasing to 10 by intention with amplitude increased. The son (age 5) did not have any detectable neurological deficits. However, the neurological testing is not designed for 5 year olds.

In conclusion, the neurological evaluation revealed multiple deficits in both parents as previously published [8, 9]. The daughter had noticeable tremors which may have resulted from exposure to tremorgenic mycotoxins [18–22] as well as others described here in after (see Section 9 and Tables 4 and 5).

4. MRI

MRIs were performed at Oak Tree Medical Imaging, Pasadena, California, for each family member with special reference to the sinuses.

Father. The father had mild diffuse thickening- bi-ethmoid, bi-maxillary, right sphenoid and frontal sinuses.

Mother. The cavernous and paranasal sinuses were normal. Prior to the MRI, she had been prescribed corticosteroids, antibiotics, and antifungals.

Daughter. The daughter had mild fluid within the bilateral mastoid air cells. There is moderate to severe mucosal thickening in the maxillary and ethmoid sinuses without evidence of air fluid level.

Son. The bifrontal and sphenoid sinuses have not developed. Maxillary sinuses are unremarkable. There is slight mucosal thickening within the bilateral sphenoid sinuses, right greater than left without air fluid level.

In conclusion, the results of the MRI studies demonstrated mucosal thickening of the sinuses of the father and two children. The absence of findings in the mother most likely resulted from the use of corticosteroids and medications to treat her sinusitis.

5. ENT Evaluation

The father and daughter were evaluated at the Atlanta Center for ENT & Facial Plastic surgery according to procedures previously published [5, 6]. The results of the evaluation are briefly summarized as follows.

Father. Nasal endoscopy revealed (a) nasal polyps and (b) the ethmoid, sphenoid, and frontal sinuses were edematous with visible thick mucoid material (mucin) bilaterally, confirming the results of earlier MRI and CT scans (data not described). Total IgE was 76.9 IU/mL with a positive IgE score at level IV for *Alternaria*. He was tested for IgG antibodies for ten fungi and was positive for *Epiccocum* and *Cladosporium* at level I, *Penicillium, Aspergillus, Alternaria, Fusarium,* and *Acremonium* at level III, and *Candida* at level III. Recommended treatment was saline nasal wash, intranasal amphotericin B, oral fluconazole, Nystatin, intranasal glutathione, and oxygen via a face mask. Surgery was performed to remove nasal polyps and inflamed sinus tissues. Tissue samples were sent to RealTime Laboratories, Carrollton, Texas, for RT-PCR DNA probes (10 species of fungi), and mycotoxin testing.

The RT-PCR-DNA probes were negative for the following fungi: *Aspergillus flavus, fumigatus, niger,* and *versicolor; Eurotium amstelodami; Fusarium solani; Penicillium chrysogenum* and *verrucosum;* and *Stachybotrys chartarum* and *echinata*. Cultures for bacteria (SBA) and fungi (MEA) in nasal secretions were positive for *Pseudomonas aeuroginos*a and *Penicillium spp*.

Daughter. Endoscopic examination revealed that left maxillary, ethmoid, sphenoid, and frontal recesses were edematous. The turbinates were 4+ enlarged. The nasal septum was deviated to the left. On the right side there was some white material on the middle turbinate. The adenoids were hypertrophied. In addition, small white flecks were present in the soft tissue of the left maxillary, ethmoid, and left sphenoid sinuses. Medications include fluconazole, liposomal glutathione, amphotericin B, inhaled corticosteroid, Nystatin, and oxygen via face mask. The patient required left sphenoidotomy. Also, the previous MRI and CT scans showed opacification of the left infundibulum and left maxillary sinus os. Surgical specimens were sent to RealTime Laboratories tor RT-PCR DNA probe (10 species of fungi) and mycotoxin detection.

The RT-PCR tests were negative for the same species as done on the father (see above). However, cultures for bacteria (SBA) and molds (MEA) on nasal secretions revealed *Acinetobacter spp*. and *Aspergillus fumigatus*.

In conclusion, the nasal endoscopic examinations of the father and daughter revealed edematous inflammation of the paranasal sinuses that required surgery. The RT-PCR tests were negative for 10 species of fungi, which did not eliminate the presence of fungi other than those tested. Finally, bacterial and fungal cultures of nasal mucous secretions did reveal the presence of bacteria (*Pseudomonas* and *Acinetobacter*) as well as fungi (*Penicillium* and *Aspergillus*). Thus both patients had severe chronic rhinosinusitis most likely related to microbes (bacteria and fungi) detected in their water-damaged home [1–3, 5, 6, 23–25].

6. The Home

The home was inspected for construction defects and dampness by two independent services: Barkman Inspection Services [26] and Engineering Dynamics Corp [27]. The results of the two inspections are briefly summarized.

6.1. Barkman Report. A serious moisture/mold problem is observed in the crawlspace directly below the bedrooms. Moisture is penetrating the walls of the foundation. The HVAC system is designed to force air into the crawl space, forcing crawl space air into the bedrooms and other areas above. Moisture intrusion also results from the master shower into the crawl space as well as from sprinklers, damp soil against the foundation, lack of roof gutters, and poor grading.

6.2. Engineering Dynamics Report. This is a two-story house with a crawl space. Lower level has a family room, guest bedroom, bathroom, powder room, arts and crafts room, storage closet, garage, and crawl space, which are under upper level bedrooms and bathrooms. Upper level has 3 bedrooms, 3 bathrooms, entertainment room, living room, kitchen, office, and powder room.

The crawl space had water intrusion, musty mold odor, and visible mold on floor joists. The yard sprinklers were directed towards the house and the eaves did not have rain gutters, permitting the pooling of water. Water entered the crawl space through cement walls and followed piping present in the crawl space. Smoke testing revealed communication between the crawl space and upper level bedrooms via electrical outlets and electrical ducts and plumbing. The conduit holes were not sealed, permitting observance of light coming through spaces in the floor joists. A musty odor was present in the master bathroom and noted to get stronger when the fan coil was turned on.

7. Identification of Mold

All air and bulk samples were sent under chain of custody to EMSL Analytical, Inc., Westmont, NJ. The ERMI Q-PCR 36 for mold species was performed on 5 different bulk samples. The data are summarized in Table 1. The identified species of mold varied according to source but included species of *Aspergillus, Penicillium, Eurotium amstelodami, A pullulans, C. globosum*, and *T. viride*, among others. The ERMI interpretation level ranged from 2 to 3, indicating moderate contamination.

Airborne viable spores were determined by Air-O-Cell cassettes and cultured and identified by EMSL Method M050 and the data are summarized in Table 2. The viable airborne spores (Table 2) showed the presence of toxic fungi inside of the home and none outdoors. The viable spores included species of *Aspergillus* and *Penicillium*, which varied according

Table 1: This table summarizes the results of the E.P.A. ERMI PCR-DNA tests performed on 5 mg dust samples from basement and master bedroom carpeting and master bedroom wall insulation. Only the species detected are listed.

Sample 36 ERMI Q-PCR test	Carpet basement	Carpet, master Bdrm	Insulation master Bdrm[1]	Insulation return air duct	Moist fiberglass
Group 1 Molds					
Asp. penicillioides	77	26	ND	ND	
A. restrictus	ND	ND	ND	40	40
A. versicolor	ND	ND	ND	ND	50
*E. amstelod*ami	ND	ND	ND	4	4
Aur. pullulans	189	20	ND	ND	ND
Ch. globosum	ND	14	ND	ND	2
Cl. Sphaerospermum	9	3	ND	ND	ND
Pae. variotii	ND	2	87	ND	734
P. brevicompactum	ND	19	ND	ND	ND
P. corylophilum	ND	ND	ND	ND	85
P. crustosum	ND	ND	3	ND	ND
P. purpurogenum	ND	2	ND	ND	ND
P. spinulosum	15	ND	3	ND	ND
P. variabile	ND	ND	ND	136	3
T. viride	ND	ND	NS	ND	15
Sum of the Logs	6.6	6.2	2.8	2.8	10.6
Group 2 Molds					
A. ustus	2	4	187	ND	226
Cl. cladosporioides II	1	ND	ND	65	2
Ep. nigrum	15	17	ND	65	8
Ep. nigrum	15	17	ND	14	5
Mucor/Rhizopus	9	21	ND	ND	ND
P. chrysogenum	5	4	8.738	ND	14.013
Sum of the logs	3.3	3.7	6.2	3.0	8.1
ERMI Value	3	2	−3	0	3
ERMI Interpretation	Level 3	Level 3	Level 2	Level 2	Level 3

ND: Not detected.
[1]RT-PCR detected *Aspergillus fumigatus* in a towel taken from the master bathroom.
All values are in Spores E.−/mg dust.

to the sample area, for example, crawl space versus bedroom air and wall space cavity.

In conclusion, these data demonstrated that testing for fungal contamination must include several different sample locations involving dust and bulk materials as well as airborne viable spores [28].

8. Identification of Bacteria and Endotoxins

Bulk samples of crawl space dirt, gravel, plastic sheeting, wood, and a sandal from under the master bed were sent to EMSL Analytical, Inc., Westmont, NJ and RealTime Laboratories, Carrolton, TX, to culture and identify bacteria using sheep blood agar (SBA) plates. In addition, two swab samples from the kitchen were analyzed for endotoxins by EMSL. The results are summarized in Table 3.

Bacteria detected by both laboratories included Gram negative and positive organisms. The primary Gram positive bacteria included *Bacillus spp*, *Actinomycetes* (e.g., *Streptomyces* sp., *Mycobacterium hominis*), and *Staphylococcus* (non aureus). The Gram negative bacteria were species of *Pseudomonas* and *Proteus spp.* Both groups of bacteria are potential human pathogens. For example, *Mycobacterium* and *Streptomyces spp.* are capable of causing lung abscesses and granulomatous mycetomas, while *Pseudomonas* species can cause respiratory and other infections [29–31].

Endotoxins were tested in only two areas of the home. The J-tube under the kitchen sink, a relatively protected area, had a concentration of 4.930 EU per swab. In contrast, the top of the kitchen cabinet had a concentration of 24.800 EU/swab. The two control swabs were negative. These observations indicate that additional testing was probably warranted, since endotoxins cause respiratory inflammation, sensitizers, and exacerbation of asthma [32–35]. In conclusion, bacterial cultures identified potentially pathogenic Gram negative and positive bacteria. In addition, these bacteria are known to produce toxic secondary metabolites

Table 2: This table summarizes the identification and enumeration of culturable air-borne fungi collected by Aerotech cassettes (including speciation of *Penicillium*, *Aspergillus, Cladosporium, and Stachybotrys*) by EMSL Method M050.

Sample location	Media	Temp (°C)	Sensitivity & dilution	Fungal identification	Colon count	CFU per cassette
Swimming pool deck	MEA	25	100 & 100	None detected	0	0
Master bedroom	MEA	25	100 & 100	*Asp. sydowii*	1	100
			100 & 100	*Cl. sphaerospermum*	1	100
				P. chrysogenum	1	100
				Total	**3**	**300**
Crawl space	MEA	25	100 & 100	*Asp. ochraceus*	5	500
			100 & 100	*Asp. sydowii*	2	200
			100 & 100	*P. chrysogenum*	1	100
			1000 & 1000	*P. citreonigrum*	1	1000
			1000 & 1000	*Phialophora sp.*	1	1000
			1000 & 1000	Sterile (dark) sp.	1	1000
				Total	**11**	**3.800**
Wall space master bedroom	MEA	25	100 & 100	*Asp. fumigatus*	1	100
			100 & 100	*Asp. ustus*	3	300
			100 & 100	*Paecilomyces sp.*	2	200
			1000 & 1000	*P. chrysogenum*	1	1000
				Total	**7**	**1.600**

of which Valinomycin is a mitochondrial toxin and is synergistic with macrocyclic trichothecenes [36–39]. Recently, several toxic bacterial metabolites have been demonstrated to cooccur with mycotoxins in moisture-damaged indoor environments [15].

9. Identification of Mycotoxins in Environmental Samples and Body Fluids

Bulk samples were sent to RealTime Laboratories, Carrollton, TX, to test for the presence of mycotoxins. In addition, urine and nasal mucous were collected in sterile cups, sealed and sent to RealTime Laboratories to test for the presence of mycotoxins. The tests for macrocyclic trichothecenes, aflatoxins, and ochratoxin A were performed as previously reported [40].

9.1. Environmental Samples. The data for mycotoxins detected in bulk samples are summarized in Table 4. Trichothecenes and ochratoxin A were detected in the bathroom towel (11.71 and 4.9 ppb), respectively, and the sandal (0.47 and 3.4 ppb), respectively. Mycotoxins were identified in the samples from the crawl space as follows: Wood truss: trichothecenes (1.69 ppb), aflatoxins (3.5 ppb), ochratoxin A (5.8 ppb); Gravel: trichothecenes (7.7 ppb), ochratoxin A (7.7 ppb); Dirt: trichothecenes (2.1 ppb), ochratoxin A (2.1 ppb); and Plastic sheeting: ochratoxin A (2.8 ppb).

9.2. Body Fluids. Mycotoxins detected in body fluids of family members and the pet dog are summarized in Table 5. The father was positive for ochratoxin A in his urine (18.2 ppb), while two separate nasal mucous samples were positive for both aflatoxins (0.5 and 11.2 ppb) and ochratoxin A (18.2 ppb). The mother's urine contained ochratoxin A (18.2 ppb), while nasal mucous contained the three mycotoxins aflatoxin, ochratoxin A, and trichothecenes at 1.02, 1.2, and 1.5 ppb, respectively. The daughter's urine had trichothecenes (0.23 ppb) and ochratoxin (28 ppb), while nasal mucosa had trichothcenes (4.68 ppb) and ochratoxin A (3.8 ppb). The urine sample from the son was positive for ochratoxin A (18.9 ppb), while tests on nasal mucous were not performed. The urine from the pet dog was positive for trichothecenes (1.49 ppb) and ochratoxin A (25.9 ppb).

10. Newborn Baby

The mother gave birth to a girl who was born with a total body flare 3 months after vacating the home (Figure 1). The infant was born with pigmented skin identified as Cafe-au-lait. They are currently distributed as follows: Face (2), neck (6), right axilla (9), left axilla (10), left and right arms (4), abdomen (16), back (28), buttocks (9), right leg (8), and left leg (2) for a total of 84. As a result, breast milk, placenta, umbilical cord, and the baby's urine were tested for the presence of mycotoxins. Ochratoxin A was detected in the breast milk (2.7 ppb), placenta (4.2 ppb), and the umbilical cord (7 ppb). The newborn's urine was negative for mycotoxins. In retrospect, the amniotic fluid (lost during birth) should have been tested.

11. Pet Dog

The pet dog had approximately 72 skin lesions on its legs, trunk, and ears (Figure 2). The lesions were surgically removed. Pathology of the ear mass described it as a sebaceous gland, while the other lesions were lipomas. Tests

Table 3: This table summarizes the bacteria and endotoxins identified in various bulk samples taken from the home (EMSL Method M009) and by RealTime Laboratories (RTL), Dallas, TX.

(a)

Sample	Sample #	Media	Temp (°C)	Analytical sensitivity CFU/g	Bacteria	Colony count	CFU/g
Plastic sheeting, crawl space	#34	SBA	35	98.000	*Bacillus sp* *Streptomyces sp.* *Actinomycetes*	25	2.450.000
Moist gravel, crawl space	#27	SBA[1]	35	885	*B. megaterium* *Bacillus sp.* **Total**	10 7 **17**	8.850 6.190 **15.000**
Moist dirt, crawl space	#28	SBA[1]	35	8130	*B. megaterium* *Bacillus sp.* **Total**	4 6 **10**	32.500 48.800 **81.300**
Swab of wood, crawl space	#25	SBA[2]	35	10.000	*Microbacterium hominis* *Staphylococcus sp (not aureus)* **Total**	972 2 **974**	9.720.000 20.000 **9.740.000**
Dirt crawl space	#28	Blood Agar	35[3]	—	*Bacillus sp.* *Proteus sp* *Pseudomonas sp.*	TNC[4]	TNC[4]
Gravel, crawl space	#27	Blood Agar	35[3]	—	*Bacillus sp.* *Proteus sp.* *Pseudomonas sp.*	TNC[4]	TNC[4]
Sandal, under master bed	#36	Blood Agar	35[3]	—	*Bacillus sp.* *Proteus sp.* *Pseudomonas sp.*	TNC[4]	TNC[4]

(b)

Endotoxins	Sample #	Sample type	Location	Concentration (EU/Swab)[5]
	#3	Swab	J-Tube, Under Sink	4930
	#4	Swab	Top, Kitchen Cabinet	24.800
	Blank	Swab	Field Blank	None Detected
	Blank	Swab	Lab Blank	None Detected

[1]These samples were tested to determine the major species of *Bacillus*.
[2]This sample was tested for *Actinomycetes* because of white mycelia type growth on wood truss.
[3]These samples were tested by RealTime Laboratories for the presence of bacteria species on samples tested for mycotoxins.
[4]CFU was not determined. TNTC: too numerous to count.
[5]Endotoxins were analyzed by ESML using LAL Kinetic Chromogenic Assay.

for mycotoxins in the surgical specimens revealed the following: Ear mass—trichothecenes (23.07 ppb) and ochratoxin A (2.2 ppb); and Lipoma—trichothecenes (20.9 ppb) and ochratoxin A (1.4 ppb). The veterinarian stated that lipomas in dogs are normal; however, the presence of multiple lipomas is a rare occurrence.

12. Discussion

We have presented a family of five who had no history of health problems until they moved into a water-damaged home in Hawaii. Shortly after the move in they began to develop multiple symptoms, sought medical consultation for the health problems involving the upper and lower respiratory tract, headaches, neurocognitive deficits, and severe sinusitis. Neurological evaluation revealed 17 areas of neurological abnormalities in the two adults, consistent with previous reports [8, 9]. The daughter developed tremors that could be related to exposure to tremorgenic and other mycotoxins [18–22]. The son, age 5 at the time of examination, did not have neurological deficits. However, he did have a variety of symptoms (e.g., nose bleeds, cough, wheeze, and headaches) consistent with exposure to water-damaged indoor environments. In addition, when he began school, the teacher reported lack of concentration while in class. Perhaps he was showing signs of autistic spectrum disorder and/or ADD/ADHD as previously reported in children exposed to water-damaged home environments [10].

The parents and the two children have chronic sinusitis and nasal inflammation. The isolation of bacteria (*Pseudomonas* and *Acinetobacter*) and molds (*Penicillium* and *Aspergillus*) from nasal secretions from the father and daughter is consistent with the literature. Bacterial and fungal sinusitis has been reported [1, 5, 6, 23–25]. In

addition, the detection of mycotoxins in the nasal secretions from the family points towards fungal rhinosinusitis. Finally, the culture of surgical specimens taken from the daughter's sphenoid/ethmoid mucosa identified *Aspergillus fumigatus*.

Macrocyclic trichothecenes and tremorgens have been detected in airborne fungal fragments less than the size of conidia [22, 41–43]. Furthermore, trichothecenes, aflatoxins, sterigmatocystin, ochratoxin A, and other mycotoxins are present in the dust of water-damaged buildings [13, 16]. In addition, indoor microbial growth fragments, releasing particulates less than one micron that penetrate deep into the alveolar spaces [44–46]. Thus, the presence of trichothecenes, ochratoxin A, and aflatoxins in bulk samples (Table 4) and body fluids of the family (Table 5) is interpreted as an inhalation exposure resulting in uptake of mycotoxins attached to dust and fine microbial particulates. Moreover, it is reported in this issue and elsewhere that these mycotoxins are present in the urine and tissue biopsy/necropsy materials taken from individuals residing in water-damaged homes and buildings [42, 47–50].

The newborn girl had a total body flare at birth that began to clear at 10–12weeks after birth, which may have been associated with mast cell/eosinophil activity. However, medical workup was not done in this area. The body flaring periodically appeared until approximately 55 months of age. The majority of the Cafe-au-Lait spots were apparent soon after delivery and continued to develop after birth and continue to be present (Figure 1). She was diagnosed with NF1 by Dr. Frieden at U.C.S.F. at age 2, and additional diagnostics are anticipated. The placenta, umbilical, breast milk, urine, and nasal secretion of the mother were positive for Ochratoxin A (Table 5), while a urine sample from the infant was negative. It is reasoned that amniotic fluid (lost at birth) would have been a better choice for mycotoxin testing. However, the presence of ochratoxin A in the placenta and umbilical cord suggests that the infant most likely was exposed in utero. There is no family history of NF1 leading Dr. Frieden with conclusion that the mutation to NF1 gene most likely occurred sometime during in utero development. It is possible that her condition could be related to ochratoxin A or other toxins known to be present in water-damaged buildings.

A few comments are in order regarding the pet dog. The dog developed 72 cutaneous lesions that were distributed over its body, including the ears (Figure 2). The dog's urine was positive for ochratoxin A and trichothecenes. In addition, surgical specimens of the ear (sebaceous gland) and body tumors (lipomas) were also positive for trichothecenes and ochratoxin A. The question that arises is were the growths caused by the mycotoxins or were they storage sites for the toxins.

In conclusion, a family of five (one *in utero*) was exposed to several species of mold and bacteria while occupying a water-damaged home. They presented with multiple symptoms, including chronic sinusitis, fatigue, and neurological complaints. Testing of the home revealed the presence of both mold and bacteria. Differential diagnostic procedures demonstrated in up to seventeen areas of central nervous system deficits as well as chronic fungal/bacterial sinusitis.

Table 4: This table summarizes the detection of trichothecenes, aflatoxins and ochratoxin A present in bulk samples taken from the master bath, master bedroom (sandal), and crawl space. The reported data are in ppb per mycotoxin.

Sample	Trichothecenes	Aflatoxins	Ochratoxin A
Towel—master bath	11.71	NP	4.9
Sandal—master bdrm	0.47	NP	3.4
Wood truss—crawl space	1.68	3.5	5.8
Gravel—crawl space	7.7	NP	7.7
Dirt—crawl space	2.1	NP	2.1
Plastic sheet—crawl space	NP	NP	2.8

Reported data are ppb.
NP: Not present.
Limit of Detection: Trichothecenes (0.2 ppb); Aflatoxins (1.0 ppb); Ochratoxin A (2.0 ppb).

Table 5: Mycotoxins present in body fluid of the five members of the family and the pet dog.

Patient specimen	Trichothecenes (ppb)	Aflatoxins (ppb)	Ochratoxin (ppb)
Father-Urine	NP	NP	18.2
Father-Nasal[1] Secretion	NP	0.5 11.2	13 7.7
Mother-Urine	NP	NP	18.2
Mother-Nasal Secretion	1.02	1.2	1.6
Daughter-Urine	0.23	NP	28.0
Daughter-Nasal[2] Secretion	4.68	NP	3.8
Son-Urine	0.2	NP	18.9
Son-Nasal Secretion	ND	ND	ND
Breast Milk	0.18	0.9	2.7
Placenta	NP	NP	4.2
Umbilical Cord	NP	NP	7
New Born-Urine	NP	NP	NP
Dog-Urine	1.49	NP	25.9
Dog-Ear Mass	23.07	0	2.2
Dog-Lipoma	20.9	0	1.4

Limits of Detection: Trichothecenes (0.2 ppb); Aflatoxins (1.0 ppb); Ochratoxin A (2.0 ppb).
ND: Not done.
NP: Not present.
[1] *Pseudomonas aueroginosa* and *Penicillium* were cultured from the nasal secretions. These data represent two different tests.
[2] *Acinetobacter sp.* was cultured from nasal secretion at too numerous to count. In addition, *Aspergillus fumigatus* was cultured from left ethmoid and sphenoid mucosal surgical specimen.

Mycotoxins testing demonstrated that ochratoxin A was the predominant mycotoxin in samples of urine, nasal secretions, breast milk, placenta, and umbilical cord. Lesser concentrations of macrocyclic trichothecenes were also detected. A newborn girl had a total body flare and had Cafe-au-Lait pigmentation spots. The infant is scheduled for further evaluation for her NF1 condition. This case study

indicates that mold and bacteria and by-products in water-damaged homes are most likely the cause of the adverse health conditions of these occupants.

Disclosure

Jack Dwayne Thrasher, Ph.D, is semiretired. He has consulted to the practice of Dr. Gray. He has been an expert witness in both defense and plaintiff cases regarding toxic exposures. Michael A. Gray, M.D, is in private practice in Benson, Arizona. He has been an expert witness in both defense and plaintiff cases involving toxic exposures. Kaye H. Kilburn, M.D, is a Professor Emeritus, USC Keck School of Medicine. He has been an expert witness in plaintiff cases. Donald P. Dennis, M.D, is in private practice specializing in ENT. He has no other conflict of interest. Archie Yu MS, CIH, is the owner and operator of Compliance Solution. He performs industrial hygiene evaluations for both industry and private citizens.

References

[1] W. J. Fisk, E. A. Eliseeva, and M. J. Mendel, "Association of residential dampness and mold with respiratory tract infections and bronchitis: a meta-analysis," *Environmental Health*, vol. 9, no. 72, 2010.

[2] M. J. Mendell, A. G. Mirer, K. Cheung, M. Tong, and J. Douwes, "Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence," *Environmental Health Perspectives*, vol. 119, no. 6, pp. 748–756, 2011.

[3] D. Mudarri and W. J. Fisk, "Public health and economic impact of dampness and mold," *Indoor Air*, vol. 17, no. 3, pp. 226–235, 2007.

[4] J. H. Park and J. M. Cox-Ganser, "Mold exposure and respiratory health in damp indoor environments," *Frontiers in Bioscience E*, vol. 3, pp. 575–571, 2011.

[5] D. P. Dennis, "Chronic sinusitis: defective T-cells responding to superantigen treated by reduction of fungi in the nose and air," *Archives of Environmental Health*, vol. 58, no. 7, pp. 433–441, 2004.

[6] D. Dennis, D. Robertson, L. Curtis, and J. Black, "Fungal exposure endocrinopathy in sinusitis with growth hormone deficiency: Dennis-Robertson syndrome," *Toxicology and Industrial Health*, vol. 25, no. 9-10, pp. 669–680, 2009.

[7] L. Empting, "Neurologic and neuropsychiatric syndrome features of mold and mycotoxin exposure," *Toxicology and Industrial Health*, vol. 25, no. 9-10, pp. 577–582, 2009.

[8] K. H. Kilburn, "Role of molds and mycotoxins in being sick in buildings: neurobehavioral and pulmonary impairment," *Advances in Applied Microbiology*, vol. 55, pp. 339–359, 2004.

[9] K. H. Kilburn, "Neurobehavioral and pulmonary impairment in 105 adults with indoor exposure to molds compared to 100 exposed to chemicals," *Toxicology and Industrial Health*, vol. 25, no. 9-10, pp. 681–692, 2009.

[10] K. H. Kilburn, J. D. Thrasher, and N. B. Immers, "Do terbutaline- and mold-associated impairments of the brain and lung relate to autism?" *Toxicology and Industrial Health*, vol. 25, no. 9-10, pp. 703–710, 2009.

[11] W. J. Rea, N. Didriksen, T. R. Simon, Y. Pan, E. J. Fenyves, and B. Griffiths, "Effects of toxic exposure to molds and mycotoxins in building-related illnesses," *Archives of Environmental Health*, vol. 58, no. 7, pp. 399–405, 2004.

[12] W. J. Rea, Y. Pan, and B. Griffiths, "The treatment of patients with mycotoxin-induced disease," *Toxicology and Industrial Health*, vol. 25, no. 9-10, pp. 711–714, 2009.

[13] E. Bloom, E. Nyman, A. Must, C. Pehrson, and L. Larsson, "Molds and mycotoxins in indoor environments–a survey in water-damaged buildings," *Journal of Occupational and Environmental Hygiene*, vol. 6, no. 11, pp. 671–678, 2009.

[14] J. D. Thrasher and S. Crawley, "The biocontaminants and complexity of damp indoor spaces: more than what meets the eyes," *Toxicology and Industrial Health*, vol. 25, no. 9-10, pp. 583–615, 2009.

[15] M. Täubel, M. Sulyok, V. Vishwanath et al., "Co-occurrence of toxic bacterial and fungal secondary metabolites in moisture-damaged indoor environments," *Indoor Air*, vol. 21, no. 5, pp. 368–375, 2011.

[16] V. Polizzi, B. Delmulle, A. Adams et al., "JEM spotlight: fungi, mycotoxins and microbial volatile organic compounds in mouldy interiors from water-damaged buildings," *Journal of Environmental Monitoring*, vol. 11, no. 10, pp. 1849–1858, 2009.

[17] WHO, *Dampness and Mould: WHO Guidelines for Indoor Air Quality*, Euro Non Serial Publications, 2009.

[18] A. Ciegler, R. F. Vesonder, and R. J. Cole, "Tremorgenic mycotoxins," in *Mycotoxins and Other Fungal Related Food Problems*, J. V. Rodricks, Ed., Advances in Chemistry Series no. 149, pp. 163–177, American Chemical Society, 1976.

[19] N. Kyriakidis, E. S. Waight, J. B. Day, and P. G. Mantle, "Novel metabolites from Penicillium crustosum, including penitrem E, a tremorgenic mycotoxin," *Applied and Environmental Microbiology*, vol. 42, no. 1, pp. 61–62, 1981.

[20] W. E. Braselton and P. C. Rumler, "MS/MS screen for the tremorgenic mycotoxins roquefortine and penitrem a," *Journal of Veterinary Diagnostic Investigation*, vol. 85, no. 4, pp. 515–518, 1996.

[21] K. Khoufache, O. Puel, N. Loiseau et al., "Verruculogen associated with *Aspergillus fumigatus* hyphae and conidia modifies the electrophysiological properties of human nasal epithelial cells," *BMC Microbiology*, vol. 7, article 11, 2007.

[22] I. Kosalec, M. Š. Klarić, and S. Pepeljnjak, "Verruculogen production in airborne and clinical isolates of *Aspergillus fumigatus* Fres," *Acta Pharmaceutica*, vol. 55, no. 4, pp. 357–364, 2005.

[23] C. S. Coffey, R. E. Sonnenburg, C. T. Melroy, M. G. Dubin, and B. A. Senior, "Endoscopically guided aerobic cultures in postsurgical patients with chronic rhinosinusitis," *American Journal of Rhinology*, vol. 20, no. 1, pp. 72–76, 2006.

[24] A. Chakrabarti, D. W. Denning, B. J. Ferguson et al., "Fungal rhinosinusitis: a categorization and definitional schema addressing current controversies," *Laryngoscope*, vol. 119, no. 9, pp. 1809–1818, 2009.

[25] K. Mantovani, A. A. Bisanha, R. C. Demarco, E. Tamashiro, R. Martinez, and W. T. Anselmo-Lima, "Maxillary sinuses microbiology from patients with chronic rhinosinusitis," *Brazilian Journal of Otorhinolaryngology*, vol. 76, no. 5, pp. 548–551, 2010.

[26] J. Barkman, *Home Inspection Report*, Kihei, Hawaii, USA, 2009.

[27] Engineering Dynamics Corp, "Dizy Residence Report," Maui, Hawaii, USA.

[28] Pugliese and Thrasher, "Multipletesting parameters of water damaged buildings," *The Canadian Journal of Environmental Education*. In press.

[29] H. Feltman, G. Schubert, S. Khan, M. Jain et al., "Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*," *Journal of Microbiology*, vol. 147, pp. 2659–2669, 2001.

[30] D. E. Griffith, T. Aksamit, B. A. Brown-Elliott et al., "An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases," *American Journal of Respiratory and Critical Care Medicine*, vol. 175, no. 4, pp. 367–416, 2007.

[31] M. Kapadia, K. V. I. Rolston, and X. Y. Han, "Invasive *Streptomyces infections*: six cases and literature review," *American Journal of Clinical Pathology*, vol. 127, no. 4, pp. 619–624, 2007.

[32] S. Copeland, H. S. Warren, S. F. Lowery et al., "Acute respiratory response to endotoxin in mice and humans," *Clinical and Diagnostic Laboratory Immunology*, vol. 12, pp. 60–57, 2005.

[33] F. D. Martinez, "CD14, endotoxin, and asthma risk: actions and interactions," *Proceedings of the American Thoracic Society*, vol. 4, no. 3, pp. 221–225, 2007.

[34] R. Rylander, "Endotoxin and occupational airway disease," *Current Opinion in Allergy and Clinical Immunology*, vol. 6, no. 1, pp. 62–68, 2006.

[35] J. Thorn and R. Rylander, "Inflammatory response after inhalation of bacterial endotoxin assessed by the induced sputum technique," *Thorax*, vol. 53, no. 12, pp. 1047–1052, 1998.

[36] M. A. Andersson, R. Mikkola, R. M. Kroppenstedt et al., "The mitochondrial toxin produced by Streptomyces griseus strains isolated from an indoor environment is valinomycin," *Applied and Environmental Microbiology*, vol. 64, no. 12, pp. 46–67, 1998.

[37] Z. Islam, C. J. Amuzie, J. R. Harkema, and J. J. Pestka, "Neurotoxicity and inflammation in the nasal airways of mice exposed to the macrocyclic trichothecene mycotoxin roridin A: kinetics and potentiation by bacterial lipopolysaccharide coexposure," *Toxicological Sciences*, vol. 98, no. 2, pp. 526–541, 2007.

[38] A. Roy-Burman, R. H. Savel, S. Racine et al., "Type IIIprotein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections," *Journal of Infectious Diseases*, vol. 183, no. 12, pp. 1767–1774, 2001.

[39] K. Huttunen, J. Pelkonen, K. F. Nielsen, U. Nuutinen, J. Jussila, and M. R. Hirvonen, "Synergistic interaction in simultaneous exposure to Streptomyces californicus and *Stachybotrys chartarum*," *Environmental Health Perspectives*, vol. 112, no. 6, pp. 659–665, 2004.

[40] D. G. Hooper, V. E. Bolton, F. T. Guilford, and D. C. Straus, "Mycotoxin detection in human samples from patients exposed to environmental molds," *International Journal of Molecular Sciences*, vol. 10, no. 4, pp. 1465–1475, 2009.

[41] T. L. Brasel, D. R. Douglas, S. C. Wilson, and D. C. Straus, "Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia," *Applied and Environmental Microbiology*, vol. 71, no. 1, pp. 114–122, 2005.

[42] T. L. Brasel, A. W. Campbell, R. E. Demers et al., "Detection of trichothecene mycotoxins in sera from individuals exposed to *Stachybotrys chartarum* in indoor environments," *Archives of Environmental Health*, vol. 59, no. 6, pp. 317–323, 2004.

[43] D. C. Straus, "The possible role of fungal contamination in sick building syndrome," *Frontiers in Bioscience E*, vol. 3, pp. 562–580, 2011.

[44] T. Reponen, S. C. Seo, F. Grimsley, T. Lee, C. Crawford, and S. A. Grinshpun, "Fungal fragments in moldy houses: a field study in homes in New Orleans and Southern Ohio," *Atmospheric Environment*, vol. 41, no. 37, pp. 8140–8149, 2007.

[45] S. H. Cho, S. C. Seo, D. Schmechel, S. A. Grinshpun, and T. Reponen, "Aerodynamic characteristics and respiratory deposition of fungal fragments," *Atmospheric Environment*, vol. 39, no. 30, pp. 5454–5465, 2005.

[46] R. L. Górny, "Filamentous microorganisms and their fragments in indoor air—a review," *Annals of Agricultural and Environmental Medicine*, vol. 11, no. 2, pp. 185–197, 2004.

[47] J. H. Hope and B. E. Hope, "Ochratoxin-A from inhalation exposure associated with focal segmental glomerulosclerosis: two human cases," *The Canadian Journal of Environmental Education*. In Press.

[48] D. Hibi, Y. Susuki, Y. Ishi et al., "Site-specific in vivo mutagenicity in the kidney of bpt deta rats given a carcinogenic dose of Ochratoxin A.," *Toxicological Sciences*, vol. 122, pp. 406–414, 2011.

[49] N. Palma, S. Cinelli, O. Sapora, S. H. Wilson, and E. Dogliotti, "Ochratoxin A-induced mutagenesis in mamalian cells is consistent with the production of oxidative stress," *Chemical Research in Toxicology*, vol. 20, pp. 1031–1037, 2007.

[50] W. Karmus, P. Dimitrov, V. Simeonov, S. Tsolova, and V. Batuman, "Offspring of parents with Balkan Endemic Nephropathy have igher C-reactive protein levels suggestive of inflammatory processes: a longitudinal study," *BMC Nephrology*, vol. 10, article 10, 2009.

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Clinical Study

Objective Assessment of an Ionic Footbath (IonCleanse): Testing Its Ability to Remove Potentially Toxic Elements from the Body

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Ionic footbaths are often used in holistic health centres and spas to aid in detoxification; however, claims that these machines eliminate toxins from the body have not been rigorously evaluated. In this proof-of-principle study, we sought to measure the release of potentially toxic elements from ionic footbaths into distilled and tap water with and without feet. Water samples were collected and analyzed following 30-minute ionic footbath sessions without feet using both distilled ($n = 1$) and tap water ($n = 6$) and following four ionic footbaths using tap water (once/week for 4 weeks) in six healthy participants. Urine collection samples were analyzed at four points during the study. Hair samples were analyzed for element concentrations at baseline and study conclusion. Contrary to claims made for the machine, there does not appear to be any specific induction of toxic element release through the feet when running the machine according to specifications.

1. Introduction

With the advent of the industrial revolution, the levels of toxicants in our water, air, and soil have risen dramatically such that even newborn infants are born with toxic elements and chemical pollutants in their bodies [1]. There are a host of illnesses attributed to toxin exposure that have arisen in the 20th century that were not previously recognized. Sick building syndrome and multiple chemical sensitivity are attributed, in part, to bioaccumulation of toxins and pollutants [2]. As well, the rate of increase in cancers is greater for those born after 1940 [2, 3]. While causative links are difficult to prove, it is hypothesized that the burden of toxic elements is linked to a number of health conditions including mental health [4], ADHD [5], cancer [3, 6–9], reproductive health [10, 11], and autoimmune conditions [12].

Currently, many methods of detoxification are available, such as dimercaptosuccinic acid (DMSA), which is known to bind to heavy metals and aid in their elimination from the body [13–15]. Infrared and dry heat saunas can also detoxify, partly by breaking down body fat which liberates fat-soluble substances, medications, and heavy metals stored in adipose tissue [16, 17]. More recently, ionic footbaths have been promoted as a means of eliminating toxins and heavy metals from the body in the lay literature and worldwide web [18].

Consumer use of ionic footbaths appears to come predominantly from holistic health centres, hair salons, and health food stores which often promote ionic footbaths as a means to rid the body of toxins such as heavy metals and often charge upwards of $75 per session [19–21].

Following an empty search of Medline, EMBASE, AMED, Alt Health Watch, and CINAHL using the search terms "ionic," "footbath," and "detoxification," a search on Google found one study conducted by the Centre for Research Strategies [22]. That study found a statistically significant reduction in aluminum and arsenic, but no changes in lead, mercury, or cadmium in whole blood of the participants

after 12 weekly sessions [22]. Concomitant nutrition and meditation techniques were used, making the contribution from footbaths impossible to isolate. In addition there was a risk of bias for this study demonstrated by poor quality reporting (12-week results reported only yet the protocol described a 6-month study), a lack of scientific rigor in the methods, and potential for conflict of interest, the research was conducted by "The Centre for Research Strategies," an arm of the IonCleanse manufacturer. Unbiased, reliable information on prevalence of consumer use, as well as scientific investigation of the methods and purported effects of these devices, remains scarce.

In this proof-of-principle study, we evaluated the IonCleanse Solo footbath. This product has been available in the market since 2002 [18] and has successfully undergone electrical appliance safety testing. It received both Federal Communications Commission (FCC) and Conformité Européenne (CE) approvals [18, 23–25].

This was a two-phase project. The objective of Phase I was to establish a baseline for the contribution of the ionic footbath machine to release potentially toxic elements (PTEs) when either distilled or tap water was used without feet present. Phase II had several objectives including whether the ionic footbath could (1) effectively remove PTEs through the feet of participants; (2) increase PTE release through the urine; (3) increase PTE release as measured through hair mineral analysis (HMA).

2. Materials and Methods

2.1. Study Design. This was a proof-of-principle, nonrandomized, nonblinded comparative, no feet versus feet, trial conducted from the week of May 17, 2010 (Week 0) through to August 9, 2010 (Week 12). Ethics approval was given by Research Ethics Board of the Canadian College of Naturopathic Medicine (CCNM) according to the ethical standards set forth in the 1975 Helsinki Declaration. All participants enrolled gave written informed consent to participate in the study. This study was funded through a grant from the Holistic Health Research Foundation. The trial registry number is NCT01125592.

2.1.1. Participants. Between April and May 2010, healthy participants were recruited through e-mail to CCNM staff and students, website-based advertisements, and posters. The e-mail summarized the requirements for the study and asked interested individuals to respond to the study coordinator. The study was also open to the general public.

Inclusion criteria required participants over 18 years of age, in good health, and with a stable medication/supplementation regimen for at least six weeks prior to and during participation in the study. Individuals were excluded if they were not legally competent; were pregnant or nursing mothers; had a pacemaker; were organ transplant or metal joint implant recipients; took antiarrhythmic, anticoagulant or chelating medication; or took any medication whose absence could mentally or physically incapacitate them (antipsychotics, antiepileptics, etc.). Participants were excluded if they had used a sauna within two weeks prior to beginning the study. Participants were also instructed to avoid sauna use during the study.

Figure 1: Initial setup of IonCleanse SOLO footbath.

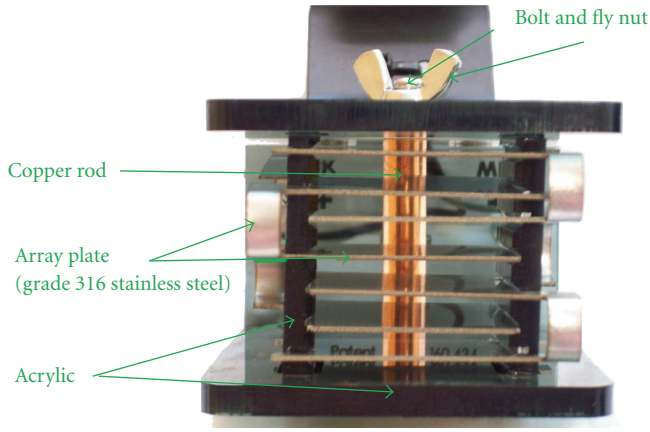


Figure 2: Close up of a new IonCleanse SOLO footbath array.

2.1.2. Ionic Footbath Device. IonCleanse SOLO (A Major Difference Inc., Aurora, Colo) ionic footbath was used for all sessions in the study. With knowledge of the trial to be conducted, A Major Difference Inc. donated an IonCleanse SOLO machine for the duration of this study. The components of the ionic footbath include the SOLO device, an array, a power cord, plastic foot tub liners, and a plastic foot tub container (Figure 1). The SOLO device has a single preset program to generate a 70/30 mix of positive/negative polarity in a standard 30-minute session.

The array is composed of an acrylic housing, a copper rod held in place with a bolt and fly nut, and a metal plate folded on itself several times (Figure 2) [18]. The side of the array is stamped with "316 SS" which we interpreted to indicate that the metal is composed of "316 grade stainless steel." The metal plates of the array have a limited lifespan and must be replaced after 30–50 sessions, with the "life" of a metal plate dependent on the mineral concentration of the water source [18].

2.1.3. Setup and Running of the Footbath Device. The IonCleanse SOLO footbath was set up according to manufacturer's instructions as follows. A new plastic liner lined the foot tub and the "source" water was used to fill the foot tub

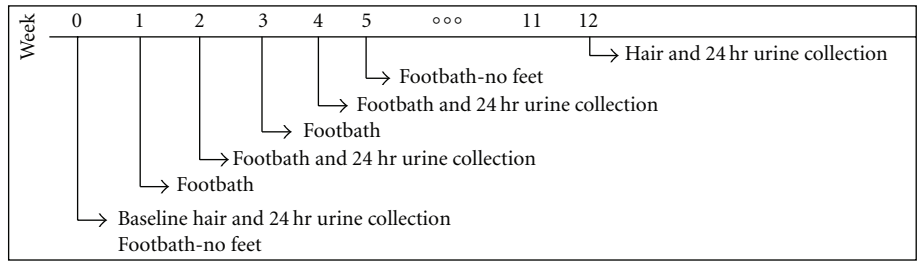


Figure 3: Study schedule.

(approximately 3.75 litres of water per session). The array was plugged into the SOLO device and placed in the foot tub, ensuring that there was sufficient water to cover the copper bar of the array. The device was turned on, and both voltage and amperage, displayed on the front of the machine, were monitored to ensure they stayed within optimal operating range, 13–20 volts and 1.8–2.2 amperes, respectively. This range was maintained for all footbath sessions, and no changes were made to the preset program on the SOLO device. Each session ran for 30 minutes, indicated by a buzzer at the end of the session.

2.2. Setting. All footbath sessions were conducted at the Robert Schad Naturopathic Clinic (RSNC) located within CCNM.

2.3. Phase I: Establishment of Baseline and Potential Confounders

Distilled Water Procedure. Three independent footbath sessions using two brands of distilled water (Life Brand and Longo's, 4-litre plastic container, steam-distilled water) were run. A sample of the distilled water was placed in the 100 mL sample bottle and labelled. The footbath was prepared as described above using distilled water. The machine was turned on, and 1/8 tsp of salt (Baleine Sea Salt, 30220 Aigues-Mortes, France), according to the manufacturer's instructions, was placed in the footbath water. At the end of the session, the water was stirred and a sample taken. This procedure was used for the first two footbath sessions with distilled water. In the last session, the sample was obtained after the salt had been added to the foot tub. The footbath session continued as described above.

Tap Water Baseline and Postsession Procedures. The following procedure was used for all tap water footbath sessions. At the outset, it was determined that 50 L of water would be required to conduct the six footbath sessions. A 105 L plastic container (Storage Solutions, Gracious Living, Woodbridge, ON) was used for all tap water tests. The level of 50 L was predetermined and marked on two of the outside walls of the 105 L plastic container. The hot and cold tap water was run for 30 seconds to ensure that no stagnant water remained in the pipes. The 105 L container was filled to the predetermined level with a mixture of hot and cold water, and, according to the manufacturer's instructions, $6 \times 1/8$ tsp of sea salt (Baleine Sea Salt, 30220 Aigues-Mortes, France) were added and stirred 20 times. A water temperature of approximately 39-40°C was used. A 100 mL sample of the tap water was obtained and labelled with the identifier "CCNM" and a sequential number. The samples were numbered in the sequence in which they were obtained to blind the laboratory to the source of the water sample. Samples were placed in the refrigerator overnight and couriered to the laboratory the following day.

On Week 0, the baseline parameters of the footbath device were established as follows: daily, for three consecutive days, the SOLO device was set up as before and run for 30 minutes with no feet in the footbath water. Samples were taken. On Week 5, after all participant footbath sessions had been completed, three additional postsession "no feet" sessions were conducted on the same day and samples obtained.

2.4. Phase II: Assessment for Efficacy in Removal of Potentially Toxic Elements.

An overview of the study schedule is provided in Figure 3.

Establishment of Baseline and Postsession Parameters for Participants. At baseline and Week 12, participants were requested to provide a hair and 24-hour urine sample for analysis following instructions provided by the laboratory for obtaining these samples. Hair is a very stable medium [41] and therefore regular mail (Letter, Canada Post, Ottawa, Canada) was used to send the hair samples in sealed envelopes to the laboratory for analysis. Participants were instructed to obtain their second hair sample from the same location as the first, so that the second sample better represented what had been circulating in the blood during the previous 3-month period. For the urine samples, participants were provided with courier forms and packaging materials and asked to contact the courier company (Xpress-post, Purolator, Mississauga, ON) for shipment pickup and overnight delivery to the laboratory.

Assessment of Detoxification through Urine. Twenty-four-hour urine collections were also collected during the 24 hours following the second and fourth footbath sessions. Collection began the day of the footbath session and continued until the first morning void the day after.

Table 1: Categorization of reported elements by group.

Array components	Essential elements	Potentially toxic elements
(i) Chromium (Cr)	(i) Boron (Bo)	(i) aluminum (Al) [26–28]
(ii) Cobalt (Co)	(ii) Calcium (Ca)	(ii) Antimony (Sb) [26, 29, 30]
(iii) Copper (Cu)	(iii) Lithium (Li)	(iii) Arsenic (As) [26, 29, 31, 32]
(iv) Iron (Fe)	(iv) Magnesium (Mg)	(iv) Barium (Ba) [26, 33]
(v) Manganese (Mn)	(v) Phosphorus (P)	(v) Cadmium (Cd) [26, 34, 35]
(vi) Molybdenum (Mo)	(vi) Potassium (K)	(vi) Lead (Pb) [26, 28, 36]
(vii) Nickel (Ni)	(vii) Selenium (Se)	(vii) Silver (Ag) [26, 37, 38]
(viii) Silicon (Si)	(viii) Sodium (Na)	(viii) Uranium (U) [26, 39, 40]
	(ix) Strontium (Sr)	
	(x) Sulphur (S)	
	(xi) Vanadium (Vn)	
	(xii) Zinc (Zn)	

Footbath Sessions with Participants. Footbath sessions were scheduled weekly on the same weekday and time. To decrease any residual particulate matter or mineral-containing excretions participant's feet were rinsed under running water prior to placing their feet in the foot tub. The tap water and footbath device were set up as previously described for the initial footbath session. For all footbaths conducted within the day, the 105 L container was used as a consistent water source. Participants placed their washed feet into the prefilled foot tub and the SOLO device turned on. At the end of 30 minutes, participants removed their feet from the footbath, the footbath water was stirred and a sample taken and labelled. At the end of the day, all samples were collected and couriered to the laboratory. The array was removed from the footbath and rinsed with clean water. Once the visible residue was removed, a disinfectant (Ultra-Safe Plus commercial cleaner, Safer Soaps, Traveler's Rest, SC) was sprayed on the array as per the manufacturer's recommendations. Several minutes later, the array was rinsed and dried with a clean towel.

Each week, the array was soaked in a dilute solution of ascorbic acid (A Major Difference Inc., Aurora, Colo) and water according to manufacturer's instructions.

2.5. Laboratory Analysis. Water, hair, and urine analyses were performed using Inductively Coupled Plasma Source Mass Spectroscopy (ICP-MS) by CanAlt Health Laboratory Inc., Concord ON, Canada. Calibration of the method has been carried out using at least two internationally recognized National Institute of Standards and Technology (NIST) standards for each element and is validated by analysis of Certified Reference Material (CRM). CanAlt Health Laboratory follows and documents Good Laboratory Practice Standards for handling of materials, quality control, and standardization of instruments to control for determinate error and to provide quality assurance.

2.6. Statistical Analysis

Water Samples. The water reports provided by CanAlt Health Laboratory Inc. list the concentrations of 28 individual elements. Descriptive statistics (total, mean, standard deviation) were calculated for each element. In addition, to facilitate reporting, elements tested were categorized into three groups and subtotals determined for "array components," "essential elements," and "PTEs" (Table 1).

The change in each element's concentration was calculated by subtracting the concentration in the postfootbath session (Post-FBS) from the concentration in the source sample (Pre-FBS) to derive the difference (Diff-FBS). There were 3 distinct groups of water samples: (1) distilled water with no feet, (2) tap water with no feet, and (3) tap water with feet. Mann-Whitney tests compared the Post-FBS to the Pre-FBS element concentration to determine whether the Diff-FBS element concentration was statistically significant. This analysis was done for both the tap water with no feet and the tap water with feet groups. One valid observation was sufficient for the highly controlled distilled water source to act as a comparison group, and this precluded use of the Mann-Whitney test. Also, Mann-Whitney test compared the Diff-FBS (no feet/feet) to determine whether the presence of participants' feet affected results. A Kruskal-Wallis test comparing the total concentrations of the Pre-FBS and Post-FBS tap water with no feet and Post-FBS tap water with feet was used to determine whether a significant difference existed between the groups.

Hair Mineral Analysis (HMA). HMA reports list the concentration of 40 individual elements. Total PTEs, defined as Al, Sb, As, Ba, Beryllium (Be) [26, 42, 43], Cd, Mercury (Hg) [26, 44–46], Pb, and U, were summed for HMA results.

Urine Analysis (UA). The UA reports list the concentrations of 40 individual elements. Total PTEs, defined as Al, Sb, As, Ba, Be, Cd, Hg, Pb, and U, were summed for UA results. Microsoft Office Excel-2007 was used for all data manipulations and descriptive statistics. StatsDirect version 2.7.7 was used for the nonparametric statistics.

Table 2: Characteristics of the participants.

	Number	Mean age (years)	Age range	Medication use (n)	Supplement use (n)
Gender					
Male	3	56.3	54–59	0	2
Female	3	36.6	30–45	3	0
Total	6	46.5	30–59	3	2

3. Results and Discussion

Participants. An e-mail request was sent out to all the staff ($n \sim 100$) at the CCNM to solicit possible recruits. The first participants who responded were assessed for eligibility leading to three people excluded due to (1) an inability to commit to the schedule of footbaths; (2) not able to maintain a stable medication/supplement regime; (3) presence of a metal implant. Table 2 summarizes the characteristics of the six study participants that were included. Participants received no compensation for involvement in the study but were provided with copies of the results from their laboratory tests.

While participants' schedules necessitated some minor adjustments of appointment times, all but one of the footbath sessions occurred on the same weekday between 10 AM and 4 PM. One participant's second footbath was performed two days after the usually scheduled session due to an illness unrelated to the study. Participants were requested to maintain a stable lifestyle and medication/supplementation regime throughout; however one participant, during Week 3, needed to take antibiotics for 11 days for an illness unrelated to the study.

The footbath sessions were well tolerated by all of the participants. There were no adverse events reported during the course of the study.

3.1. Phase I

3.1.1. Footbath Sessions without Feet Using Distilled Water as Source ($n = 4$) (Table 3). Though two different sources were used, it is evident from these results that Al, Cu, Fe, and Na were present in the distilled water in small amounts at the outset. In the Post-FBS, the largest changes in element concentrations were for Cr, Co, Cu, Fe, Mn, Mo, Ni, and Si. Total PTEs increased 17 μg/L after running the machine with greatest increases in Al, Sb, As, and Cd.

3.1.2. Footbath Sessions without Feet Using Tap Water as Source ($n = 6$) (Table 4). The concentration of essential elements predominates in the tap water prior to the footbath. There are also PTEs in the tap water, with Al representing the largest concentration. In the Post-FBS, as with the distilled water results, the largest changes in element concentrations occur within the array elements ($P = 0.010$). Mean total PTE concentrations also increased by 30.50 μg/L ($P = 0.133$) with nonsignificant increases in Al, Ba, and Pb and significant increases in Sb ($P = 0.038$), As ($P = 0.010$), and Cd ($P = 0.010$).

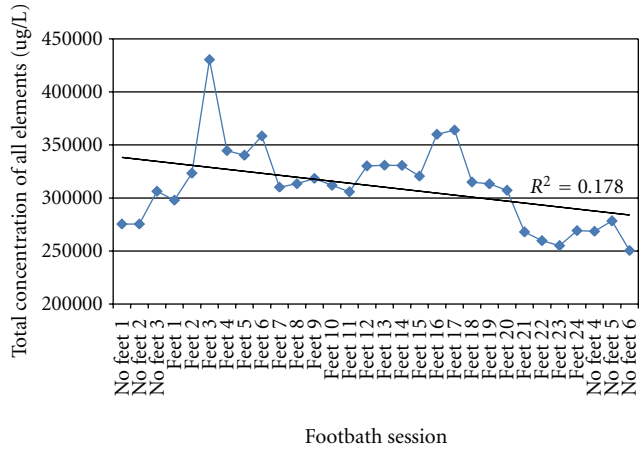


Figure 4: Post-footbath session: total concentration of all elements in order of session occurrence.

3.2. Phase II

3.2.1. Footbath Sessions with Feet Using Tap Water as Source ($n = 24$) (Table 5). The concentration of essential elements (98.9%) vastly outweighs that of PTEs (<1%) in the tap water prior to the footbath. Although present in very low quantities, Al had the highest concentration of all of the PTEs present in baseline tap water. Statistically significant differences were found in Diff-FBS for both array components ($P < 0.0001$) and toxic elements ($P = 0.042$).

We also compared the change in element concentrations (Diff-FBS in tap water with feet versus Diff-FBS in tap water without feet, Table 5). The increase in As was found to be significantly different ($P = 0.016$); however, the differences in total PTE concentration were not ($P = 0.869$), indicating that addition of a person's feet did not significantly alter PTE composition of the water.

To assess leeching as a factor in the change of concentration of elements, we plotted the total element concentration in μg/L from Post-FBS in sequence (Figure 4). More elements are discharged into the water when the array is new versus after 40+ sessions ($R^2 = 0.178$). Figure 5 graphically represents the average total elements concentration in μg/L in three groups of results using tap water: Pre-FBS, Post-FBS without feet, and Post-FBS with feet. The Kruskal-Wallis test found no significant differences between the three groups ($P = 0.524$).

3.2.2. 24 Hour Urine Analysis. Four samples were obtained from the participants: at baseline (Week 0), during the second (Week 2) and fourth (Week 4) footbath sessions, and Week 12 (Figure 3). The total PTEs (Hg, Pb, Al, Cd, Sb, As, Ba, Be, and U) excreted by each participant were graphed (Figure 6). Elimination of PTEs was substantially higher in Participant-1 overall, with initially a reduction in the second footbath followed by an increase in PTE

Table 3: Changes in element concentrations in distilled water after running the machine without feet.

Elements (ug/L)	Distilled water + salt (pre-FBS)	Distilled water + salt (post-FBS)	Mean difference	%change
Aluminum§	25.0	26.0	1.0	4.0
Antimony§	0.0	2.0	2.0	200.0
Arsenic§	0.0	6.0	6.0	600.0
Barium§	0.0	0.0	0.0	0.0
Boron†	0.0	1.0	1.0	100.0
Cadmium§	0.0	9.0	9.0	900.0
Calcium†	30.0	150.0	120.0	400.0
Chromium‡	4.0	23,634.0	23,630.0	590,750.0
Cobalt‡	0.0	320.0	320.0	320.0
Copper‡	40.0	280.0	240.0	600.0
Iron‡	31.0	116,421.0	116,390.0	375,451.6
Lead§	1.0	0.0	−1.0	−100.0
Lithium†	0.0	0.0	0.0	0.0
Magnesium	570.0	570.0	0.0	0.0
Manganese‡	0.0	1,566.0	1,566.0	1566.0
Molybdenum	50.0	3,155.0	3,105.0	6,210.0
Nickel‡	2.0	15,179.0	15,177.0	758,850.0
Phosphorus†	21.0	59.0	38.0	180.9
Potassium†	60.0	50.0	−10.0	−16.7
Selenium†	0.0	1.0	1.0	100.0
Silicon‡	20.0	1,170.0	1,150.0	5,750.0
Silver§	0.0	0.0	0.0	0.0
Sodium†	136,740.0	141,860.0	5,120.0	3.7
Strontium†	5.0	6.0	1.0	20.0
Sulfur	0.0	0.0	0.0	0.0
Uranium§	0.0	0.0	0.0	0.0
Vanadium †	1.0	59.0	58.0	5,800.0
Zinc†	10.0	30.0	20.0	200.0
Total	**137,610.0**	**304,554.0**	**166,944.0**	**121.3**
Array component‡	**147.0**	**161,725.0**	**161,578.0**	**1,09,917.0**
Essential elements†	**137,437.0**	**142,786.0**	**5,349.0**	**3.9**
PTEs§	**26.0**	**43.0**	**17.0**	**65.4**

§PTEs: potentially toxic elements are defined to be aluminium, antimony, arsenic, barium, cadmium, lead, silver and uranium.
†Essential elements are defined to be boron, calcium, lithium, magnesium, phosphorus, potassium, selenium, sodium, strontium, sulphur, vanadium, and zinc.
‡Array component elements are to be chromium, cobalt, copper, iron, manganese, molybdenum, nickel, and silicon.

elimination during fourth footbath. Baseline elimination of PTEs was highest for Participant-4 and remained low for each subsequent sample. For the remaining participants, the elimination of PTEs remained stable during the course of the study. The second urine sample for Participant-2 was lost in transit.

3.2.3. Hair Mineral Analysis. Hair samples were taken at baseline and at Week 12 of the study. PTEs analyzed included Hg, Pb, Al, Cd, Sb, As, Ba, Be, and U. The difference (μg/g) between baseline and Week 12 results of HMA for total toxic elements was graphed (Figure 7). The baseline sample for Participant-2 was lost in transit. For Participant-6, there was a significant change that was highly discrepant from the minimal change in hair PTEs observed for any of the other participants.

3.3. Discussion. We found that the IonCleanse SOLO device did not induce the elimination of PTEs through the feet of study participants. There is no evidence that the device stimulates pathways of PTE elimination through either the kidneys, via urine, or through the hair after receiving four 30-minute footbath sessions given weekly.

3.3.1. Ionic Footbath Effectiveness. The manufacturers of the IonCleanse device claim that their product's effectiveness lies

Table 4: Changes in element concentrations in tap water after running the machine without feet.

Elements (μg/L)	Pre-FBS ($n = 4$)	Post-FBS ($n = 6$)	Post-FBS–Pre-FBS		P value
	Mean ± Std dev	Mean ± Std dev	Difference ± Std dev	%change	
Aluminum§	93.75 ± 11.35	105.00 ± 18.95	14.17 ± 12.22	15.1	0.257
Antimony§	**0.75 ± 0.50**	**1.83 ± 0.41**	**1.00 ± 0.63**	**133.3**	**0.038**
Arsenic§	**1.00 ± 0.00**	**5.50 ± 0.84**	**4.50 ± 0.84**	**450.0**	**0.010**
Barium§	20.00 ± 0.00	25.00 ± 5.48	5.00 ± 5.48	25.0	0.333
Boron†	35.00 ± 5.77	36.67 ± 5.16	3.33 ± 5.16	9.5	0.905
Cadmium§	**0.50 ± 1.00**	**6.50 ± 1.64**	**5.50 ± 2.43**	**1,100.0**	**0.010**
Calcium†	39,255.00 ± 1,354.51	39,843.33 ± 906.15	1,206.67 ± 893.37	3.1	0.609
Chromium‡	**3.50 ± 1.29**	**17,289.67 ± 4,240.36**	**17,286.67 ± 4,239.65**	**493,904.7**	**0.010**
Cobalt‡	**1.00 ± 0.00**	**249.17 ± 44.02**	**248.17 ± 44.02**	**24,816.6**	**0.010**
Copper‡	**465.00 ± 46.55**	**723.33 ± 93.31**	**253.33 ± 64.39**	**54.5**	**0.010**
Iron‡	**213.50 ± 183.72**	**88,689.17 ± 17,460.59**	**88,388.17 ± 17,532.11**	**41,399.6**	**0.010**
Lead§	2.75 ± 1.71	3.17 ± 1.17	0.33 ± 1.37	12.1	0.676
Lithium†	0.00 ± 0.00	1.17 ± 2.86	1.17 ± 2.86	0.0	0.800
Magnesium†	10,720.00 ± 437.34	11,025.00 ± 561.31	405.00 ± 427.73	3.7	0.476
Manganese‡	**5.25 ± 0.50**	**1,240.17 ± 212.04**	**1,235.00 ± 211.83**	**23,523.8**	**0.010**
Molybdenum‡	**47.50 ± 18.36**	**2,559.83 ± 440.07**	**2,505.17 ± 438.10**	**5,274.0**	**0.010**
Nickel‡	**3.25 ± 2.50**	**11,623.17 ± 2,076.03**	**11,621.00 ± 2,075.55**	**357,569.2**	**0.010**
Phosphorus†	16.75 ± 15.73	48.50 ± 28.03	37.33 ± 21.73	222.9	0.114
Potassium†	2,052.50 ± 235.28	2,146.67 ± 190.23	−11.67 ± 163.64	−0.6	0.610
Selenium†	0.75 ± 0.50	0.50 ± 0.55	0.00 ± 0.00	0.0	0.905
Silicon‡	**742.50 ± 120.93**	**1,805.00 ± 204.52**	**1,003.33 ± 170.37**	**135.1**	**0.010**
Silver§	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0	0.000
Sodium†	77,622.50 ± 41,701.87	101,911.67 ± 9,916.69	19,190.00 ± 38,454.88	24.7	0.114
Strontium†	200.50 ± 4.80	202.83 ± 7.88	3.17 ± 10.94	1.6	0.114
Sulfur†	6,245.00 ± 1,537.15	6,178.33 ± 1,234.35	−628.33 ± 1,253.99	−10.1	0.914
Uranium§	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0	0.000
Vanadium†	**1.00 ± 0.00**	**43.50 ± 11.71**	**42.50 ± 11.71**	**4,250.0**	**0.010**
Zinc†	22.50 ± 9.57	35.00 ± 17.61	16.67 ± 10.33	74.1	0.543
Total	**137,771.75 ± 43,704.77**	**285,799.67 ± 27,823.80**	**142,837.17 ± 36,748.30**	**103.7**	**0.010**
Array components‡	**1,481.50 ± 334.61**	**124,179.50 ± 24,547.41**	**122,540.83 ± 24,659.98**	**8,271.4**	**0.010**
Essential elements†	136,171.50 ± 43,506.28	161,473.17 ± 10,605.21	20,265.83 ± 39,878.15	14.9	0.171
PTEs§	118.75 ± 11.53	147.00 ± 25.42	30.50 ± 19.85	25.7	0.133

§PTEs: potentially toxic elements were defined to be aluminium, antimony, arsenic, barium, cadmium, lead, silver, and uranium.
†Essential elements were defined to be boron, calcium, lithium, magnesium, phosphorus, potassium, selenium, sodium, strontium, sulphur, vanadium, and zinc.
‡Array components were defined to be chromium, cobalt, copper, iron, manganese, molybdenum, nickel, and silicon.
Bold indicates a statistically significant difference, $P < 0.05$ (Mann-Whitney U-test).

in its ability to generate positively and negatively charged ions (H+, OH−) via electrolysis in water. Purportedly, these ions cause the neutralization and subsequent removal of charged particles from the body via osmosis and diffusion through the skin that is in contact with the ion gradient created in the water. While much attention in the claim is given to the impact this gradient may have on a person whose feet are immersed in this water, little is given towards the impact this gradient may have on the array itself.

Stainless steel is a composite of different elements with Fe as the basic element. The composition of the steel varies, with 316 grade having a higher amount of chromium in order to provide increased resistance to corrosion [48, 49]. The usual composition of 316 grade stainless steel is summarized in Table 7 [48]. The elements with the greatest change in concentration after running the device, with or without feet, were Fe, Cr, Ni, Mo, Mn, and Si. These elements align very closely to those elements common to 316 grade stainless steel.

Corrosion can be defined as "deterioration of a material due to interaction with its environment. It is the process in which metallic atoms leave the metal or form compounds in the presence of water and gases" [49, 50]. The use of direct current and salt in the water will accelerate the corrosion of

Table 5: Changes in element concentrations in tap water after running the machine with participants feet.

Elements (μg/L)	Pre-FBS ($n = 6$)	Post-FBS ($n = 24$)	Post-FBS FF–Pre-FBS		P value
	Mean ± Std dev	Mean ± Std dev	Difference ± Std dev	%change	
Aluminum[§]	110.80 ± 51.79	126.75 ± 44.05	5.00 ± 58.43	4.5	0.239
Antimony[§]	1.00 ± 1.41	1.71 ± 1.52	1.21 ± 1.50	120.8	0.056
Arsenic[§]	**1.00 ± 0.00**	**6.58 ± 1.02**	**5.58 ± 1.02**	**558.3**	**0.0001**
Barium[§]	**22.00 ± 4.47**	**26.04 ± 4.89**	**6.04 ± 4.89**	**27.5**	**0.034**
Boron[†]	40.00 ± 10.00	41.13 ± 6.31	4.04 ± 9.12	10.1	0.118
Cadmium[§]	**0.80 ± 1.10**	**8.54 ± 2.55**	**8.04 ± 2.80**	**1,005.2**	**< 0.0001**
Calcium[†]	39,316.00 ± 617.36	39,091.46 ± 1,198.06	−231.04 ± 1,354.99	−0.6	0.250
Chromium[‡]	**1.60 ± 2.07**	**23,548.21 ± 4,783.09**	**23,546.21 ± 4,782.52**	**1,471,638.0**	**< 0.0001**
Cobalt[‡]	**1.00 ± 0.00**	**332.05 ± 57.69**	**331.05 ± 57.69**	**33,105.0**	**< 0.0001**
Copper[‡]	**406.00 ± 190.34**	**836.71 ± 152.62**	**456.71 ± 318.01**	**112.5**	**< 0.0001**
Iron[‡]	**388.60 ± 178.33**	**114,629.83 ± 23,404.94**	**114,200.08 ± 23,447.22**	**29,387.6**	**< 0.0001**
Lead[§]	2.40 ± 1.14	2.50 ± 0.78	0.00 ± 0.78	0.0	0.607
Lithium[†]	1.40 ± 1.95	2.00 ± 2.30	0.25 ± 0.94	17.9	0.985
Magnesium[†]	10,647.60 ± 315.70	10,600.83 ± 398.56	−128.67 ± 509.11	−1.2	0.218
Manganese[‡]	**5.20 ± 0.45**	**1,643.83 ± 275.35**	**1,638.58 ± 275.26**	**31,511.2**	**< 0.0001**
Molybdenum[‡]	**48.00 ± 48.79**	**3,575.50 ± 569.09**	**3,515.50 ± 567.16**	**7,324.0**	**< 0.0001**
Nickel[‡]	**2.00 ± 2.12**	**15,946.42 ± 2,770.42**	**15,943.92 ± 2,769.51**	**797,195.8**	**< 0.0001**
Phosphorus[†]	**26.60 ± 9.81**	**70.29 ± 26.79**	**44.29 ± 26.82**	**166.5**	**0.004**
Potassium[†]	**2,118.00 ± 132.36**	**2,586.71 ± 171.82**	**489.21 ± 162.18**	**23.1**	**< 0.0001**
Selenium[†]	**0.22 ± 0.44**	**1.04 ± 0.69**	**0.77 ± 0.64**	**348.5**	**0.032**
Silicon[‡]	**634.00 ± 217.55**	**1,933.38 ± 250.56**	**1,338.38 ± 238.36**	**211.1**	**< 0.0001**
Silver[§]	0.00	0.00	0.00	0.0	0.000
Sodium[†]	**96,458.00 ± 8,298.75**	**95,941.42 ± 8,473.96**	**−1,958.58 ± 6,064.23**	**−2.0**	**< 0.0001**
Strontium[†]	204.80 ± 14.24	190.83 ± 12.16	−11.67 ± 9.95	−5.7	0.070
Sulfur[†]	9,858.00 ± 2,707.58	8,685.71 ± 1,471.65	−496.79 ± 2,448.86	−5.0	0.512
Uranium[§]	0.20 ± 0.45	0.29 ± 0.46	0.04 ± 0.36	20.8	0.851
Vanadium[†]	**0.60 ± 0.55**	**59.96 ± 12.70**	**59.04 ± 12.66**	**9,840.3**	**< 0.0001**
Zinc[†]	**18.00 ± 8.37**	**34.13 ± 8.78**	**16.63 ± 7.65**	**92.4**	**0.001**
Total	**160,313.82 ± 7,685.23**	**319,923.84 ± 37,781.00**	**158,783.82 ± 34,556.21**	**99.0**	**< 0.0001**
Array components[‡]	**1,486.40 ± 7,702.91**	**160,512.55 ± 31,630.27**	**160,970.43 ± 31,896.70**	**10,829.5**	**< 0.0001**
Essential elements[†]	158,689.22 ± 368.55	159,238.88 ± 9,413.96	−2,212.53 ± 8,230.33	−1.4	0.8013
PTEs[§]	**138.20 ± 49.37**	**172.42 ± 41.38**	**25.92 ± 58.46**	**18.8**	**0.0423**

[§] PTEs: potentially toxic elements were defined to be aluminium, antimony, arsenic, barium, cadmium, lead, silver, and uranium.
[†] Essential elements were defined to be boron, calcium, lithium, magnesium, phosphorus, potassium, selenium, sodium, strontium, sulphur, vanadium, and zinc.
[‡] Array components were defined to be chromium, cobalt, copper, iron, manganese, molybdenum, nickel, and silicon.
Bold indicates a statistically significant difference, $P < 0.05$ (Mann-Whitney U test).

Table 6: Summary of differences between element concentrations after footbath runs with feet and without feet.

Elements (μg/L)	Post-FBS–Pre-FBS no feet Mean ± Std dev	Post-FBS–Pre-FBS with feet Mean ± Std dev	P value
Aluminum[§]	14.17 ± 12.22	5.00 ± 58.43	0.487
Antimony[§]	1.00 ± 0.63	1.21 ± 1.50	0.8859
Arsenic[§]	**4.50 ± 0.84**	**5.58 ± 1.02**	**0.016**
Barium[§]	5.00 ± 5.48	6.04 ± 4.89	0.911
Boron[†]	3.33 ± 5.16	4.04 ± 9.12	0.814
Cadmium[§]	5.50 ± 2.43	8.04 ± 2.80	0.064
Calcium[†]	**1,206.67 ± 893.37**	**−231.04 ± 1,354.99**	**0.02**
Chromium[‡]	**17,286.67 ± 4,239.65**	**23,546.21 ± 4,782.52**	**0.003**
Cobalt[‡]	**248.17 ± 44.02**	**331.05 ± 57.69**	**0.001**
Copper[‡]	253.33 ± 64.39	456.71 ± 318.01	0.162
Iron[‡]	**88,388.17 ± 17,532.11**	**114,200.08 ± 23,447.22**	**0.008**
Lead[§]	0.33 ± 1.37	0.00 ± 0.78	0.909
Lithium[†]	1.17 ± 2.86	0.25 ± 0.94	0.994
Magnesium[†]	**405.00 ± 427.73**	**−128.67 ± 509.11**	**0.024**
Manganese[‡]	**1,235.00 ± 211.83**	**1,638.58 ± 275.26**	**0.001**
Molybdenum[‡]	**2,505.17 ± 438.10**	**3,515.50 ± 567.16**	**0.001**
Nickel[‡]	**11,621.00 ± 2,075.55**	**15,943.92 ± 2,769.51**	**0.001**
Phosphorus[†]	37.33 ± 21.73	44.29 ± 26.82	0.502
Potassium[†]	**−11.67 ± 163.64**	**489.21 ± 162.18**	**< 0.0001**
Selenium[†]	**0.00 ± 0.00**	**0.77 ± 0.64**	**0.010**
Silicon[‡]	**1,003.33 ± 170.37**	**1,338.38 ± 238.36**	**0.003**
Silver[§]	0.00	0.00	
Sodium[†]	**19,190.00 ± 38,454.88**	**−1,958.58 ± 6,064.23**	**0.016**
Strontium[†]	**3.17 ± 10.94**	**−11.67 ± 9.95**	**0.009**
Sulfur[†]	−628.33 ± 1,253.99	−496.79 ± 2,448.86	0.490
Uranium[§]	0.00 ± 0.00	0.04 ± 0.36	0.731
Vanadium[†]	**42.50 ± 11.71**	**59.04 ± 12.66**	**0.005**
Zinc[†]	16.67 ± 10.33	16.63 ± 7.65	0.956
Total	142,837.17 ± 36,748.30	158,783.82 ± 34,556.21	0.2962
Array components[‡]	**122,540.83 ± 24,659.98**	**160,970.43 ± 31,896.70**	**0.0051**
Essential elements[†]	20,265.83 ± 39,878.15	−2,212.53 ± 8,230.33	0.1011
PTEs[§]	30.50 ± 19.85	25.92 ± 58.46	0.8697

[§] PTEs: potentially toxic elements were defined to be aluminium, antimony, arsenic, barium, cadmium, lead, silver, and uranium.
[†] Essential elements were defined to be boron, calcium, lithium, magnesium, phosphorus, potassium, selenium, sodium, strontium, sulphur, vanadium, and zinc.
[‡] Array components were defined to be chromium, cobalt, copper, iron, manganese, molybdenum, nickel, and silicon.
Bold indicates a statistically significant difference, $P < 0.05$ (Mann-Whitney U-test).

the stainless steel. There are PTEs in all of the footbath water Post-FBS regardless of the presence or absence of feet. Sb, As, and Cd were significantly different from the tap water in the Post-FBS without feet sessions; As, Ba, and Cd were significantly different in the Post-FBS with feet sessions. It is difficult to identify the source for the increased elements. Other components of the footbath apparatus represent possible sources. However, since materials analysis of these components was not performed it is difficult to be certain. Regardless, the elevation of PTEs in the sessions without feet strongly suggests that the participants are not the source of PTE elevation in the sessions with feet. This is further supported by the lack of statistically significant change in mean PTEs when with and without feet sessions are compared (Table 6). The overall reduction in total elements present in Post-FBS with each subsequent running of the machine further supports the corrosion idea, as there is less material available to dissociate into the water.

3.3.2. Elimination through Urine. One hypothesis whereby PTE elimination could be supported using the ionic footbath device is through stimulation of an alternate detoxification pathway through the kidneys. To test this hypothesis, 24-hour urine collections were obtained concurrent with the

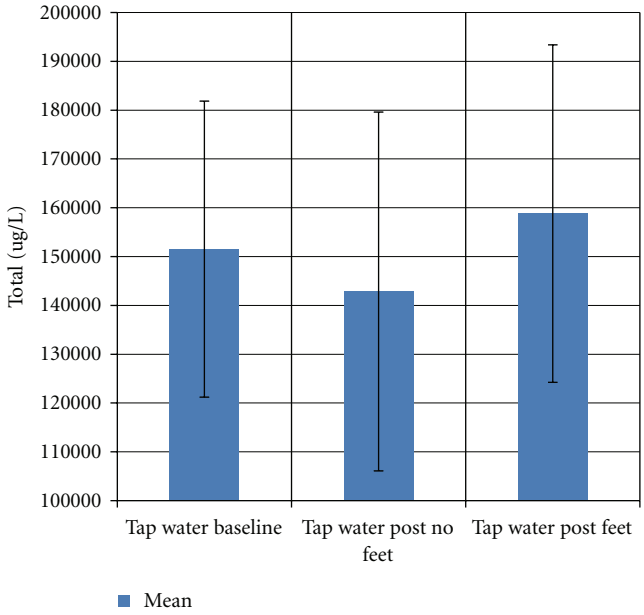
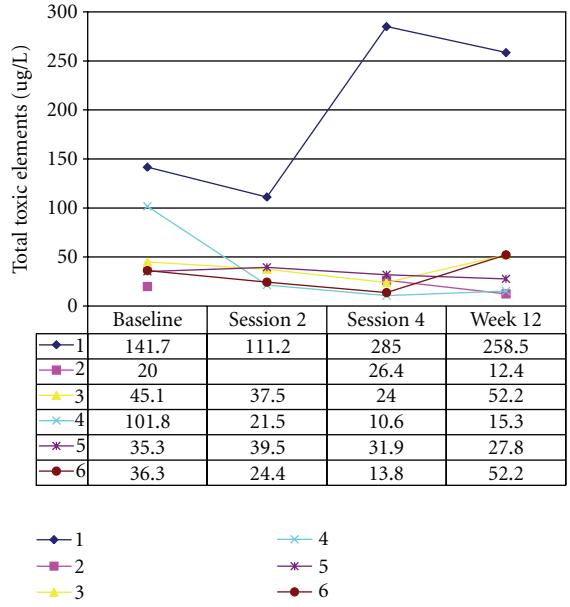


Figure 5: Comparison of mean total PTEs§ (μg/L) in tap water: baseline versus Post-FBS no feet versus and Post-FBS with feet. **Error bars represent ± standard deviation from the mean. §Potentially toxic elements (PTEs) were defined to be aluminium, antimony, arsenic, barium, cadmium, lead, silver, and uranium. **Kruskal-Wallis test found no difference between the three groups ($P = 0.524$).



	Baseline	Session 2	Session 4	Week 12
1	141.7	111.2	285	258.5
2	20		26.4	12.4
3	45.1	37.5	24	52.2
4	101.8	21.5	10.6	15.3
5	35.3	39.5	31.9	27.8
6	36.3	24.4	13.8	52.2

Figure 6: Total PTEs§ excreted in urine for each participant. §Total potentially toxic elements (PTEs) were defined to include aluminium, antimony, arsenic, barium, beryllium, cadmium, mercury, lead, and uranium.

second and fourth footbath sessions. If the hypothesis was correct, increased elimination resulting in elevated urinary total PTEs in sessions two and four should have been evident over and above baseline. This was not found to be the case. While some variance between participants is evident, during the 4 weeks where participants were receiving footbaths there were no clinically relevant changes in the elimination of PTEs that cannot be differentiated from normal fluctuations in excretion via urinary pathways. It is unclear why results for Participant-1 appeared as an outlier to the general trend in the other participants. Given these results, exposure to four sessions of ionic footbath did not appear to have any substantive influence over the body's ability to eliminate PTEs through the urine.

3.3.3. Hair Mineral Analysis. Hair is a stable medium that records which elements are circulating in the blood, and there is evidence that toxic elements in hair are representative of toxic element levels in the internal organs [51, 52]. Hair grows at the rate of approximately 1 cm per month [41]. Hair also represents a meagre but still possible route of excretion as elements incorporated into the hair shaft are removed from circulation. To test for any changes in PTEs in the hair of participants having the ionic footbath, hair samples for analysis were provided at baseline and Week 12 of the study. We hypothesized that if, because of the ionic footbaths, detoxification pathways related to PTEs were stimulated, there would be elevated levels of these elements in the hair at Week 12 compared to baseline. The difference in

Table 7: Composition of grade 316 stainless steel.

Element	Percentage composition
Chromium	16–18%
Nickel	10–14%
Molybdenum	2-3%
Manganese	2%
Silicon	1%
Carbon	0.08%
Phosphorus	0.045%
Sulfur	0.03%

[47]

toxic elements at Week 12 for all participants but one showed essentially no change. Participant-6's total PTEs at Week 12 was substantially higher over baseline. When compared to Participant-6's urine, the increased level of PTEs in the hair was not offset by a concomitant increase in urinary excretion of toxic elements. The high toxic element findings in the hair may have reflected a redistribution of toxic elements in the body or contamination of the hair sample that we were unable to identify.

3.3.4. Strengths and Limitations. In this trial, we tested the application of the IonCleanse SOLO ionic footbath across the lifespan of an array amongst six individuals. Each participant was exposed to four footbath sessions. It is conceivable that a larger number of sessions are required to see an overall detoxification effect in the individual; however, the lack of observable changes in PTEs in the water that might be attributed to a person seems unlikely. If there was any

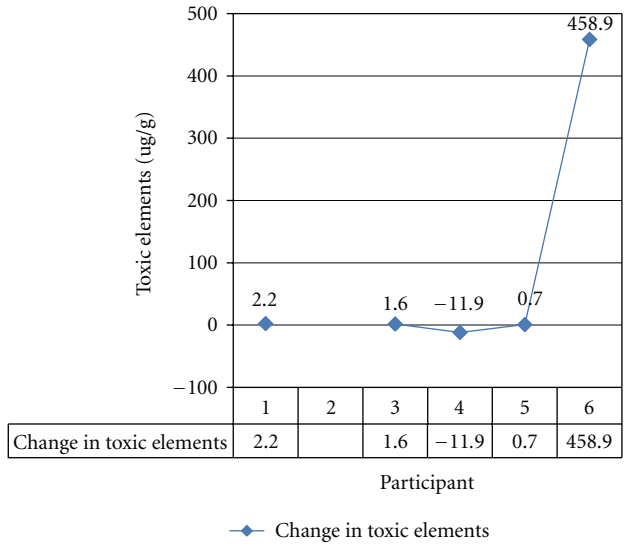


Figure 7: Change in total PTE[§] (μg/L) in hair: baseline to Week 12. [§]Total potentially toxic elements (PTEs) were defined to include aluminium, antimony, arsenic, barium, beryllium, cadmium, mercury, lead, and uranium.

resistance to effect from a single exposure this was accounted for with multiple exposures over the course of one month.

In addition to testing for possible stimulation of physiological detoxification pathways, we also analyzed pre- and postexposure samples for both urine and hair in each participant. By testing and comparing three possible routes of elimination (feet, urine, and hair) we went beyond the implied claims of direct elimination through the feet by exploring other possible routes of elimination. Budgetary constraints precluded us from examining elimination through the colon as stool. It is possible that detoxification through the liver and bile could have been augmented with exposure. However, as both urinary excretion and HMA did not uncover any significant changes in these routes of elimination over the course of treatment and due to a lack of biological rationale it is unlikely that a liver specific elimination would be stimulated either.

The outcome of primary importance in this study, toxic element concentrations, depends on accurate measurements with low intertest variability. A strength of this study was the quality analysis performed by an independent laboratory following good laboratory practices with expertise in water, urine, and hair mineral analysis. The laboratory was blinded to the source of the water being tested and to the protocol from which sequential participant urine and hair samples were taken.

This was a proof-of-principle study with a small sample size. The small sample size would not permit us to identify small shifts in the elimination of PTEs through the utilization of the ionic footbath device. It is possible that a larger study may be able to identify clinical significant differences. Further, we tested healthy participants (self-defined and suffering from no major diseases), and it is conceivable that, in people with high levels of toxicity, application of the ionic footbath could have led to increased elimination either directly or indirectly.

We did not perform materials testing on all of the components of the ionic footbath device. As such, we were not able to confirm other potential sources of PTEs that might be contributing to the changes in toxic elements observed between Pre-FBS and Post-FBS without feet. We hypothesized that the elements found in the residual water could come from the array, salt, plastic storage container, or the plastic liner of the foot tub.

4. Conclusions

In this proof-of-principle study we found no evidence to suggest that ionic footbaths help promote the elimination of toxic elements from the body through the feet, urine, or hair. While unlikely to cause harm or result in any increased uptake, the use of ionic footbaths may release minute quantities of PTEs into the aqueous environment.

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References

[1] Environmental Working Group, "Study: Chemicals, Pollutants Found in Newborns 2009," December 2009, http://www.ewg.org/node/28365.

[2] W. J. Crinnion, "Environmental medicine, Part 1: the human burden of environmental toxins and their common health effects," *Alternative Medicine Review*, vol. 5, no. 1, pp. 52–63, 2000.

[3] D. L. Davis, G. E. Dinse, and D. G. Hoel, "Decreasing cardiovascular disease and increasing cancer among whites in the United States from 1973 through 1987: good news and bad news," *Journal of the American Medical Association*, vol. 271, no. 6, pp. 431–437, 1994.

[4] J. Brinkel, M. H. Khan, and A. Kraemer, "A systematic review of arsenic exposure and its social and mental health effects with special reference to Bangladesh," *International Journal of Environmental Research and Public Health*, vol. 6, no. 5, pp. 1609–1619, 2009.

[5] D. Seely, K. Cooley, and H. Fritz, "Links between ADHD and environmental pollutants: implications for preventative

naturopathic clinical practice," *Townsend Letter*, vol. 307/308, pp. 83–88, 2009.

[6] A. Adenugba, S. A. Khan, S. D. Taylor-Robinson et al., "Polychlorinated biphenyls in bile of patients with biliary tract cancer," *Chemosphere*, vol. 76, no. 6, pp. 841–846, 2009.

[7] F. Falck Jr., A. Ricci Jr., M. S. Wolff, J. Godbold, and P. Deckers, "Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer," *Archives of Environmental Health*, vol. 47, no. 2, pp. 143–146, 1992.

[8] H. Mussalo-Rauhamaa, E. Hasanen, H. Pyysalo, K. Antervo, R. Kauppila, and P. Pantzar, "Occurrence of beta-hexachlorocyclohexane in breast cancer patients," *Cancer*, vol. 66, no. 10, pp. 2124–2128, 1990.

[9] G. N. Wogan, S. S. Hecht, J. S. Felton, A. H. Conney, and L. A. Loeb, "Environmental and chemical carcinogenesis," *Seminars in Cancer Biology*, vol. 14, no. 6, pp. 473–486, 2004.

[10] J. M. Weiss, O. Bauer, A. Blüthgen et al., "Distribution of persistent organochlorine contaminants in infertile patients from Tanzania and Germany," *Journal of Assisted Reproduction and Genetics*, vol. 23, no. 9-10, pp. 393–399, 2006.

[11] R. Kumar, N. Pant, and S. P. Srivastava, "Chlorinated pesticides and heavy metals in human semen," *International Journal of Andrology*, vol. 23, no. 3, pp. 145–149, 2000.

[12] G. S. Cooper, D. Germolec, J. Heindel, and M. Selgrade, "Linking environmental agents and autoimmune diseases," *Environmental Health Perspectives*, vol. 107, supplement 5, pp. 659–660, 1999.

[13] S. Bradberry, T. Sheehan, and A. Vale, "Use of oral dimercaptosuccinic acid (succimer) in adult patients with inorganic lead poisoning," *QJM*, vol. 102, no. 10, pp. 721–732, 2009.

[14] S. Bradberry and A. Vale, "Dimercaptosuccinic acid (succimer; DMSA) in inorganic lead poisoning," *Clinical Toxicology*, vol. 47, no. 7, pp. 617–631, 2009.

[15] Gold Standard Inc, "Succimer (Chemet—DMSA) Monograph.," 2009, http://www.mdconsult.com/das/pharm/body/280300990-809/0/full/1569.

[16] H. L. Johnson and H. I. Maibach, "Drug excretion in human eccrine sweat," *Journal of Investigative Dermatology*, vol. 56, no. 3, pp. 182–188, 1971.

[17] J. R. Cohn and E. A. Emmett, "The excretion of trace metals in human sweat," *Annals of Clinical and Laboratory Science*, vol. 8, no. 4, pp. 270–275, 1978.

[18] A Major Difference Inc., "AMD Making a Difference in your health. [brochure]," December 2010.

[19] Detox Foot Spas, October 2010, http://www.reikitoronto.com/detox.htm.

[20] Home of the Aqua Chi, October 2010, http://www.aquachifootbath.com/.

[21] Why are Seawalk arrays the best on the market?, October 2010, http://www.seawalkspa.ca/array.html.

[22] Center for Research Strategies Inc, "Evaluation of heavy metal levels in relation to ionic foot bath session with Ion Cleanse," 2008, http://www.ahrfoundation.net/dlfiles/study_results.pdf.

[23] EMC Integrity, Inc., "Ion Cleanse CE Safety Test Report," Longmount 2008, http://www.amajordifference.com/store/item.asp?ITEM_ID=4.

[24] EMC Integrity, Inc., Full Compliance Immunity Test Report. Longmount, Colorado April 2008, http://www.amajordifference.com/store/item.asp?ITEM_ID=4.

[25] EMC Integrity, Inc., Radiation and Conduction emissions. Longmount 2008, http://www.amajordifference.com/store/item.asp?ITEM_ID=4.

[26] G. Nordberg, *Handbook on the Toxicology of Metals*, Academic Press, Boston, Mass, USA, 2007.

[27] S. C. Bondy, "The neurotoxicity of environmental aluminum is still an issue," *NeuroToxicology*, vol. 31, no. 5, pp. 575–581, 2010.

[28] S. V. Verstraeten, L. Aimo, and P. I. Oteiza, "Aluminium and lead: molecular mechanisms of brain toxicity," *Archives of Toxicology*, vol. 82, no. 11, pp. 789–802, 2008.

[29] T. Gebel, "Aresnic and antimony: comparative approach on mechanistic toxicology," *Chemico-Biological Interactions*, vol. 107, no. 3, pp. 131–144, 1997.

[30] M. D. Pulido and A. R. Parrish, "Metal-induced apoptosis: mechanisms," *Mutation Research*, vol. 533, no. 1-2, pp. 227–241, 2003.

[31] S. Khandpur, A. K. Malhotra, V. Bhatia et al., "Chronic arsenic toxicity from Ayurvedic medicines," *International Journal of Dermatology*, vol. 47, no. 6, pp. 618–621, 2008.

[32] V. K. Sharma and M. Sohn, "Aquatic arsenic: toxicity, speciation, transformations, and remediation," *Environment International*, vol. 35, no. 4, pp. 743–759, 2009.

[33] S. S. Wise, J. H. C. Schuler, S. P. Katsifis, and J. P. Wise, "Barium Chromate Is Cytotoxic and Genotoxic to Human Lung Cells," *Environmental and Molecular Mutagenesis*, vol. 42, no. 4, pp. 274–278, 2003.

[34] G. Cannino, E. Ferruggia, C. Luparello, and A. M. Rinaldi, "Cadmium and mitochondria," *Mitochondrion*, vol. 9, no. 6, pp. 377–384, 2009.

[35] H. Czeczot and M. Skrzycki, "Cadmium–element completely unnecessary for the organism," *Postpy Higieny i Medycyny Doświadczalnej*, vol. 64, pp. 38–49, 2010.

[36] H. C. Gonick, "Nephrotoxicity of cadmium & lead," *Indian Journal of Medical Research*, vol. 128, no. 4, pp. 335–352, 2008.

[37] P. L. Drake and K. J. Hazelwood, "Exposure-related health effects of silver and silver compounds: a review," *Annals of Occupational Hygiene*, vol. 49, no. 7, pp. 575–585, 2005.

[38] A. B. G. Lansdown, "Critical observations on the neurotoxicity of silver," *Critical Reviews in Toxicology*, vol. 37, no. 3, pp. 237–250, 2007.

[39] X. Arzuaga, S. H. Rieth, A. Bathija, and G. S. Cooper, "Renal effects of exposure to natural and depleted uranium: a review of the epidemiologic and experimental data," *Journal of Toxicology and Environmental Health—Part B*, vol. 13, no. 7-8, pp. 527–545, 2010.

[40] W. Briner, "The toxicity of depleted uranium," *International Journal of Environmental Research and Public Health*, vol. 7, no. 1, pp. 303–313, 2010.

[41] M. Villain, V. Cirimele, and P. Kintz, "Hair analysis in toxicology," *Clinical Chemistry and Laboratory Medicine*, vol. 42, no. 11, pp. 1265–1272, 2004.

[42] K. J. Cummings, A. B. Stefaniak, M. A. Virji, and K. Kreiss, "A reconsideration of acute beryllium disease," *Environmental Health Perspectives*, vol. 117, no. 8, pp. 1250–1256, 2009.

[43] D. M. Hollins, M. A. McKinley, C. Williams et al., "Beryllium and lung cancer: a weight of evidence evaluation of the toxicological and epidemiological literature," *Critical Reviews in Toxicology*, vol. 39, no. 1, supplement, pp. S1–S32, 2009.

[44] W. J. Crinnion, "Environmental medicine, part three: long-term effects of chronic low- dose mercury exposure," *Alternative Medicine Review*, vol. 5, no. 3, pp. 209–223, 2000.

[45] C. Freire, R. Ramos, M. J. Lopez-Espinosa et al., "Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain," *Environmental Research*, vol. 110, no. 1, pp. 96–104, 2010.

[46] J. J. Wirth and R. S. Mijal, "Adverse effects of low level heavy metal exposure on male reproductive function," *Systems*

Biology in Reproductive Medicine, vol. 56, no. 2, pp. 147–167, 2010.

[47] R. A. Lula, "Stainless steel," *American Society for Metals*, 1986.

[48] R. A. Lula and J. G. Parr, *Stainless Steel*, Edited by R. A. Lula and J. G. Parr, American Society for Metals, Materials Park, Ohio, USA, 1986.

[49] B. A. Shaw and R. G. Kelly, "What is corrosion?" *Electrochemical Society Interface*, vol. 15, no. 1, pp. 24–26, 2006.

[50] Department of Energy, *DOE Fundamentals Handbook*, Department of Energy, 1993.

[51] C. R. Robbins, *Chemical and Physical Behavior of Human Hair*, Springer, New York, NY, USA, 4th edition, 2002.

[52] T. H. Maugh II, "Hair: a diagnostic tool to complement blood serum and urine," *Science*, vol. 202, no. 4374, pp. 1271–1273, 1978.

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Review Article

What's Out There Making Us Sick?

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Throughout the continuum of medical and scientific history, repeated evidence has confirmed that the main etiological determinants of disease are nutritional deficiency, toxicant exposures, genetic predisposition, infectious agents, and psychological dysfunction. Contemporary conventional medicine generally operates within a genetic predestination paradigm, attributing most chronic and degenerative illness to genomic factors, while incorporating pathogens and psychological disorder in specific situations. Toxicity and deficiency states often receive insufficient attention as common source causes of chronic disease in the developed world. Recent scientific evidence in health disciplines including molecular medicine, epigenetics, and environmental health sciences, however, reveal ineluctable evidence that deficiency and toxicity states feature prominently as common etiological determinants of contemporary ill-health. Incorporating evidence from historical and emerging science, it is evident that a reevaluation of conventional wisdom on the current construct of disease origins should be considered and that new knowledge should receive expeditious translation into clinical strategies for disease management and health promotion.

An analysis of almost any scientific problem leads automatically to a study of its history.
—Ernst Mayr

1. Introduction

Greek philosophers including Thales and Aristotle sought logical, sensible, and cogent explanations for the spectrum of human experience, for everyday events, and for the way the world works [1]. From the times of ancient Greek civilization, through the middle ages, and into our current technological age, thinkers and scientists have pondered and sought answers as to why people get sick. From its fundamental genesis in philosophy, with emphasis on skepticism and critical thought, modern medical science has emerged with conclusions about the etiology of suffering and disease.

In this paper, a few snapshots of medical history illuminating the origins of illness will initially be presented. Through the lens of history and emerging science, current conventional wisdom about disease etiology will then be examined. Finally, evidence in disciplines such as molecular medicine, epigenetics, and environmental health will be explored to explain the root cause of chronic and degenerative disease, a problem that afflicts so many in the world today.

2. Historical Perspective on Disease Etiology

2.1. The Origins of Modern Medicine. As with every culture including the present, the ancient Greeks believed that they embodied the ultimate in sophistication [1]. As world leaders of progressive thought in philosophy, education, and science, academics in early Greece assumed that events of life including calamity and illness were the result of metaphysical forces and mystical powers. Apollo would send, it was thought, his invisible arrows to inflict pain and suffering on the condemned.

Amidst this milieu, however, a young Greek physician named Hippocrates challenged the popular paradigm that supernatural factors were the driving force behind disease [2]. With a skeptical mindset, Hippocrates scrutinized

conventional medicine of the day, he challenged disease attribution to paranormal factors, and he rejected the accepted medical standard of care—appeasement of mystical forces with chemical concoctions. Searching for rational evidence to explain origins of illness that could be demonstrated through reproducible observation and experimentation, Hippocrates endeavored to convert the field of medicine from a religion to a science [2].

Witnessing health demise after some patients consumed dispensed poisons from bribed practitioners, he penned the Hippocratic Oath to challenge the ethics of corrupt physicians [3]. With observation of divergent population health in differing locales, dissimilar individual constitutions from birth, and variations in health related to diet and sun exposure, Hippocrates concluded that nutrition, inborn factors, and environmental influences were major determinants of sickness and health [2]. Building on the fundamental scientific premise that every effect has to have a source cause, he surmised that perhaps if the cause of illness was found, then disease might be cured. Writing in the Hippocratic Corpus, this young physician and his followers defied both the spirit and the practice of metaphysical traditional medicine.

2.1.1. The Early Years. The basics of science—vigilant observation, empirical experimentation, and reproducible research—were brought into the ethos of medical practice, a monumental accomplishment which earned Hippocrates the worthy title of "Father of Modern Medicine" [4]. Although some of his interpretations were primitive and misguided, the substantive basis of his scientific approach to understanding the etiology of illness remains credible to this day. He came to believe that disease commences because of a cause, disease persists because the cause persists, and that disease can only desist when the cause desists [2].

Notable scientists in early Common Era centuries continued to observe and explore causes of disease. Galen (circa 130–200 AD), for example, spent his early career doctoring gladiators and noted that those with wounds often became ill and frequently succumbed. Hypothesizing that wounds provided "windows to the body," Galen deduced that unhealthy vapors rising from the ground formed poisonous gases which entered through wounds to cause illness [5]. Although various theories and ideas emerged over the next few centuries, limited original contribution relating to disease causation was recorded until the Middle Ages [4, 5].

Throughout the early centuries, however, the metaphysical construct of disease causation, a mindset engrained in the fabric of many cultures, continued to pervade medical practice. Some afflicted individuals, for example, were executed as demonic possession was often considered the source of mental illness and aberrant behavior. Black Death, the plague which consumed countless lives in the fourteenth century, was oft blamed on the Jews—an attribution which spurred violence and prompted reigning Pope Clement VI to issue an edict pronouncing a misalignment of Mars, Jupiter, and Saturn as the true culprit. Commencing in the 16th century, however, a number of notable discoveries confirming Hippocrates' notion about natural causes of illness began to emerge.

(1) Toxicant Exposures. Immortalized as Paracelsus, the "Father of Toxicology," Auroleus Phillipus Theostratus Bombastus von Hohenheim worked in the 1500s as an alchemist, astrologer, and physician. With the observation that use of chemicals such as mercury and opium could change the mental and physical status of individuals, Paracelsus introduced the idea that disease was the result of a chemical imbalance [6]. With much experimentation, he pioneered the use of elements and chemical compounds in medicine.

Treated as an outcast and heretic by the established medical community, Paracelsus noted that, at low dose, certain compounds appeared to be therapeutic, while at larger dose they acted as poisons [6]. The emergence of medicine by alchemy increasingly became the standard of care with assorted toxic elements including mercury, lead, and arsenic being used by practitioners to deal with myriad afflictions from fatigue to syphilis.

Paracelsus affirmed Hippocrates' observation, however, that chemical toxins had the potential to act as a poison and to induce illness if a threshold dose was exceeded. Paracelsus' defining publication, *On the Miners' Sickness and Other Diseases of Miners,* documented occupational risks associated with exposures during metalworking [7]. In conclusion, exposure to chemical toxins was identified as a cause of sickness and death.

(2) Nutritional Deficiency. A major breakthrough in medicine occurred on the high seas with the British Royal Navy. Initially described by Hippocrates more than two thousand years ago, a disease called scurvy consumed many passengers and crew on long-distance voyages. A Scottish surgeon, Dr. James Lind, puzzled as to why some of his crew would succumb to this treacherous disease while others did not. Wondering whether dietary habits might be a factor in illness, Lind prescribed different diets for individuals deteriorating with scurvy, and, as described in his 1753 book, *A Treatise of the Scurvy,* he found that citrus fruit rapidly and consistently cured this previously fatal malady [8].

But as consistently occurs in medicine when new ideas and scientific discoveries regarding disease causation are offered—no matter how compelling the evidence—Lind's findings were initially mocked and disregarded. Only after decades passed did the British navy and the medical world at large accept his evidence in order to stop the flood of needless scurvy deaths. In conclusion, deficiency of some essential nutrient or nutrients was recognized as a cause of sickness and death.

(3) Genetics. Initially described as a monster for his findings, Austrian monk and scientist Gregor Johann Mendel observed evidence of logical transmission of inherited traits from one generation to the next in his experiments with pea plants. Mendel, subsequently titled the "Father of Modern Genetics,"

repeatedly demonstrated in the nineteenth century that inheritance patterns were consistent and followed particular laws [9].

Although Mendel's work was initially met with disdain and rejection, subsequent research after his death demonstrated a logical bond that transmitted through generations, not only in plants but also in the animal kingdom. Mendel's findings spurred further study and eventually became the foundation of modern genetics—a discipline which has repeatedly confirmed "genetic predisposition" as an important factor in the causation of illness.

(4) The Germ Theory. One of the most remarkable discoveries contributing to the discourse on disease etiology relates to the finding of pathogens or disease-causing germs [4]. At a time when more than 20% of women died in childbirth, a young Hungarian obstetrician named Ignaz Philipp Semmelweis noted that impoverished women delivering outside of hospitals had a maternal mortality rate only a fraction of that for women receiving hospital care. Also observing that maternal death rates plummeted when medical students were absent and birthing was assisted by midwives, Semmelweis comparatively investigated approaches by students and midwives [10].

Noting that medical trainees proceeded from anatomy labs to obstetric suites, he hypothesized that some pathogenic agent may be carried to the maternity area and thus introduced a hand washing technique. When maternal deaths precipitously fell overnight, this pioneer realized that he had uncovered the cause of puerperal fever. Careful documentation of evidence and desperate appeals to colleagues to replicate his work only evoked scorn and contempt.

Witnessing sickness and death from infection complicating surgery or open fractures, work in the nineteenth century by French chemist and microbiologist Louis Pasteur, "Father of the Germ Theory," added to mounting evidence of the link between microbial agents and sickness [11]. Along with other pioneers in microbiology, including Ferdinand Cohn and Robert Koch, it became apparent in the late 19th century, that pathogens were a common cause of sickness—a realization that provoked a temporary shift in conventional wisdom whereby the causation of most disease was attributed to germs [4].

(5) Psychological Determinants. During the 19th and early 20th centuries, Sigmund Freud, Carl Young, Abraham Maslow, Ivan Pavlov, and other innovators theorized at length on psychological mechanisms leading to ill-health [12]. Although many of the specific mechanisms proposed such as Freud's theory of psychosexual stages of development are now in question, the idea that psychological pathology can contribute to ill-health has repeatedly been confirmed. More recent laboratory study has found dramatic changes in physiological parameters and indices in response to psychological states, leading the medical community to accept disordered psychology as a potential source of sickness.

2.1.2. Historical Overview of Disease Causation. There are many other notable heroes in medical history who have contributed to the understanding of health and disease [4, 5]. For example, Christiaan Eijkman won the Physiology and Medicine Nobel Prize in 1929 for his discovery, at a time when everyone was looking for a germ, that beriberi resulted from deficiency of an essential nutrient (thiamine) absent in the polished white rice of European settlers stationed in the Orient [13]. On careful analysis, however, each of the other findings and discoveries on disease etiology represented further developments and clarifications on these five determinant themes—nutritional deficiency, toxic exposures, genetic predisposition, infectious agents, and psychological dysfunction (Figure 1). These five pillars of disease etiology have repeatedly been demonstrated historically to be the source of all illness. So how does modern medicine view the etiology of illness in view of this body of accumulated historical science?

3. Contemporary Beliefs about Disease Etiology

To best determine how contemporary medicine views the origins of illness, it is instructive to observe how mainstream medicine is practiced and to explore underlying assumptions. A typical algorithm (Figure 2) is used when patients with chronic disease visit their physician [14], an approach which reflects clinical practice guidelines—pervasive administrative directives used to guide the actions of individual physicians [15].

Through an interview, physical examination, and laboratory testing, the physician does an assessment in order to determine the appropriate "diagnosis"—a label which indicates that the patient's signs, symptoms, and laboratory results match or fulfill common criteria for that label [16]. After diagnosis has been assigned, it is common for intervention to commence, frequently employing medications or surgery. For chronic conditions, which now form the overwhelming burden of illness globally, patients usually persist with therapy indefinitely to cope with their sickness. But what does this algorithm tell us about prevailing assumptions regarding the cause of sickness?

3.1. Predestination Construct. As the diagnosis does not assign any source cause or reason for the development of the condition, a search for cause in this algorithm remains neglected. As deliberate neglect of disease causation might be considered remiss and unscientific, why is etiology not actively pursued as a fundamental step in the approach to sick patients?

Most practitioners assume that, other than situations of infection or psychological compromise, source etiology of most chronic illness reflects genetic fate—the idea that people are predestined as hapless victims in a cosmic game of genetic roulette. This genetic predestination paradigm leaves no alternative but to provide drugs and surgery to overcome the misfortune of having the wrong parents [17]. Through

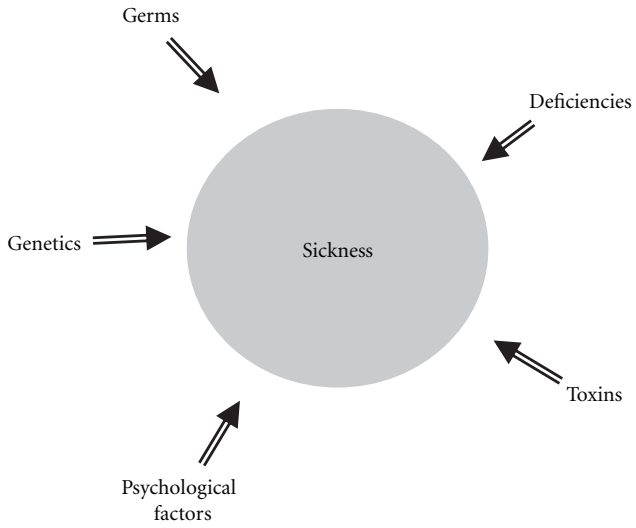

Figure 1: Sum total of etiological determinants of illness.

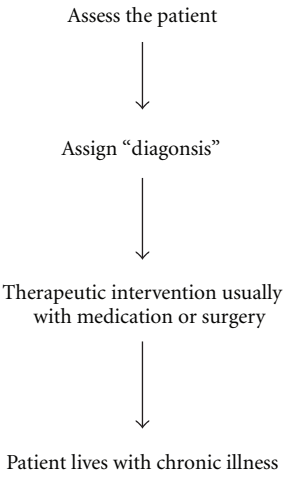

Figure 2: Common algorithm for management of contemporary chronic illness.

a historical lens, however, this contemporary fatalistic approach disregards the deficiency and toxicity components as common causative factors in disease (Figure 3) [18].

Five fundamental observations in recent science and epidemiology literature, however, have begun to challenge the inherited or genetic predestination paradigm.

(i) Identical twins with the same genome frequently have different health outcomes [19].

(ii) Many people develop chronic conditions that are absent in their ancestry.

(iii) While genomes have not changed, rates of various chronic afflictions including autism, depression, dementia, and some types of cancer have escalated considerably.

(iv) Geographic differences for various chronic diseases are evident [20].

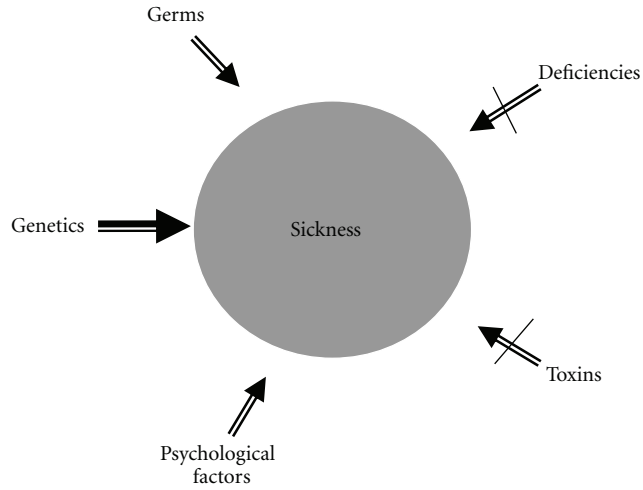

Figure 3: General perception in contemporary clinical practice about common etiological determinants of chronic illness in the Western World.

(v) Disease incidence among population groups often changes significantly with migration and adoption of new lifestyles [21].

It is unlikely that genetics accounts for the more than 2500% increase in autism [22] or the profound increase in hysterectomies performed over the last 25 years [23]. Genetics is not likely to account for the notable increase in heart disease and diabetes among Japanese immigrants settling in America or the increased likelihood of acquiring autoimmune disease for populations residing in northern latitudes. Some have attributed the prevalence of chronic illness simply to an aging population, but the recent escalation of chronic disease in pediatric populations [24] refutes this misconception. While genetics may predispose to illness, deductive reasoning suggests that other factors must be influencing health status.

4. Why Are People Getting Sick?

4.1. Molecular Medicine: Genomics. Recognizing that the human organism is fundamentally a community of specialized cells made up of countless molecules, the scientific discipline of molecular medicine endeavors to gain insights into the genetic, molecular, and cellular bases of disease. The human genome project has confirmed that each of us is unique genetically, and thus our biological functioning at a molecular level is not identical [25]. This breakthrough has spawned the expanding field of genomics.

The way we respond to our environment, medications, and stressors will depend on our specific genetic imprint. Accordingly, broad-based conclusions on the efficacy of certain treatments may be less than reliable when applied to specific individuals—each individual with a distinct biochemistry will respond differently to each medication based on their genetic makeup. The fields of pharmaco-genomics and nutrigenomics have recently emerged where medication and

nutrient interventions are personalized and tailored to the specific genomic state of the individual [26, 27].

With new laboratory investigations, the unique genetic and biochemical makeup of the individual can be assessed in order to determine irregularities at a molecular level that may be influencing health. Individual genomic assessments may provide evidence for predisposition to various afflictions. BRCA1, for example, is a genetic marker which may indicate predisposition to breast cancer [28]. The expanding repertoire of genetic markers confirms that genetic predisposition to sickness is a scientific reality. With the inability to modify human genes thus far, however, our genetic map is fixed and thus our predisposition to sickness is immutable. There is another force that will be discussed, however, which appears to control whether our predisposition to a specific sickness will manifest as disease or remain quiescent and manifest as health.

4.2. Molecular Medicine: Environmental Health Sciences. A recent edition of *Science* highlighted the emerging reality that "chronic illness is the consequence of inherited diversity of the genetic code combined with environmental biochemical influence" [29], while the Centers for Disease Control and Prevention recently concluded that "virtually all human diseases result from the interaction of genetic susceptibility and modifiable environmental factors" [30]. Ongoing scientific research has repeatedly confirmed that various modifiable factors within the environment of our body have the ability to interact with our genetic predisposition to cause sickness.

One way that environmental factors cause illness is through gene regulation. A discipline within the field of molecular medicine called epigenetics endeavors to study factors and identify determinants which regulate and control the expression of genes [31]. In other words, science is demonstrating that genes are not autonomous structures which determine fate but rather are molecules which respond to and are often regulated by modifiable environmental triggers. A loaded gun will not cause damage unless triggered; a vulnerable gene may remain dormant unless triggered by specific factors within the terrain of the body. The impact of epigenetic environmental influences makes genetic expression a dynamic reality with new evidence demonstrating the potential to transmit adverse genomic expression and clinical pathology through generations [32, 33].

The study of environmental health sciences or environmental medicine is the clinical application of molecular and epigenetic medicine. It allows for the study of the modifiable environment in order to identify and correct abnormalities that are triggering sickness. But what are these modifiable factors in our environment that have the ability to interact with genes to cause sickness?

4.3. The Blind Spots. Broadly speaking, there are only two factors in the environmental sphere: (i) requirements—are we getting what we need in order to thrive; and (ii) toxins—are we free of adverse influences. Evidence continues to accumulate that the main environmental determinants of illness are deficiency and/or toxicity states interacting with a fixed genome (Figure 4) [34]. In other words, if we are missing essential components that our human body requires in order to function, illness results; if there are adverse factors obstructing or interfering with normal biological function, illness results. This principle is eminently sensible as it applies to all machines as well as plant life. If a plant is to thrive, certain essential requirements are required, all of which are requisite to plant survival. If specific toxicants are introduced, the plant may wither.

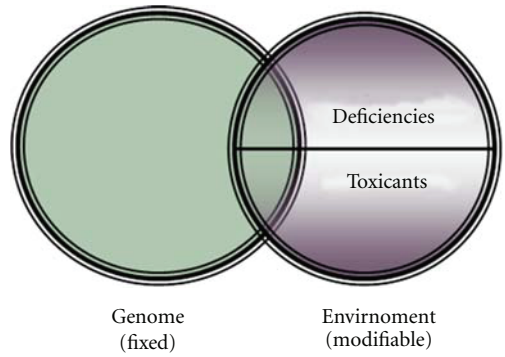

Figure 4: Etiology of illness.

Observing through the lens of history, these two determinants of health and disease are precisely the two areas neglected in much of contemporary medical practice (Figure 3). Why have deficiency and toxicity concerns, domains so clearly and repeatedly identified in medical history as causative in illness, been virtually disregarded in much of present-day conventional medicine?

4.4. Deficiency States as a Cause of Illness. Nutrient biochemicals are the building blocks of our human frame and the necessary prerequisites for ongoing physiological function [35]—we are a collection of biochemicals. Using nutrient raw materials, our body manufactures all the compounds required for life and sustenance. Our body can only thrive if we have the required nutrients to carry out our basic necessary biology. Simple logic suggests that a deficiency of essential raw nutrient materials precludes the ability of our body to make what it needs to undertake the required physiological processes of daily life—resulting in malfunction of the human machine and clinical sickness. There is an abundance of recent evidence in the scientific and laboratory literature expounding on the consequences of nutritional deficiency [36].

One could hardly imagine a student of architecture graduating from a reputable school without comprehensive knowledge of building materials, how such materials are used, how to detect problems, and how to correct irregularities. If detailed knowledge of nutritional biochemistry is so fundamental to the practice of health care, why has instruction on nutritional status assessment and nutritional remediation not been taught in most medical schools? [37]. Many in the health science community have assumed that

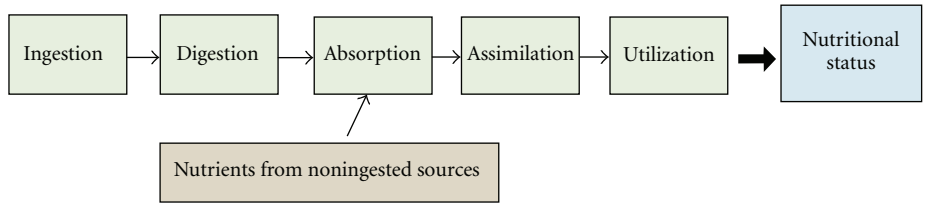

Figure 5: Determinants of Nutritional Status.

nutritional compromise cannot be a common determinant of illness in the developed world because they believe that most people are "getting all they need in their diet." Accordingly, it has not seemed prudent to waste valuable time teaching nutritional biochemistry and clinical nutrition if this is not a common cause of ill-health.

The fundamental flaw in this assumption is that nutritional status is not the same as food intake. Nutritional status commences with ingestion, but requires digestion, absorption, and assimilation—dysfunction occurring anywhere along the chain can result in metabolic compromise and disordered biology (Figure 5). Furthermore, some essential nutrients are primarily derived from noningested sources, such as vitamin D from sun exposure and vitamin K_2 from enteric organisms. Emerging research confirms that significant nutritional deficiency of required materials is much more common than recognized and a ubiquitous cause of sickness [38]. Examples include vitamin D and some required lipids, recognized regulators of hundreds of genes, which have been found in several recent epidemiological studies to be deficient in many population groups [39, 40].

To demonstrate the clinical and public health significance of addressing deficiency states, it is illuminating to comparatively consider the projected benefit of diminished morbidity and mortality associated with widespread "national bowel cancer screening programs" versus maintenance of optimal nutrient status. Recent projections suggest that early detection methods and screening will reduce colorectal cancer mortality in those screened by 12–17% over the next 20 years [41]—a figure considered to be of notable significance when applied to large-scale populations. On the other hand, a large prospective study of colon cancer risk based on levels of 25(OH)D was published in *Lancet*. Assessing more than 25,000 participants, there was a 75–80% reduction in risk of ever developing colon cancer for those with higher levels of 25(OH)D compared to those with low levels [42]. Incorporation of a vitamin D strategy might yield favorable outcomes that far exceed any bowel cancer screening program; the profound health impact of remediating deficient biochemistry is evident.

4.5. Toxicity as a Cause of Illness. Since the Second World War, a chemical revolution has emerged in an effort to provide enhanced convenience, efficiency, beauty, comfort, and safety [43]. Affecting many aspects of our everyday lives, myriad synthetic chemicals are increasingly being found in our foods, our air, our water, and our bodies. Recent population studies by the Centers for Disease Control [44] as well as cord blood research undertaken by the American Red Cross [45] confirm widespread toxicant bioaccumulation in men, women, children, and the developing unborn. Research to understand and address the impact of these compounds on human health has confirmed that accrual of various toxic agents has become a widespread cause of disease [46]. At minute levels, toxicant compounds have potential to influence critical biological function in many ways such as by hormone disruption, immune dysregulation, cell damage, genetic influence, allergy induction, liver compromise, and cancer promotion [47]. Numerous afflictions, ranging from congenital malformations to cancer to hormonal irregularities, have recently been linked to adverse toxicant exposures [46].

Agents and forces that are toxic to the human body do not only include adverse chemical compounds but also encompass other determinants including biological agents [48, 49], physical toxicants [50], metabolic irritants [51], excessive psychological stress [52], and triggers for hypersensitivity or allergic reactions [53] (Table 1). As these different stressors can coexist, it is crucial to explore the total load or total body burden of adverse factors that may be causing illness. With history and emerging science confirming toxicants as a cause of sickness, why has this field been ignored for the most part in contemporary medical education?

As physicians are no longer bribed to poison business or political rivals as occurred in Hippocrates' day, it is assumed that patients are not regularly being exposed to significant levels of toxic agents. So why spend considerable time in medical school dealing with a nonexistent problem? This assumption is misguided, however, as evidenced by a plethora of recent medical literature expounding on exposure and bioaccumulation of toxic exposures as common etiological sources of illness. Many health bodies such as the World Health Organization have recently instituted programs to educate health practitioners about this growing concern [54, 55]. Emerging techniques and interventions to diagnose and eliminate accrued or persistent toxicants can have a profound impact on human health [56].

5. Quo Vadis: Science-Based Medicine

With the realization that irregularities in the modifiable environment of our bodies are the source cause of most chronic illness, the choice to change correctable factors can transform individual destiny. Health providers can facilitate health by

Table 1: Categories comprising the total body burden of potential toxicants.

(1) Chemical toxicants—for example, heavy metals, mycotoxins, and so forth
(2) Biological toxicants—for example, viral agents, fungal exposures, and so forth
(3) Physical toxicants—for example, radiation, trauma, and so forth
(4) Metabolic toxicants—for example, hyperinsulinemia, elevated uric acid, and so forth
(5) Psychological toxicants—for example, inordinate chronic stress, abuse, and so forth
(6) Hypersensitivity toxicants—for example, intolerances such as peanut allergy, and so forth

uncovering factors responsible for disease and advising on a path to prevent illness and restore health [18]. Perhaps at some juncture in the future, technology will deliver humanity to a place where therapeutic epigenetic interventions will be used to suppress pathological genetic expression; at this point in history, however, addressing disease etiology still remains the best opportunity to prevent and overcome chronic affliction.

Is this alternative medicine? Hardly. It is scientific medicine based on perspicacious understanding of medical history, biochemistry, toxicology, infectious disease, immunology, and other mainstream scientific disciplines [18]. Finding out what is causing illness is fundamental to logical scientific medicine. One might expect a mechanic to find the cause of the knock in your engine; patients should expect at least as much from their doctors. Originating from the Latin verb "docere" meaning to teach, the term "doctor" might appropriately describe a trained scientist who educates patients on the cause of their illness and empowers them with instruction on solutions to prevent and overcome health afflictions.

Based on the tried-and-true model of clinical medicine—history, physical, laboratory investigations (including detailed nutritional status and toxicological assessment)—source causes of illness can be discovered and interventions to prevent and address molecular and biochemical irregularities can be implemented. Preventing birth defects by securing adequate folic acid [57], relieving post-partum depression by correcting fatty acid deficiency [40], restoring mental health by eliminating stockpiled toxicants [58–60], reversing some cases of autism by removing incitants and addressing nutritional deficiencies [22, 61], treating pediatric arthritis by managing food intolerances [62], overcoming impairment resulting from some chromosomal anomalies by remediating biochemistry [63], resolving inflammatory bowel disease with avoidance of sensitivities [53], relieving asthma and chronic fatigue by mold remediation [48], ending the tragedy of habitual abortion by addressing electromagnetic toxicity [50], and the author's experience of achieving remission from leukemia in a patient by eradicating retained aflatoxin are all examples of what can possibly be realized if underlying causes of sickness are explored, identified, and properly managed.

There are some who feel that change in health care may soon happen. Von Eschenbach, a former Commissioner in the US Food and Drug Administration recently stated that "[the] transformation… is so profound and so radical that I call it a metamorphosis: a molecular metamorphosis in which the future of health and healthcare will be no more like the past than a butterfly is like a caterpillar… it will alter and change not just one thing; it will change everything" [64]. Some are not so optimistic. It is well recognized historically that progress along the path to change in health care proceeds lethargically and often occurs only after education and empowerment of subsequent generations [65–70]. Many researchers, clinicians, and health administrators of each epoch steadfastly refuse to consider iconoclastic evidence, no matter how compelling; some remain immune to the power of facts—no matter how true, no matter how precise. Indeed, when considering the actualities of evidence-based medicine, the stark reality of trying to persuade clinicians to open their minds to evidence contrary to entrenched beliefs and practices has been likened to the challenge of "teaching old dogs new tricks" [66], leaving some pioneers wondering whether medicine is more about ideology and religion than science [68]. As a result, knowledge translation remains notoriously slow which accounts for Nobel Prize winner Max Planck's sobering observation that science progresses funeral by funeral [71].

In a one-week course in medical school, however, it would be possible to convey the necessary information to competent medical trainees in order to establish the required foundations to investigate and manage patients presenting with chronic illness and to educate aspiring public health candidates to implement programs to prevent illness in population groups. In an era doused by the chemical revolution, medical students need to learn how to explore toxicant categories and to acquire clinical skills to investigate for and eliminate toxic factors. Instruction in nutritional and metabolic biochemistry with practical clinical applications is fundamental to competent medical practice. If a patient is depressed, is there a problem with her/his serotonin pathway such as tryptophan deficiency? Does she/he lack coenzymes or cofactors required to convert tryptophan to 5-HTP and then to serotonin? If so, why, and what can be done about it? If chronic metabolic dysfunction is clinically apparent, is there an acquired error of metabolism because of a toxicant induced enzyme malfunction? What do the laboratory results from the urinary organics testing demonstrate? This is science. The reflexive "have an ill, pop a pill" approach to chronic illness without investigating etiology is hardly consistent with perspicacious medical or clinical science.

6. Concluding Thoughts

Since the dawn of civilization, humankind has sought to explain the phenomenon of illness and affliction. Why is it that one person enjoys robust health, while another suffers?

What is it that transforms a healthy individual from vigor and vitality to pain and chronic disability? Rather than the fatalistic outlook of genetic destiny, ongoing scientific evidence confirms that virtually all illness commences because of modifiable environmental causes, it persists because such environmental causes persist, and it can only abate when such causes are addressed.

So, frankly, why do we get sick? The evidence shows that, although there are myriad ways in which people manifest sickness, there are only a limited number of ways in which people get sick. People get sick because of vulnerable genetics interacting with potentially modifiable factors in their environment. What are these changeable environmental determinants? The expanding body of scientific research in epigenetics, environmental health, and molecular medicine verifies what medical history has repeatedly and consistently confirmed—that deficiency and toxicity states cause disease. These two causative origins, however, remain blind spots in the contemporary approach to ill-health by much of the conventional medical community. Remediation of deficient biochemistry and elimination of toxicants has enormous potential to preclude chronic illness and restore health. Rather than fashionable wisdom that may be obsolete a year or a generation from now, these observations about disease origins and the associated clinical approach represent the accumulated wisdom of cutting-edge medical science through the ages.

Conflict of Interests

There are no conflict of interests.

References

[1] R. E. E Allen, *Greek Philosophy: Thales to Aristotle*, The Free Press, New York, NY, USA, 3rd edition, 1991.

[2] F. Adams, *The Genuine Works of Hippocrates*, William Wood and Company, New York, NY, USA, 1891.

[3] L. Edelstein, *The Hippocratic Oath: Text, Translation and Interpretation*, vol. 19 of *Bulletin of the History of Medicine*, supplement, 1943.

[4] R. Porter, *The Greatest Benefit to Mankind: A Medical History of Humanity from Antiquity to the Present*, Harper Collins, London, UK, 1997.

[5] L. I. Conrad, M. Neve, V. Nutton, R. Porter, and A. Wear, *The Western Medical Tradition: 800 BC to AD 1800*, Cambridge University Press, Cambridge, UK, 1995.

[6] W. Pagel, *Paracelsus:An Introduction to Philosophical Medicine in the Era of the Renaissance*, Karger, New York, NY, USA, 1982.

[7] H. D. Crone, *Paracelsus: The Man Who Defied Medicine : His Real Contribution to Medicine*, Albarello Press, Melbourne, VIC, Australia, 2004.

[8] M. Bartholomew, "James Lind's treatise of the scurvy (1753)," *Postgraduate Medical Journal*, vol. 78, no. 925, pp. 695–696, 2002.

[9] R. M. Henig, *The Monk in the Garden: The Lost and Found Genius of Gregor Mendel, the Father of Genetics*, First Mariner Books, New York, NY, USA, 2000.

[10] H. F. Spirer and L. Spirer, *Death and Numbers: Semmelweis the Statistician*, vol. 1, PSR Quarterly, 1991.

[11] G. L. Geison, *The Private Science of Louis Pasteur*, Princeton University Press, Princeton, NJ, USA, 1995.

[12] D. Hothersall, *History of Psychology*, McGraw–Hill, New York, NY, USA, 4th edition, 2004.

[13] T. N. Raju, "The Nobel chronicles. 1929: Christiaan Eijkman (1858–1930) and Frederick Hopkins (1861–1947)," *The Lancet*, vol. 352, no. 9143, p. 1868, 1998.

[14] S. J. Genuis, "An ounce of prevention: a pound of cure for an ailing health care system," *Canadian Family Physician*, vol. 53, no. 4, pp. 597–599, 2007.

[15] S. J. Genuis, "The proliferation of clinical practice guidelines: professional development or medicine-by-numbers?" *Journal of the American Board of Family Practice*, vol. 18, no. 5, pp. 419–425, 2005.

[16] S. J. Genuis, "Back to the future of healthcare: aetiology-centred medicine," *New Zealand Medical Journal*, vol. 118, no. 1215, p. U1467, 2005.

[17] S. J. Genuis and S. K. Genuis, "Exploring the continuum: medical information to effective clinical practice. Paper II. Towards aetiology-centred clinical practice," *Journal of Evaluation in Clinical Practice*, vol. 12, no. 1, pp. 63–75, 2006.

[18] S. J. Genuis, "Medical practice and community health care in the 21st century: a time of change," *Public Health*, vol. 122, no. 7, pp. 671–680, 2008.

[19] G. Rogler, "Interaction between susceptibility and environment: examples from the digestive tract. Digestive diseases," *Digestive Diseases*, vol. 29, no. 2, pp. 136–143, 2011.

[20] S. O'Neill and R. Cervera, "Systemic lupus erythematosus," *Best Practice & Research. Clinical Rheumatology*, vol. 24, no. 6, pp. 841–855, 2010.

[21] R. Sinha, D. E. Anderson, S. S. McDonald, and P. Greenwald, "Cancer risk and diet in India," *Journal of Postgraduate Medicine*, vol. 49, no. 3, pp. 222–228, 2003.

[22] S. J. Genuis, "Is autism reversible?" *Acta Paediatrica*, vol. 98, no. 10, pp. 1575–1578, 2009.

[23] M. Candiani, S. Izzo, A. Bulfoni, J. Riparini, S. Ronzoni, and A. Marconi, "Laparoscopic vs vaginal hysterectomy for benign pathology," *American Journal of Obstetrics and Gynecology*, vol. 200, no. 4, pp. 368.e1–368.e7, 2009.

[24] J. M. Perrin, S. R. Bloom, and S. L. Gortmaker, "The increase of childhood chronic conditions in the United States," *Journal of the American Medical Association*, vol. 297, no. 24, pp. 2755–2759, 2007.

[25] E. S. Lander, "Initial impact of the sequencing of the human genome," *Nature*, vol. 470, no. 7333, pp. 187–197, 2011.

[26] J. F. Carlquist and J. L. Anderson, "Pharmacogenetic mechanisms underlying unanticipated drug responses," *Discovery Medicine*, vol. 11, no. 60, pp. 469–478, 2011.

[27] P. Tajik and P. M. Bossuyt, "Genomic markers to tailor treatments: waiting or initiating?" *Human Genetics*, vol. 130, no. 1, pp. 15–18, 2011.

[28] A. Nasir, R. E. Shackelford, F. Anwar, and T. J. Yeatman, "Genetic risk of breast cancer," *Minerva Endocrinologica*, vol. 34, no. 4, pp. 295–309, 2009.

[29] P. Kiberstis and L. Roberts, "The puzzle of complex diseases," *Science*, vol. 296, pp. 685–703, 2002.

[30] Office of Genomics and Disease Prevention: Centers for Disease Control and Prevention. Department of Health and Human, S., *Gene-Environment Interaction Fact Sheet*, 2000.

[31] D. Rodenhiser and M. Mann, "Epigenetics and human disease: translating basic biology into clinical applications," *Canadian Medical Association Journal*, vol. 174, no. 3, pp. 341–348, 2006.

[32] M. D. Anway, A. S. Cupp, N. Uzumcu, and M. K. Skinner, "Epigenetic transgenerational actions of endocrine disruptors

and male fertility," *Science*, vol. 308, no. 5727, pp. 1466–1469, 2005.

[33] M. D. Anway and M. K. Skinner, "Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease," *Reproductive BioMedicine Online*, vol. 16, no. 1, pp. 23–25, 2008.

[34] S. J. Genuis, "Evolution in pediatric health care," *Pediatrics International*, vol. 52, no. 4, pp. 640–643, 2010.

[35] S. J. Genuis, "Nutritional transition: a determinant of global health," *Journal of Epidemiology and Community Health*, vol. 59, no. 8, pp. 615–617, 2005.

[36] J. A. Bralley and R. S. Lord, *Laboratory Evaluations in Molecular Medicine: Nutrients, Toxicants, and Cell Regulators*, The Institute for Advances in Molecular Medicine, Norcross, Ga, USA, 2005.

[37] C. Lo, "Integrating nutrition as a theme throughout the medical school curriculum," *American Journal of Clinical Nutrition*, vol. 72, no. 3, supplement, pp. 882S–889S, 2000.

[38] Centers for Disease and C. and P. National Center for Chronic Disease Prevention and Health, "Physical activity and good nutrition: essential elements to prevent chronic diseases and obesity," *Nutrition in Clinical Care*, vol. 6, no. 3, pp. 135–138, 2003.

[39] G. Schwalfenberg, "Not enough vitamin D: health consequences for Canadians," *Canadian Family Physician*, vol. 53, no. 5, pp. 841–854, 2007.

[40] S. J. Genuis and G. Schwalfenberg, "Time for an oil check: the role of essential omega-3 fatty acids in maternal and pediatric health," *Journal of Perinatology*, vol. 26, no. 6, pp. 359–365, 2006.

[41] D. M. Parkin, P. Tappenden, A. H. Olsen, J. Patnick, and P. Sasieni, "Predicting the impact of the screening programme for colorectal cancer in the UK," *Journal of Medical Screening*, vol. 15, no. 4, pp. 163–174, 2008.

[42] C. F. Garland, F. C. Garland, E. K. Shaw, G. W. Comstock, K. J. Helsing, and E. D. Gorham, "Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study," *The Lancet*, vol. 2, no. 8673, pp. 1176–1178, 1989.

[43] S. J. Genuis, "Nowhere to hide: chemical toxicants and the unborn child," *Reproductive Toxicology*, vol. 28, no. 1, pp. 115–116, 2009.

[44] Centers for Disease Control and Department of Health and Human Services, "Fourth National Report on Human Exposure to Environmental Chemicals," Atlanta, Georgia, pp.1–529, 2009, http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf.

[45] Environmental Working Group, Body burden—the pollution in newborns: A benchmark investigation of industrial chemicals, pollutants and pesticides in umbilical cord blood, 2005, http://ewg.org/reports/bodyburden2/execsumm.php/.

[46] S. J. Genuis, "The chemical erosion of human health: adverse environmental exposure and in-utero pollution—determinants of congenital disorders and chronic disease," *Journal of Perinatal Medicine*, vol. 34, no. 3, pp. 185–195, 2006.

[47] S. J. Genuis, "Health issues and the environment—an emerging paradigm for providers of obstetrical and gynaecological health care," *Human Reproduction*, vol. 21, no. 9, pp. 2201–2208, 2006.

[48] S. J. Genuis, "Clinical medicine and the budding science of indoor mold exposure," *European Journal of Internal Medicine*, vol. 18, no. 7, pp. 516–523, 2007.

[49] B. Lorber, "Are all diseases infectious? Another look," *Annals of Internal Medicine*, vol. 131, no. 12, pp. 989–990, 1999.

[50] S. J. Genuis, "Fielding a current idea: exploring the public health impact of electromagnetic radiation," *Public Health*, vol. 122, no. 2, pp. 113–124, 2008.

[51] M. Kanbay, M. I. Yilmaz, A. Sonmez et al., "Serum uric acid level and endothelial dysfunction in patients with nondiabetic chronic kidney disease," *American Journal of Nephrology*, vol. 33, no. 4, pp. 298–304, 2011.

[52] G. E. Simon, W. J. Katon, and P. J. Sparks, "Allergic to life: psychological factors in environmental illness," *American Journal of Psychiatry*, vol. 147, no. 7, pp. 901–906, 1990.

[53] S. J. Genuis, "Sensitivity-related illness: the escalating pandemic of allergy, food intolerance and chemical sensitivity," *Science of the Total Environment*, vol. 408, no. 24, pp. 6047–6061, 2010.

[54] World Health Organization, "International Programme on Chemical Safety: Environmental Health Criteria 101: Methylmercury," Geneva, Switzerland, United Nations Environment Programme, 1990.

[55] World Health Organization, Children's Health and the Environment. WHO Training Package for the Health Sector World Health Organization, 2009, http://www.who.int/ceh/en/.

[56] S. J. Genuis, "Elimination of persistent toxicants from the human body," *Human & Experimental Toxicology*, vol. 30, no. 1, pp. 3–18, 2011.

[57] G. Barkai, S. Arbuzova, M. Berkenstadt, S. Heifetz, and H. Cuckle, "Frequency of down's syndrome and neural-tube defects in the same family," *The Lancet*, vol. 361, no. 9366, pp. 1331–1335, 2003.

[58] S. J. Genuis, "Toxic causes of mental illness are overlooked," *NeuroToxicology*, vol. 29, no. 6, pp. 1147–1149, 2008.

[59] M. P. Dumont, "Psychotoxicology: the return of the mad hatter," *Social Science and Medicine*, vol. 29, no. 9, pp. 1077–1082, 1989.

[60] S. J. Genuis, "Toxicant exposure and mental health—individual, social, and public health considerations," *Journal of Forensic Sciences*, vol. 54, no. 2, pp. 474–477, 2009.

[61] S. J. Genuis and T. P. Bouchard, "Celiac disease presenting as autism," *Journal of Child Neurology*, vol. 25, no. 1, pp. 114–119, 2010.

[62] S. J. Genuis and A. K. Siy, "Nutritional supplementation and dietary restriction in the resolution of enthesitis-related arthritis," *JRSM Short Reports*, vol. 2, no. 4, p. 32, 2011.

[63] S. J. Genuis and R. A. Lobo, "Potential amelioration of morbidity in patients with chromosomal anomalies: relevance to Bardet-Biedl syndrome," *Clinical Genetics*, vol. 79, no. 5, pp. 482–488, 2011.

[64] A. C. von Eschenbach, Speech given on Dec 18, 2008. U.S. Food and Drug Administration, 2008, http://www.fda.gov/NewsEvents/Speeches/ucm177387.htm.

[65] R. Grol and J. Grimshaw, "From best evidence to best practice: effective implementation of change in patients' care," *The Lancet*, vol. 362, no. 9391, pp. 1225–1230, 2003.

[66] S. Doherty, "History of evidence-based medicine. Oranges, chloride of lime and leeches: barriers to teaching old dogs new tricks," *Emergency Medicine Australasia*, vol. 17, no. 4, pp. 314–321, 2005.

[67] S. K. Genuis and S. J. Genuis, "Exploring the continuum: medical information to effective clinical practice: paper I: the translation of knowledge into clinical practice," *Journal of Evaluation in Clinical Practice*, vol. 12, no. 1, pp. 49–62, 2006.

[68] B. Marshall, *Helicobacter Pioneers: Firsthand Accounts from the Scientists who Discovered Helicobacters*, Blackwell Publishing, Victoria, Australia, 2002.

[69] S. B. Nuland, "The enigma of semmelweis-an interpretation," *Journal of the History of Medicine and Allied Sciences*, vol. 34, no. 3, pp. 255–272, 1979.

[70] C. Duncan, S. Langlais, J. Danyluk-Hall, and K. Simonson, "Knowledge translation: empowering health professionals to take the lead," *Journal of Continuing Education in the Health Professions*, vol. 28, no. 4, pp. 282–283, 2008.

[71] J. L. Heilbron, *Dilemmas of an Upright Man: Max Planck and the Fortunes of German Science*, University of California Press, Berkeley, Calif, USA, 1986.

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Review Article

The Alkaline Diet: Is There Evidence That an Alkaline pH Diet Benefits Health?

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This review looks at the role of an alkaline diet in health. Pubmed was searched looking for articles on pH, potential renal acid loads, bone health, muscle, growth hormone, back pain, vitamin D and chemotherapy. Many books written in the lay literature on the alkaline diet were also reviewed and evaluated in light of the published medical literature. There may be some value in considering an alkaline diet in reducing morbidity and mortality from chronic diseases and further studies are warranted in this area of medicine.

1. Background

Life on earth depends on appropriate pH levels in and around living organisms and cells. Human life requires a tightly controlled pH level in the serum of about 7.4 (a slightly alkaline range of 7.35 to 7.45) to survive [1].

As a comparison, in the past 100 years with increasing industrialization, the pH of the ocean has dropped from 8.2 to 8.1 because of increasing CO_2 deposition. This has a negative impact on life in the ocean [1, 2] and may lead to the collapse of the coral reefs [3]. Even the pH of the soil in which plants are grown can have considerable influence on the mineral content of the food we eat (as minerals are used as buffers to maintain pH). The ideal pH of soil for the best overall availability of essential nutrients is between 6 and 7. Acidic soils below pH of 6 may have reduced calcium and magnesium, and soil above pH 7 may result in chemically unavailable iron, manganese, copper and zinc. Adding dolomite and manure are ways of raising pH in an acid soil environment when the pH is below 6 [4].

When it comes to the pH and net acid load in the human diet, there has been considerable change from the hunter gather civilization to the present [5]. With the agricultural revolution (last 10,000 years) and even more recently with industrialization (last 200 years), there has been an decrease in potassium (K) compared to sodium (Na) and an increase in chloride compared to bicarbonate found in the diet [6]. The ratio of potassium to sodium has reversed, K/Na pre-viously was 10 to 1 whereas the modern diet has a ratio of 1 to 3 [7]. It is generally accepted that agricultural humans today have a diet poor in magnesium and potassium as well as fiber and rich in saturated fat, simple sugars, sodium, and chloride as compared to the pre-agricultural period [6]. This results in a diet that may induce metabolic acidosis which is mismatched to the genetically determined nutritional requirements [8]. With aging, there is a gradual loss of renal acid-base regulatory function and a resultant increase in diet-induced metabolic acidosis while on the modern diet [9]. A low-carbohydrate high-pro-tein diet with its increased acid load results in very little change in blood chemistry, and pH, but results in many changes in urinary chemistry. Urinary magnesium levels, urinary citrate and pH are decreased, urinary calcium, undissociated uric acid, and phosphate are in-creased. All of these result in an increased risk for kidney stones [10].

Much has been written in the lay literature as well as many online sites expounding on the benefits of the alkaline diet. This paper is an attempt to balance the evidence that is found in the scientific literature.

Table 1: Ph of selected fluids, organs, and membranes.

Organ, fluid or membrane	pH	Function of pH
(1) Skin	Natural pH is between 4 and 6.5 [17]	Barrier protection from microbes
(2) Urine	4.6 to 8.0 [18]	Limit overgrowth of microbes
(3) Gastric	1.35 to 3.5	Break down protein
(4) Bile	7.6 to 8.8	Neutralize stomach acid, aid in digestion
(5) Pancreatic fluid	8.8	Neutralize stomach acid, aid in digestion
(6) Vaginal fluid	<4.7 [13]	Limit overgrowth of opportunistic microbes
(7) Cerebrospinal fluid	7.3	Bathes the exterior of the brain
(8) Intracellular fluid	6.0–7.2 [19]	Due to acid production in cells
(9) Serum venous	7.35	Tightly regulated
(10) Serum arterial	7.4	Tightly regulated

2. The Role of pH in Various Cells, Organs, and Membranes

The pH in our body may vary considerably from one area to another with the highest acidity in the stomach (pH of 1.35 to 3.5) to aid in digestion and protect against opportunistic microbial organisms. But even in the stomach, the layer just outside the epithelium is quite basic to prevent mucosal injury. It has been suggested that decreased gastric lining secretion of bicarbonates and a decrease in the alkaline/acid secretion in duodenal ulcer patients may play a significant role in duodenal ulcers [11]. The skin is quite acidic (pH 4–6.5) to provide an acid mantle as a protective barrier to the environment against microbial overgrowth. There is a gradient from the outer horny layer (pH 4) to the basal layer (pH 6.9) [12]. This is also seen in the vagina where a pH of less than 4.7 protects against microbial overgrowth [13].

The urine may have a variable pH from acid to alkaline depending on the need for balancing the internal environment. Acid excretion in the urine can be estimated by a formula described by Remer (sulfate + chloride + 1.8x phosphate + organic acids) minus (sodium + potassium + 2x calcium + 2x magnesium) mEq [14]. Foods can be categorized by the potential renal acid loads (PRALs) see Table 2. Fruits, vegetables, fruit juices, potatoes, and alkali-rich and low phosphorus beverages (red and white wine, mineral soda waters) having a negative acid load. Whereas, grain products, meats, dairy products, fish, and alkali poor and low phosphorus beverages (e.g., pale beers, cocoa) have relatively high acid loads [15]. Measurement of pH of the urine (reviewed in a recent study with two morning specimens done over a five-year span) did not predict bone fractures or loss of bone mineral density [16]. However, this may not be reflective of being on an alkaline or acid diet throughout this time. For more details, see Table 1.

3. Chronic Acidosis and Bone Disease

Calcium in the form of phosphates and carbonates represents a large reservoir of base in our body. In response to an acid load such as the modern diet these salts are released into the systemic circulation to bring about pH homeostasis [7]. It has been estimated that the quantity of calcium lost in the urine with the modern diet over time could be as high as almost 480 gm over 20 years or almost half the skeletal mass of calcium [21]. However, urinary losses of cal-cium are not a direct measure of osteoporosis. There are many regulatory factors that may compensate for the urinary calcium loss. When the arterial pH is in the normal range, a mild reduction of plasma bicarbonate results in a negative calcium balance which could benefit from supplementing bicarbonate in the form of potassium bicarbonate [22]. It has been found that bicarbonate, which increases the alkali content of a diet, but not potassium may attenuate bone loss in healthy older adults [23]. The bone minerals that are wasted in the urine may not have complete compensation through intestinal absorption, which is thought to result in osteoporosis. However, adequate vitamin D with a 25(OH)D level of >80 nmol/L may allow for appropriate intestinal absorption of calcium and magnesium and phosphate when needed [24]. Sadly, most populations are generally deficient in vitamin D especially in northern climates [25]. In chronic renal failure, correction of metabolic acidosis with bicarbonate significantly improves parathyroid levels and levels of the active form of vitamin D 1,25(OH)2D_3 [26]. Recently, a study has shown the importance of phosphate in Remer's PRAL formula. According to the formula it would be expected that an increase in phosphate should result in an increase in urinary calcium loss and a negative calcium balance in bone [27]. It should be noted that supplementation with phosphate in patients with bed rest reduced urinary calcium excretion but did not prevent bone loss [28]. The most recent systematic review and meta-analysis has shown that calcium balance is maintained and improved with phosphate which is quite contrary to the acid-ash hypothesis [29]. As well a recent study looking at soda intake (which has a significant amount of phosphate) and osteoporosis in postmenopausal American first nations women did not find a correlation [30]. It is quite possible that the high acid content according to Remer's classification needs to be looked at again in light of compensatory phosphate intake. There is online information promoting an alkaline diet for bone health as well as a number of books. However, a recent systematic review of the literature looking for evidence supporting the alkaline diet for bone health found no protective role of dietary acid load in osteoporosis [31].

Table 2: Potential renal acid loads (PRALs) of selected foods [20].

Food or food group	PRAL mEq of: $Cl + P0_4 + SO_4 - Na - K - Ca - Mg$
Dairy	
Parmesan cheese	34.2
Processed cheese plain	28.7
Cheddar reduced fat	26.4
Hard cheese (average)	19.2
Fresh cheese (quark)	11.3
Cottage cheese plain	8.7
Yogurt whole milk	1.5
Ice Cream	0.8
Whole milk	0.7
Buttermilk	0.5
Eggs	
Eggs yolk	23.4
Eggs white	1.1
Eggs chicken whole	8.2
Meats	
Corned beef	13.2
Luncheon meat canned	10.2
Turkey	9.9
Veal	9.0
Lean beef	7.8
Frankfurters	6.7
Sugars	
Sugar white	−0.1
Honey	−0.3
Vegetables	
Cucumber	−0.8
Broccoli	−1.2
Tomato	−3.1
Eggplant	−3.4
Celery	−5.2
Spinach	−14.0
Fats and Oils	
Butter	0.6
Margarine	−0.5
Olive oil	0.0
Fruits and nuts and fruit juices	
Peanuts	8.3
Walnuts	6.8
Grape juice unsweetened	−1.0
Orange juice unsweetened	−2.9
Apples or apple juice unsweetened	−2.2
Apricots	−4.8
Banana	−5.5
Black currents	−6.5
Raisins	−21.0
Grains and grain products	
Brown Rice	12.5
Rolled Oats	10.7
Spaghetti whole meal	7.3
Spaghetti white	6.5

Table 2: Continued.

Food or food group	PRAL mEq of: $Cl + P0_4 + SO_4 - Na - K - Ca - Mg$
Cornflakes	6.0
Rice white	4.6
Bread rye flower	4.1
Bread whole wheat	1.8
Legumes	
Lentils green and brown	3.5
Green beans	−3.1
Fish	
Trout brown	10.8
Cod fillets	7.1
Beverages	
Beer pale	0.9
Coca-Cola	0.4
Beer draft	−0.2
Wine white	−1.2
Coffee infusion	−1.4
Wine red	−2.4

Another element of the modern diet is the excess of sodium in the diet. There is evidence that in healthy humans the increased sodium in the diet can predict the degree of hyperchloremic metabolic acidosis when consuming a net acid producing diet [32]. As well, there is evidence that there are adverse effects of sodium chloride in the aging population. A high sodium diet will exacerbate disuse-induced bone and muscle loss during immobilization by increasing bone resorption and protein wasting [33]. Excess dietary sodium has been shown to result in hypertension and osteoporosis in women [34, 35]. As well, dietary potassium which is lacking in the modern diet would modulate pressor and hypercalciuric effects of excess of sodium chloride [36].

Excess dietary protein with high acid renal load may decrease bone density if not buffered by ingestion of supplements or foods that are alkali rich [37]. However, adequate protein is necessary for prevention of osteoporosis and sarcopenia; therefore, increasing the amount of fruit and vegetables may be necessary rather than reducing protein [38].

4. Alkaline Diets and Muscle

As we age, there is a loss of muscle mass, which may predispose to falls and fractures. A three-year study looking at a diet rich in potassium, such as fruits and vegetables, as well as a reduced acid load, resulted in preservation of muscle mass in older men and women [39]. Conditions such as chronic renal failure that result in chronic metabolic acidosis result in accelerated breakdown in skeletal muscle [40]. Correction of acidosis may preserve muscle mass in conditions where muscle wasting is common such as diabetic ketosis, trauma, sepsis, chronic obstructive lung disease, and renal failure [41]. In situations that result in acute acidosis, supplementing younger patients with sodium bicarbonate prior to exhaustive exercise resulted in significantly less acidosis in the blood than those that were not supplemented with sodium bicarbonate [42].

5. Alkaline Supplementation and Growth Hormone

It has long been known that severe forms of metabolic acidosis in children, such as renal tubular acidosis, are associated with low levels of growth hormone with resultant short stature. Correction of the acidosis with bicarbonate [7] or potassium citrate [43] increases growth hormone significantly and improved growth. The use of enough potassium bicarbonate in the diet to neutralize the daily net acid load in postmenopausal women resulted in a significant increase in growth hormone and resultant osteocalcin [44]. Improving growth hormone levels may improve quality of life, reduce cardiovascular risk factors, improve body composition, and even improve memory and cognition [45]. As well this results in a reduction of urinary calcium loss equivalent to 5% of bone calcium content over a period of 3 years [46].

6. Alkaline Diet and Back Pain

There is some evidence that chronic low back pain improves with the supplementation of alkaline minerals [47]. With supplementation there was a slight but significant increase in blood pH and intracellular magnesium. Ensuring that there is enough intracellular magnesium allows for the proper function of enzyme systems and also allows for activation of vitamin D [48]. This in turn has been shown to improve back pain [49].

7. Alkalinity and Chemotherapy

The effectiveness of chemotherapeutic agents is markedly influenced by pH. Numerous agents such as epirubicin and adriamycin require an alkaline media to be more effective. Others, such as cisplatin, mitomycin C, and thiotepa, are more cytotoxic in an acid media [50]. Cell death correlates with acidosis and intracellular pH shifts higher (more alkaline) after chemotherapy may reflect response to chemotherapy [51]. It has been suggested that inducing metabolic alkalosis may be useful in enhancing some treatment regimes by using sodium bicarbonate, carbicab, and furosemide [52]. Extracellular alkalinization by using bicarbonate may result in improvements in therapeutic effectiveness [53]. There is no scientific literature establishing the benefit of an alkaline diet for the prevention of cancer at this time.

8. Discussion

The human body has an amazing ability to maintain a steady pH in the blood with the main compensatory mechanisms being renal and respiratory. Many of the membranes in our body require an acid pH to protect us and to help us digest food. It has been suggested that an alkaline diet may prevent a number of diseases and result in significant health benefits. Looking at the above discussion on bone health alone, certain aspects have doubtful benefit. There does not seem to be enough evidence that milk or cheese may be as detrimental as Remer's formula suggests since phosphate does benefit bone health and result in a positive calcium balance. However, another mechanism for the alkaline diet to benefit bone health may be the increase in growth hormone and resultant increase in osteocalcin. There is some evidence that the K/Na ratio does matter and that the significant amount of salt in our diet is detrimental. Even some governments are demanding that the food industry reduce the salt load in our diet. High-protein diets may also affect bone health but some protein is also needed for good bone health. Muscle wasting however seems to be reduced with an alkaline diet and back pain may benefit from this as well. An alkaline environment may improve the efficacy of some chemotherapy agents but not others.

9. Conclusion

Alkaline diets result in a more alkaline urine pH and may result in reduced calcium in the urine, however, as seen in some recent reports, this may not reflect total calcium balance because of other buffers such as phosphate. There is no substantial evidence that this improves bone health or protects from osteoporosis. However, alkaline diets may result in a number of health benefits as outlined below

(1) Increased fruits and vegetables in an alkaline diet would improve the K/Na ratio and may benefit bone health, reduce muscle wasting, as well as mitigate other chronic diseases such as hypertension and strokes.

(2) The resultant increase in growth hormone with an alkaline diet may improve many outcomes from cardiovascular health to memory and cognition.

(3) An increase in intracellular magnesium, which is required for the function of many enzyme systems, is another added benefit of the alkaline diet. Available magnesium, which is required to activate vitamin D, would result in numerous added benefits in the vitamin D apocrine/exocrine systems.

(4) Alkalinity may result in added benefit for some chemotherapeutic agents that require a higher pH.

From the evidence outlined above, it would be prudent to consider an alkaline diet to reduce morbidity and mortality of chronic disease that are plaguing our aging population. One of the first considerations in an alkaline diet, which includes more fruits and vegetables, is to know what type of soil they were grown in since this may significantly influence the mineral content. At this time, there are limited scientific studies in this area, and many more studies are indicated in regards to muscle effects, growth hormone, and interaction with vitamin D.

References

[1] A. Waugh and A. Grant, *Anatomy and Physiology in Healthand Illness*, Churchill Livingstone Elsevier, Philadelphia, Pa, USA, 10th edition, 2007.

[2] University, Birmingham oAa, "Oceans reveal further impacts of climate change," ScienceDaily, 2010.

[3] O. Hoegh-Guldberg, P. J. Mumby, A. J. Hooten et al., "Coral reefs under rapid climate change and ocean acidification," *Science*, vol. 318, no. 5857, pp. 1737–1742, 2007.

[4] J. Dam-ampai SO and C. Nilnond, "Effect of cattle manure and dolomite on soil properties and plant growth in acid upland soils," *Songklanakarin Journal of Science and Technologh*, vol. 27, supplement 3, pp. 727–737, 2005.

[5] A. Ströhle, A. Hahn, and A. Sebastian, "Estimation of the diet-dependent net acid load in 229 worldwide historically studied hunter-gatherer societies," *American Journal of Clinical Nutrition*, vol. 91, no. 2, pp. 406–412, 2010.

[6] A. Sebastian, L. A. Frassetto, D. E. Sellmeyer, R. L. Merriam, and R. C. Morris Jr., "Estimation of the net acid load of the diet of ancestral preagricultural Homo sapiens and their hominid ancestors," *American Journal of Clinical Nutrition*, vol. 76, no. 6, pp. 1308–1316, 2002.

[7] L. Frassetto, R. C. Morris, Jr. R.C. Jr., D. E. Sellmeyer, K. Todd, and A. Sebastian, "Diet, evolution and aging—the pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet," *European Journal of Nutrition*, vol. 40, no. 5, pp. 200–213, 2001.

[8] M. Konner and S. Boyd Eaton, "Paleolithic nutrition: twenty-five years later," *Nutrition in Clinical Practice*, vol. 25, no. 6, pp. 594–602, 2010.

[9] R. D. Lindeman and R. Goldman, "Anatomic and physiologic age changes in the kidney," *Experimental Gerontology*, vol. 21, no. 4-5, pp. 379–406, 1986.

[10] S. T. Reddy, C. Y. Wang, K. Sakhaee, L. Brinkley, and C. Y. Pak, "Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism,"

American Journal of Kidney Diseases, vol. 40, no. 2, pp. 265–274, 2002.

[11] Y. S. Malov and A. N. Kulikov, "Bicarbonate deficiency and duodenal ulcer," *Terapevticheskii Arkhiv*, vol. 70, no. 2, pp. 28–32, 1998.

[12] H. Ohman and A. Vahlquist, "In vivo studies concerning a pH gradient in human stratum corneum and upper epidermis," *Acta Dermato-Venereologica*, vol. 74, no. 5, pp. 375–379, 1994.

[13] D. G. Ferris, S. L. Francis, E. D. Dickman, K. Miler-Miles, J. L. Waller, and N. McClendon, "Variability of vaginal pH determination by patients and clinicians," *Journal of the American Board of Family Medicine*, vol. 19, no. 4, pp. 368–373, 2006.

[14] T. Remer and F. Manz, "Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein," *American Journal of Clinical Nutrition*, vol. 59, no. 6, pp. 1356–1361, 1994.

[15] T. Remer, "Influence of diet on acid-base balance," *Seminars in Dialysis*, vol. 13, no. 4, pp. 221–226, 2000.

[16] T. R. Fenton, M. Eliasziw, S. C. Tough, A. W. Lyon, J. P. Brown, and D. A. Hanley, "Low urine pH and acid excretion do not predict bone fractures or the loss of bone mineral density: a prospective cohort study," *BMC Musculoskeletal Disorders*, vol. 11, article 88, 2010.

[17] E. Boelsma, L. P. L. van de Vijver, R. A. Goldbohm, I. A. A. Klöpping-Ketelaars, H. F. J. Hendriks, and L. Roza, "Human skin condition and its associations with nutrient concentrations in serum and diet," *American Journal of Clinical Nutrition*, vol. 77, no. 2, pp. 348–355, 2003.

[18] B. A. Ince, E. J. Anderson, and R. M. Neer, "Lowering dietary protein to U.S. recommended dietary allowance levels reduces urinary calcium excretion and bone resorption in young women," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 8, pp. 3801–3807, 2004.

[19] W. F. Boron, "Regulation of intracellular pH," *Advances in Physiology Education*, vol. 28, pp. 160–179, 2004.

[20] T. Remer and F. Manz, "Potential renal acid load of foods and its influence on urine pH," *Journal of the American Dietetic Association*, vol. 95, no. 7, pp. 791–797, 1995.

[21] T. R. Fenton, M. Eliasziw, A. W. Lyon, S. C. Tough, and D. A. Hanley, "Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid-ash diet hypothesis," *American Journal of Clinical Nutrition*, vol. 88, no. 4, pp. 1159–1166, 2008.

[22] A. Sebastian and R. C. Morris Jr., "Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate," *New England Journal of Medicine*, vol. 331, no. 4, p. 279, 1994.

[23] B. Dawson-Hughes, S. S. Harris, N. J. Palermo, C. Castaneda-Sceppa, H. M. Rasmussen, and G. E. Dallal, "Treatment with potassium bicarbonate lowers calcium excretion and bone resorption in older men and women," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 1, pp. 96–102, 2009.

[24] R. P. Heaney, M. S. Dowell, C. A. Hale, and A. Bendich, "Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D," *Journal of the American College of Nutrition*, vol. 22, no. 2, pp. 142–146, 2003.

[25] G. K. Schwalfenberg, S. J. Genuis, and M. N. Hiltz, "Addressing vitamin D deficiency in Canada: a public health innovation whose time has come," *Public Health*, vol. 124, no. 6, pp. 350–359, 2010.

[26] K. C. Lu, S. H. Lin, F. C. Yu, S. H. Chyr, and S. D. Shieh, "Influence of metabolic acidosis on serum 1,25(OH)2D3 levels in chronic renal failure," *Mineral and Electrolyte Metabolism*, vol. 21, no. 6, pp. 398–402, 1995.

[27] T. R. Fenton, A. W. Lyon, M. Eliasziw, S. C. Tough, and D. A. Hanley, "Phosphate decreases urine calcium and increases calcium balance: a meta-analysis of the osteoporosis acid-ash diet hypothesis," *Nutrition Journal*, vol. 8, article 41, 2009.

[28] S. B. Hulley, J. M. Vogel, C. L. Donaldson, J. H. Bayers, R. J. Friedman, and S. N. Rosen, "The effect of supplemental oral phosphate on the bone mineral changes during prolonged bed rest," *Journal of Clinical Investigation*, vol. 50, no. 12, pp. 2506–2518, 1971.

[29] T. R. Fenton, A. W. Lyon, M. Eliasziw, S. C. Tough, and D. A. Hanley, "Meta-analysis of the effect of the acid-ash hypothesis of osteoporosis on calcium balance," *Journal of Bone and Mineral Research*, vol. 24, no. 11, pp. 1835–1840, 2009.

[30] J. D. Supplee, G. E. Duncan, B. Bruemmer, J. Goldberg, Y. Wen, and J. A. Henderson, "Soda intake and osteoporosis risk in postmenopausal American-Indian women," *Public Health Nutrition*, pp. 1–7, 2011.

[31] T. R. Fenton, S. C. Tough, A. W. Lyon, M. Eliasziw, and D. A. Hanley, "Causal assessment of dietary acid load and bone disease: a systematic review & meta-analysis applying Hill's epidemiologic criteria for causality," *Nutrition Journal*, vol. 10, no. 1, article 41, 2011.

[32] L. A. Frassetto, R. C. Morris Jr., and A. Sebastian, "Dietary sodium chloride intake independently predicts the degree of hyperchloremic metabolic acidosis in healthy humans consuming a net acid-producing diet," *American Journal of Physiology—Renal Physiology*, vol. 293, no. 2, pp. F521–F525, 2007.

[33] P. Frings-Meuthen, J. Buehlmeier, N. Baecker et al., "High sodium chloride intake exacerbates immobilization-induced bone resorption and protein losses," *Journal of Applied Physiology*, vol. 111, no. 2, pp. 537–542, 2011.

[34] F. P. Cappuccio, E. Meilahn, J. M. Zmuda, and J. A. Cauley, "High blood pressure and bone-mineral loss in elderly white women: a prospective study," *Lancet*, vol. 354, no. 9183, pp. 971–975, 1999.

[35] A. Devine, R. A. Criddle, I. M. Dick, D. A. Kerr, and R. L. Prince, "A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women," *American Journal of Clinical Nutrition*, vol. 62, no. 4, pp. 740–745, 1995.

[36] R. C. Morris Jr., O. Schmidlin, L. A. Frassetto, and A. Sebastian, "Relationship and interaction between sodium and potassium," *Journal of the American College of Nutrition*, vol. 25, no. 3, pp. 262S–270S, 2006.

[37] U. S. Barzel and L. K. Massey, "Excess dietary protein may can adversely affect bone," *Journal of Nutrition*, vol. 128, no. 6, pp. 1051–1053, 1998.

[38] R. P. Heaney and D. K. Layman, "Amount and type of pro-tein influences bone health," *American Journal of Clinical Nutrition*, vol. 87, no. 5, pp. 156S–157S, 2008.

[39] B. Dawson-Hughes, S. S. Harris, and L. Ceglia, "Alkaline diets favor lean tissue mass in older adults," *American Journal of Clinical Nutrition*, vol. 87, no. 3, pp. 662–665, 2008.

[40] G. Garibotto, R. Russo, A. Sofia et al., "Muscle protein turnover in chronic renal failure patients with metabolic acidosis or normal acid-base balance," *Mineral and Electrolyte Metabolism*, vol. 22, no. 1–3, pp. 58–61, 1996.

[41] G. Caso and P. J. Garlick, "Control of muscle protein kinetics by acid-base balance," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 8, no. 1, pp. 73–76, 2005.

[42] M. J. Webster, M. N. Webster, R. E. Crawford, and L. B. Gladden, "Effect of sodium bicarbonate ingestion on exhaustive resistance exercise performance," *Medicine and Science in Sports and Exercise*, vol. 25, no. 8, pp. 960–965, 1993.

[43] E. McSherry and R. C. Morris Jr., "Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis," *Journal of Clinical Investigation*, vol. 61, no. 2, pp. 509–527, 1978.

[44] L. Frassetto, R. C. Morris Jr., and A. Sebastian, "Potassium bicarbonate reduces urinary nitrogen excretion in postmenopausal women," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 1, pp. 254–259, 1997.

[45] J. A. H. Wass and R. Reddy, "Growth hormone and memory," *Journal of Endocrinology*, vol. 207, no. 2, pp. 125–126, 2010.

[46] L. Frassetto, R. C. Morris Jr., and A. Sebastian, "Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 2, pp. 831–834, 2005.

[47] J. Vormann, M. Worlitschek, T. Goedecke, and B. Silver, "Supplementation with alkaline minerals reduces symptoms in patients with chronic low back pain," *Journal of Trace Elements in Medicine and Biology*, vol. 15, no. 2-3, pp. 179–183, 2001.

[48] I. Zofková and R. L. Kancheva, "The relationship between magnesium and calciotropic hormones," *Magnesium Research*, vol. 8, no. 1, pp. 77–84, 1995.

[49] G. Schwalfenberg, "Improvement of chronic back pain or failed back surgery with vitamin D repletion: a case series," *Journal of the American Board of Family Medicine*, vol. 22, no. 1, pp. 69–74, 2009.

[50] E. Groos, L. Walker, and J. R. Masters, "Intravesical chemotherapy. Studies on the relationship between pH and cytotoxicity," *Cancer*, vol. 58, no. 6, pp. 1199–1203, 1986.

[51] S. R. Smith, P. A. Martin, and R. H. T. Edwards, "Tumour pH and response to chemotherapy: an in vivo 31P magnetic resonance spectroscopy study in non-Hodgkin's lymphoma," *British Journal of Radiology*, vol. 64, no. 766, pp. 923–928, 1991.

[52] N. Raghunand and R. J. Gillies, "pH and chemotherapy," *Novartis Foundation Symposium*, vol. 240, pp. 199–211, 2001.

[53] N. Raghunand, X. He, R. Van Sluis et al., "Enhancement of chemotherapy by manipulation of tumour pH," *British Journal of Cancer*, vol. 80, no. 7, pp. 1005–1011, 1999.