March 5, 2010
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Comments on "A Scientific Review of Multiple Chemical Sensitivity"

Dear Drs.:

I see some improvements in the document over what was in the previous draft. I also see a number of areas that are in desperate need of improvement. I hope you will forgive me if I focus on the latter.

What I am going to do is to spend most of this document to discuss three important interrelated issues that are the focus of much of my concern.

My first concern is how you deal with the genetics of genes that determine rates of metabolism of xenobiotics. You have inserted the fact that the Schnakenberg study (1) found genetic roles of GSTM1 and GSTT1 genes in MCS, thank you for that. You have not inserted the other study published by the same group (2) that also showed a statistically significant role of the UGT1A1 gene, another gene that has an important role in detoxification. What you also have not commented on is the very high levels of statistical significance of these Schnakenberg studies (1,2), which were a product of the largest such study of apparent MCS patients. One of these four genes, had a $p<10^{-3}$, and the other three each had $p<10^{-4}$. In total, then, since these are multiplicative, the p value for all four of these genes showing an apparent association <u>if there is no true association</u> is $p<10^{-15}$. These are obviously **not** chance associations and the fact that we have such high levels of statistical significance in what was the largest such study argues strongly for a true causal role. The next highest levels of statistical significance were shown by the McKeown-Eyssen et al (3) study and the Hayley (4) study, the next largest such studies. The tiny Weissmuller study (5) and the only slightly larger recent Berg et al (6) study show either no (5) or low level (6) statistical significance. This is exactly the kind of pattern that one would expect to see in a genuine causal phenomenon.

There are other aspects of these studies that provide strong support for the view that these genes are acting in determining the rates of metabolism of toxic chemicals in the body. For each of the genes that

are acting to produce detoxification enzymes, the alleles with the lowest activity in detoxifying classes of compounds otherwise implicated in MCS, are associated with increased incidence of the disease. This is true for PON1 as you do note in a portion of the document, it is also true for GSTM1, GSTT1, and for UGT1A1. In other words when one looks at the chemicals otherwise implicated in MCS and the role that these genes have in their metabolism, detoxification always leads to lowered apparent activity in initiating cases of MCS. The interpretation of the CYP2D6 gene study also is consistent with its known role in metabolizing chemicals otherwise implicated in MCS. Here the action of the enzyme encoded by this gene is to act to increase toxicity. The enzyme encoded by this gene activates organophoshorus pesticides containing P=S bonds, where the S must be removed via CYP2D6 mediated oxidation in order to produce the toxic, active pesticide (you can easily find references on this by doing a PubMed search using organophosphate and CYP2D6). Chemical activation by this enzyme may also be expected for strictly hydrophobic solvents that act by binding to the TRPV1 receptor, a receptor that requires the presence of a hydrogen binding group on its agonists to be most active, and cytochrome P450s including CYP2D6 add hydroxyl groups that acts, of course, as a hydrogen binding group.

So it is not just that one can find statistically significant data, but that the data in each case argues that these genes act to determine the level of toxic responses to chemicals otherwise implicated in MCS, leading to the inescapable conclusion that these data provide substantial (I would argue compelling) evidence that chemicals act as toxicants in the body when they initiate cases of MCS.

Rather than on focussing on these important data, you have chosen to focus on the NAT2 gene, whose role in activation vs inactivation is unclear and therefore leads to a situation where its role may well depend on what chemicals are involved in a specific population. This complex role of NAT2 is documented in the role of this gene in carcinogenesis, where some carcinogens are activated by it but others are inactivated.

I have to say that by focusing on NAT2 to the exclusion of all of this other important data, you open up yourself to the charge of trying to provide a whitewash for the chemical industry. It is my hope and expectation that this is not the case, and I will be looking forward to the final document which hopefully will make the situation clear. The reported effects of each of these genes are not only supported by the statistical significance of the published data. It is also supported by

the fact that alleles predicted to increase the toxicity of chemicals implicated in MCS, all produce increased reported susceptibility

There is a second, related area where there is a similar concern. You interpret this genetic data as supporting "another postulated mechanism for MCS", altered xenobiotic metabolism.

You know, when people have studied cancer occurrence (vs non-occurrence) in people with some of these same genetic polymorphisms, the role of these polymorphisms in determining susceptibility to cancer has been interpreted in terms of the carcinogenenic roles of chemicals metabolized by these polymorphic enzymes. A similar interpretation has been given to such studies in autism or ALS, where the genetic role provides evidence for a causal role of chemicals acting as toxicants in initiating cases of these diseases. I am providing a citation where a similar such interpretation has been given for role of some of these same genes in producing susceptibility to neurological dysfunction in farmers using organophosphorus pesticides in "sheep dip" (7). <u>These are well accepted interpretations, in addition to making logical sense</u>. No one is arguing that because such genetic polymorphisms influence carcinogenesis in humans, that we have "another postulated mechanism for" carcinogenesis, of course not! Nor is anyone arguing for another postulated mechanism for autism or ALS or neurological dysfunction among sheep dip farmers, based on the genetic data. <u>The genetic studies simply confirm that these are diseases caused by chemical toxicants whose metabolism helps determine, therefore, susceptibility.</u>

So we have MCS where people from Cullen to Miller to Ziem to Meggs to Kilburn to me have all inferred that cases of chemical sensitivity are caused by toxic chemical exposure, where there are many dozens of papers describing a pattern of chemical exposure followed by initiation of chemical sensitivity and instead of interpreting these genetic data as supporting that inference, instead of using an interpretation that is well accepted for other diseases, you come up with an "alternative mechanism for MCS". With all due respect, this is frankly bizarre. I certainly hope that this is corrected in the final document – if it is not, it will fall far short, in my view, of being a "scientific review".

A third, concern is how you have completely failed to deal with the evidence for a role of chemicals implicated in MCS acting as toxicants via increased NMDA activity. Many of my concerns are summarized in the problems generated by a single sentence on p.12. You state that

“In contrast to a vast array of individual chemicals and chemical products linked with MCS in the literature, Pall (2009) in defense of a particular mechanistic theory for MCS identified seven different chemical types commonly implicated in MCS – pesticides, organic solvents, hydrogen sulphide, carbon monoxide, mercury, mercurial compounds and mould.” You know, I must congratulate you in having generated three major errors in a single sentence – that is quite an accomplishment. The seven classes of chemicals I referred to are: Organic solvents and related compounds (by far the largest group); the organophosphorus and carbamate pesticides; the organochlorine pesticides; the pyrethroid pesticides; and three specific chemicals, hydrogen sulfide, mercury (acting through its product methylmercury) and carbon monoxide. I did not lump these three pesticides together – they are after all, toxicologically distinct from each other. I did not list mercurial compounds, although it may be correct that they will eventually be listed, basically because I am unaware of any substantial data that MCS cases are caused by such other mercurials. I did not list molds, because although mold involvement is fairly well documented, molds are types of organisms, not a class of chemicals and we don’t know what mycotoxins have a role here. It is more than a bit puzzling and disturbing that you cannot accurately list what was clearly stated in my review. Error is certainly part of the human condition, but so many errors on something that can be so easily checked??

Now let’s go on to the other two serious errors in this sentence. You state that “In contrast to the vast array of individual chemicals and chemical products linked to MCS in the literature, Pall ... .” You repeat this statement three times in your “scientific” document. This is sheer nonsense. As I’ve made clear in several publications, the class of organic solvents and related compounds implicated in MCS is huge. In one quote that I provided in my MCS toxicology review I state that the response mechanism of these compounds in MCS is probably the same as that involved in the sensory irritation response, which includes but is not limited to the following set of chemicals “sensory irritation (SI) response, a response elicited by chemicals including alkanes, alkyl benzenes, halogenated benzenes, halogenated alkylbenzenes, alcohols, ketones, ethers, aldehydes, formaldehyde, isocyanates, and chlorine”. I am unaware of any pesticides implicated in MCS that is not included in the three pesticide classes that I specific include as classes of MCS chemicals. So where does this repeated, completely undocumented ridiculous claim come from?? It is completely unacceptable to have this type of statement in what is purportedly a scientific review.

The third serious error in this sentence is “Pall, in defense of a particular mechanistic theory of MCS, identified ... .” You are making a completely false and unsupported assumption about my motivation here. Even if this assumption were true, that my sole goal in identifying toxicological mechanisms for chemicals in MCS was to support my theory, and let me assure you it is not, you have no way of knowing that your unsupported claim, which you state as a fact, has any support in reality.

If science is anything, it is a search for truth, and it has been clear to me for over a decade, that an essential part in understanding MCS, is to understand the action of the various types of chemicals implicated in MCS. I have made progress in understanding this issue from the first paper I published with Satterlee (8) on MCS, focussing consideration in that paper on organophosphorus/carbamate pesticides, and have made further progress with each further publication on this topic. The Pall and Satterlee paper was published before there was anything like a NO/ONOO- cycle theory of MCS so clearly this search for understanding the action of chemicals in MCS predates the theory and so it transparently has nothing to do with defending the theory. As best I can determine, with the exception of those who have falsely assumed that MCS is an olfactory phenomenon, no one else has even asked let alone approached a complete answer to this essential question. To make a false and undocumented claim about my motivation in trying to understand the initial mechanism of action of chemicals in MCS, something that is of obvious importance to understanding this disease, is the kind of sleaze that may be fairly common in politics, unfortunately, but certainly has no place in science.

Now let’s talk about the issue of whether chemicals act as toxicants in initiating cases of MCS and producing sensitivity responses in those already sensitive. This is obviously an absolutely crucial question for our understanding of MCS. This was obvious even to Staudenmayer who repeated asked the question as to whether MCS is what he called toxicogenic, that is generated by chemicals acting as toxicants, as opposed to psychogenic. Clearly, as acknowledged by Staudenmayer, if MCS is caused by chemicals acting as toxicants, it is not a psychogenic disease. Gots, another psychogenic advocate also raised another aspect of this issue where he claimed that the diversity of chemicals implicated in MCS could not possibly produce a common response in the body. On this basis he inferred that MCS must be psychogenic because it could not possibly be due to a common toxic

response to the diversity of chemicals implicated in MCS. We have then two crucial interrelated questions: Are chemicals acting as toxicants in MCS? Can the chemicals involved produce a common response in the body? Both of these questions are of undoubted great importance to understanding MCS. We now have compelling evidence answering both of those questions. Imagine my astonishment to find that your "scientific" review of MCS completely ignores all of this evidence except for the genetic studies of susceptibility, which you have clearly misinterpreted and in so doing, hidden the obvious relevance of these genetic studies to the first of these questions.

If it can be clearly established that chemicals act as toxicants in MCS, that not only argues compellingly against any strictly psychological mechanism, is also argues equally compellingly against the notion that MCS is a response to odors, another view that has been advocated by many.

I was asked, as you know, to write a review on MCS (9) for "General and Applied Toxicology, 3rd Edition" a multivolume set for professional toxicologists, regulators and others who have important professional roles in dealing with that impact of toxicants on human health. This was to be a authoritative publication on various aspect of toxicology and human health. What should be clear, is that the editors of that publication were convinced before my paper was written that MCS is a toxicological phenomenon, otherwise they would never have solicited a paper on it. It should also be clear that they would not have asked me to write such an authoritative review on the toxicology of MCS, if they had not had substantial respect for my earlier publications on MCS. Finally, it should be clear that they would not have accepted the paper that I wrote if it had had, in their professional judgments as toxicologists, any substantial flaws. The three editors of that multivolume set, Bryan Ballantyne, Timothy C. Marrs and Tore Syversen all have had distinguished publication records in toxicology and all three have published on chemicals implicated in MCS, so it would be difficult to find a more qualified group of toxicologists whose judgments should be respected on these issues. Despite all that, and despite the very extensive documentation provided in my MCS toxicology review (9) and despite the obvious importance of this to our understanding of MCS, you have essentially dismissed this whole area of inquiry leaving your readers profoundly and unnecessarily ignorant about the scientific status of this issue.

The inference that can be drawn from the studies on toxicant action of chemicals in MCS is that all seven classes of chemicals implicated in

MCS clearly act to produce a common response: excessive activity of the NMDA receptors in regions of the body and that this excessive NMDA activity is the main, possibly sole mode of action of these chemicals in MCS.

What I am going to do here, is to outline the types of evidence leading to these inferences. Then I will copy the section in my review that documents three crucial types of evidence (rather than try to rewrite all of this). This section is supported by 115 citations from the scientific literature and is, therefore, more highly documented than is any section of your own review.

The main interpretation is that that toxicants implicated in MCS act in part or in whole by indirectly leading to excessive activity of the NMDA receptors in regions of the body. Let's look at some of the relevant evidence. There are seven classes of chemicals implicated in initiating cases of MCS in the literature. We will consider them in groups here.

Three classes of pesticides, the organophosphorus/carbamate pesticides that act as acetylcholinesterase inhibitors, the organochlorine pesticides that act as $GABA_A$ antagonists, and the pyrethroid pesticides which act in large part by keeping sodium channels open.

- The organophosphorus/carbamate pesticides are established to act as acetylcholinesterase inhibitors, leading in turn to acetylcholine accumulation and therefore increased muscarinic receptor activity. Such increased muscarinic activity act, via known mechanisms including release of glutamate, the most important physiological NMDA agonist, to produce increases in NMDA activity. This is all well established and extensively documented.

- The organochlorine pesticides, another class of chemicals implicated in MCS, are well known to act as GABAa antagonists. Because the GABAa receptors are well known to lower NMDA activity, antagonists which lower GABAergic activity produce increased NMDA activity.

- The pyrethroid pesticides, another class of chemicals implicated in MCS, act by keeping sodium channels open, a process that also is well known to produce increased NMDA activity. Some of the pyrethroid pesticides also act as GABAa antagonists, like the organochlorine pesticides and thus increase NMDA activity via

this mechanism as well. All of this is well documented in the literature.

- The huge class of organic solvents and related compounds probably act by stimulating the TRPV1 (vanilloid) receptors, as was argued and documented in the Pall and Anderson study (9). However, as indicated in my more recent MCS toxicology review (10), it is clear now that this was oversimplified and that other members of the large TRP family of receptors are involved here, especially the TRPA1 receptor, have role in responding to these xenobiotics, as well. The TRPV1 receptor, the TRPA1 receptor and some of the other TRP receptors have been shown to produce increased NMDA stimulation, acting by releasing glutamate, the main NMDA agonist.

- A fifth class of chemicals implicated in MCS has only a single member – mercury which apparently acts via its metabolic product methylmercury. Methylmercury is known to inactivate transporters that transporte glutamate into cells and by doing this methylmercury leads to increased extracellular glutamate and thus excessive NMDA activity.

So with all five of these classes of chemicals, we have well identified and well documented pathways leading to increased NMDA activity. Clearly when one finds evidence supporting an excessive NMDA role for one of these classes of chemicals, that could well be a coincidence. When one finds this pattern over and over again, it becomes much less likely that this is simply a coincidence.

The other two classes of chemicals implicated in MCS, each containing a single compound, are hydrogen sulfide and carbon monoxide. I was unable to identify a specific pathway of action for these two leading to excessive NMDA activity, but as I will show shortly, there is other evidence clearly demonstrating that these both produce increased NMDA activity, as well.

Members of each of these seven classes of chemicals have been studied for their toxic responses in animal models and it has been show in each case, that such members have their toxic responses, often including both morbidity and mortality, greatly lowered by using NMDA antagonists. This shows that each of these not only produce increased NMDA activity but that such increased activity has a predominant role in producing their toxic responses. The finding of a predominant NMDA role in the toxic responses to members of each of

these seven classes of chemicals is quite stunning, and this finding for each of the seven produces a strong argument that they are probably acting in MCS via this mechanism. Certainly, this is the only known confluence of the various pathways of action of five these seven classes of compounds. But the case is NOT limited to this.

There are six additional observations that also suggest an role for excessive NMDA activity in MCS:

1. Published and unpublished observations have been reported that the NMDA antagonist, dextromethorphan, lowers responses to low level chemical exposure in MCS.

2. Alleles of the CCK-B receptor gene that produce elevated NMDA activity have been associated with increased susceptibility to MCS (see Pall 2002 (11) for discussion). The original study reporting this (12) has been recently confirmed by the recent Berg et al (6) study.

3. MCS patients are hypersensitive to monosodium glutamate and glutamate is the main NMDA physiological agonist.

4. Bell and others have proposed that neural sensitization has a key role in MCS and the probable mechanism for such neural sensitization, called long term potentiation, is known to involve increased NMDA activity. A variety of types of evidence supporting neural sensitization in MCS have been reviewed by Bell and her colleagues and also by Ashford and Miller (13). It is difficult to see how such neural sensitization could be implicated in the central nervous system related symptoms of MCS without the chemicals involved having an excessive NMDA role.

5. Elevated NMDA activity has been shown to play an essential role in several animal models of MCS.

6. Elevated NMDA activity appears to play a role in such related illnesses as fibromyalgia, chronic fatigue syndrome and post-traumatic stress disorder, with the most extensive evidence for such a role being found in fibromyalgia. Four NMDA antagonists as well as agents that indirectly decreased NMDA activity have been shown in clinical trial studies to produce substantial improvements in fibromyalgia patients.

It should be noted that the first, third and fifth types of evidence implicate excessive NMDA activity in responses to chemicals in those already chemically sensitive. The second, fourth and all of the evidence previously discussed evidence on the action of chemicals implicated in initiating cases of MCS, implicate excessive NMDA activity in the initiation of the disease.

It should also be noted that each of these six types of evidence, are individually relatively weak, but the combination of the six produces very strong support for excessive NMDA activity in MCS. When taken in conjunction with the evidence on the role of the seven classes of chemicals each producing their toxicant responses in the body via excessive NMDA activity, the evidence becomes overwhelming.

And now, we must return to the genetic studies of susceptibility, where six genes with major roles in determining the metabolism of chemicals otherwise implicated in MCS, have important roles in determining susceptibility to MCS. Since the Nobel prize winning studies by Beadle and Tatum in the 1940's, it has been clear that genetics is THE most powerful single approach to study biological mechanisms. The MCS genetic studies clearly confirm that chemicals act as toxicants in MCS and that genes encoding enzymes that metabolize these chemicals influence, therefore, susceptibility.

None of the data producing the conclusion that chemicals act as toxicants in MCS by indirectly increasing NMDA activity <u>is dependent in any way</u> on my NO/ONOO- cycle fusion mechanism although it does provide substantial support for that mechanism, as do a variety of other types of evidence. Because these data and the inferences drawn directly from them are independent of this mechanism, it follows that conclusions drawn from them do not depend on my NO/ONOO- cycle mechanism either. Primary among these conclusions is that MCS is not psychogenic and that its mechanism is not centered on a response to odors – that is that it is not primarily an olfactory mechanism. This, in turn, argues compellingly against some of the models that you present in your review.

I cannot conceive of anyone ignoring this now easily accessible and obviously relevant data in a general review of MCS and still considering such a review to be scientific. It follows that your draft falls far short of what is needed to be considered scientific. I am requesting, therefore, that if these several important flaws in your draft are not corrected, that you include a copy of this message wherever your review is to be circulated, so that your readers will know that there are

substantial flaws in your review and and where and based on what types of evidence those flaws can be seen

Sincerely,

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Research Director, The Tenth Paradigm Research Group

Citations for the above document:

1. Schnakenberg E, Fabig KR, Stanulla M, Strobl N, Lustig M, Fabig N, Schloot W (2007) A cross-sectional study of self-reported chemical-related sensitivity is associated with gene variants of drug-metabolizing enzymes. Environ Health 6,6-16.
2. Müller KE, Schnakenberg E (2008) Die Bedeutung der Glukuronidierung bei umweltmedizinischen Erkrankungen am Beispiel der UDP-Glukuronosyltransferase 1A1. Umwelt Medizin Gesellschaft 21(4): 295-300
3. McKeown-Eyssen G, Baines C, Cole DE, Riley N, Tyndale RF, Marshall L, Jazmaji V (2004) Case-control study of genotypes in multiple chemical sensitivity: CYP2D6, NAT1, NAT2, PON1, PON2 and MTHFR. Int J Epidemiol 33,971-978.
4. Haley RW, Billecke S, La Du BN (1999) Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. Toxicol Appl Pharmacol 157,227-233.
5. Wiesmüller GA, Niggemann H, Weissbach W, Riley F, Maarouf Z, Dott W, Kunert HJ, Zerres K, Eggermann T, Blömeke B (2008)
Sequence variations in subjects with self-reported multiple chemical sensitivity (sMCS): a case-control study. J Toxicol Environ Health A 71:786-94.
6. Berg ND, Berg Rasmussen H, Linneberg A, Brasch-Andersen C, Fenger M, Dirksen A, Vesterhauge S, Werge T, Elberling J. (2010)
Genetic susceptibility factors for multiple chemical sensitivity revisited. Int J Hyg Environ Health 213:131-139.
7. Povey AC, Jury F, Dippnall WM, Smith AE, Thomson S, Mackness B, Mackness M, Durrington P, Cherry NM. (2007) GST CYP and PON1 polymorphisms in farmers attributing ill health to organophosphate-containing sheep dip. Biomarkers 12:188-202.
8. Pall ML, Satterlee JD. (2001) Elevated nitric oxide/peroxynitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome, and

posttraumatic stress disorder. Ann N Y Acad Sci 933:323-329.
9. Pall ML, Anderson JH (2004) The vanilloid receptor as a putative target of diverse chemicals in multiple chemical sensitivity. Arch Environ Health 59,363-372.
10. Pall ML (2009) Multiple Chemical Sensitivity: Toxicological Questions and Mechanisms. In General and Applied Toxicology, Bryan Ballantyne, Timothy C. Marrs, Tore Syversen, Eds., John Wiley & Sons, London, pp 2303-2352.
11. Pall ML. (2002) NMDA sensitization and stimulation by peroxynitrite, nitric oxide, and organic solvents as the mechanism of chemical sensitivity in multiple chemical sensitivity. FASEB J 16:1407-17.
12. Binkley K, King N, Poonai N, Seeman P, Ulpian C, Kennedy J. (2001) Idiopathic environmental intolerance: increased prevalence of panic disorder-associated cholecystokinin B receptor allele 7. J Allergy Clin Immunol 107:887-90.
13. Ashford N, Miller C (1998) Chemical Exposures: Low Levels and High Stakes, 2nd edition. John Wiley & Sons, New York.

Diverse chemicals are reported to apparently initiate cases of MCS

Citations provided in: Pall ML (2009) Multiple Chemical Sensitivity: Toxicological Questions and Mechanisms. In General and Applied Toxicology, Bryan Ballantyne, Timothy C. Marrs, Tore Syversen, Eds., John Wiley & Sons, London, pp 2303-2352.

There have been dozens of papers reporting a pattern of chemical exposure preceding development of cases of MCS, typically one high level exposure or multiple lower level exposures (Ashford and Miller, 1998; Sorg 1999). Pall (2007a, chapter 13) cited 24 distinct studies reporting chemical exposure preceding development of many cases of MCS and Miller (2000) cited12 additional such studies and still additional studies are cited below in this section. The types of chemicals most commonly involved are the volatile organic solvents (sometimes described as volatile organic compounds or VOCs) and pesticides, especially organophosphorus and carbamate pesticides (Ashford and Miller, 1998; Sorg 1999; Rea, 1992; Ziem and McTamney, 1997). There are a number of additional papers reporting that exposure to organic solvent chemicals that outgas in “sick building syndrome” situations also appear to initiate cases of MCS (Welch and Sokas, 1992; Davidoff and Keyl, 1996; Miller et al, 1999; Hodgson, 2000; Arnold-Llamosas et al, 2006; Redlich et al, 1997; Ross, 1997). Berglund et al (1984) reported that apparently chemically sensitive individuals reacted to air piped in from such a “sick building” in blinded fashion but did not react to uncontaminated air, suggesting that chemicals in the “sick building” air were causal in generating the reactions. Many of the chronic symptoms of the surviving victims of the Bhopal disaster may be ascribed to MCS (Ross, 2000; Nemery, 1996).

When Miller and Mitzel (1995) wanted to compare cases of MCS apparently initiated by two different classes of chemicals, they chose cases from recently remodeled sick buildings (volatile organic solvent exposure) and compared those with cases apparently initiated by organophosphorus pesticides. In their highly cited paper, Miller and Mitzel (1995) found these two groups of MCS patients were similar but not identical to each other with some differences in symptom patterns and some differences in average severity between the two groups. Because MCS cases apparently initiated in these two ways are so common, it was relatively easy for Miller and Mitzel to find substantial numbers of patients of the two types to study.

Two of the most interesting sick building cases occurred in the then recently remodeled Environmental Protection Agency building in Washington DC in which approximately 200 people were apparently sickened with cases of MCS (Miller, 2001) and in Brigham and Women's hospital in Boston, part of the Harvard Medical School complex. The latter case, was described in detail in a US government publication (Kawamoto et al., 1997), where subsequent decreases in chemical usage and increases in air flow led to substantial decreases in new cases of chemical sensitivity and related illnesses, suggesting a causal relationship between chemical exposure and illness initiation. Ashford and Miller (1998) suggested that the decreases in required air flow in buildings in the US, as a response to the energy crises of the 1970's, led to major increases in the incidence of MCS. In an important study, occupational medicine patients differed from general patients in responses to the Toronto MCS questionnaire in much the same way that self identified MCS patients did, albeit to a lesser extent (McKeown-Eyssen et al, 2001), suggesting that chemical exposure in the occupational environment may initiate substantial numbers of MCS cases. Zibrowski and Robertson, 2006 reported increased prevalence of MCS-like symptoms among laboratory technicians exposed to organic solvents as compared with similar laboratory technicians with no apparent exposure. An epidemiological study, estimating the prevalence of MCS in various occupations including those expected to have substantial chemical exposure to classes of chemicals implicated in MCS as a consequence of the occupation, reported increased prevalence of MCS in several occupations involving such chemical exposure, again suggesting a causal role of chemical exposure (Maschewsky 1996 and 2002). Yu et al, 2004 found high prevalences of MCS-like symptoms among solvent exposed printing workers as compared with non-chemically exposed controls. There are at least a dozen studies reporting high prevalences of reactive airways disease, a common aspect of MCS, among workers occupationally exposed to organic solvents.

In addition to organic solvents and related compounds and the organophosphorus and carbamate pesticides, there are additional classes of chemicals that are reported to apparently initiate cases of MCS. These include the organochlorine pesticides chlordane, lindane, dieldrin and aldrin (Corrigan et al, 1994; Ziem and McTamney, 1997; Lohmann et al, 1997;

Wallace, 1995; Pröhl et al, 1997) and also a variety of pyrethroid pesticides (Corrigan et al, 1994; Lohmann et al, 1997; Altenkirch, 1995; Altenkirch et al, 1996; Bradberry et al, 1995). Lindane has been shown to initiate apparent animal models of MCS (Gilbert, 2001; Cloutier et al, 2006) as has another GABAa antagonist (Adamec, 1994). There are reports that hydrogen sulfide exposure can initiate cases of MCS-like illnesses (Kilburn 1997 and 2003). Donnay (1999 and 2000) has reviewed evidence suggesting that carbon monoxide exposure may be able to initiate cases of MCS. Furthermore, mercury and mercurial compounds are also reported to apparently initiate some cases of MCS (Eneström and Hultman, 1995; Latini et al, 2005; Brent 2001; Stejskal et al, 1999) and dental assistants working with mercury amalgams were reported to have higher prevalences of neurological symptoms including MCS-like symptoms (Moen et al, 2008).

Mold exposure is also suggested to initiate cases of MCS in sick building situations characterized by mold infested buildings (Redlich et al, 1997; Claeson et al, 2002; Lee, 2003; Mahmoudi and Gershwin, 2000; Straus et al, 2003). Here, we cannot say much about what mycotoxins may be involved although there is some evidence that Stachybotrys molds may be often involved (Mahmoudi and Gershwin, 2000; Hintikka, 2004; Straus et al, 2003; Pestka, 2008). Hirvonen et al (1999) reported that moldy "sick" buildings produced increases in nitric oxide and inflammatory cytokines in nasal passages of exposed people and similar responses were also reported in the lungs of similarly exposed people (Akpinar-Elci et al, 2008). Nitric oxide and inflammatory cytokines are important aspects of the MCS mechanism developed in this review.

A common response to initiating chemicals: Increased NMDA activity

One of the great puzzles about MCS is how can such a diverse group of chemicals produce a common biological response? In fact, one of the MCS skeptics, Ronald Gots (1996) has argued that MCS cannot possibly be a physiological response to chemicals because the diverse chemicals implicated in MCS cannot possibly produce a common response in the human body. Clearly one needs to find such a common physiological response in order to develop a compelling model of the mechanism of MCS. An important role for excessive NMDA receptor activity in MCS was first suggested by Thomas (1998) and by Dudley (1998). Pall (2002) argued that elevated NMDA* receptor activity is likely to have a key role in MCS and that chemicals were likely to act, in most cases indirectly, to increase such activity. There were several types of evidence reviewed in that paper suggesting a role of elevated NMDA activity:

* The most important physiological agonist for the NMDA receptors is L-glutamate; NMDA stands for N-methyl-D-aspartate, a non-physiological agonist that is specific for these receptors, not acting as an agonist for other, non-NMDA glutamate receptors.

1. MCS patients are hypersensitive to monosodium glutamate and glutamate is the common physiological agonist of the NMDA receptors.
2. In studies of the genetic polymorphism of the CCK-B gene, the allele of the gene that acts indirectly to produce higher NMDA activity was associated with increased prevalence of MCS (Binkley et al, 2001; see Pall, 2002 for discussion).
3. The NMDA antagonist, dextromethorphan was reported from both clinical observations and anecdotal reports to lower reactions to chemicals in MCS patients.
4. Bell and others have proposed that neural sensitization has a key role in MCS and the probable mechanism for such neural sensitization, called long term potentiation, is known to involve increased NMDA activity.
5. Elevated NMDA activity has been shown to play an essential role in several animal models of MCS.
6. Elevated NMDA activity appears to play a role in such related illnesses as fibromyalgia, chronic fatigue syndrome and post-traumatic stress disorder, with the most extensive evidence for such a role being found in fibromyalgia (Pall, 2006 and 2007a).

It should be noted that numbers 2 and 5 above suggest that chemicals initiating cases of MCS may act to increase NMDA activity and number 3 suggests that chemicals acting in those already sensitive may also act to increase NMDA activity. In fact, these two sets of chemicals are similar or identical to each other (Ashford and Miller, 1998) so it should not be surprising if they both may act via the same mechanism(s). All of these considerations raise the question about whether there are known mechanisms by which the several classes of chemicals implicated in MCS may act to increase NMDA activity?

Pesticides and NMDA stimulation

In that Pall, 2002 review, evidence was discussed showing that organophosphorus and carbamate toxicants (including pesticides) can act to produce increases in NMDA activity via the following pathway: These toxicants are acetylcholinesterase inhibitors, producing an increase in acetylcholine which stimulates in turn, the muscarinic receptors which produce, in turn, increased glutamate release leading to increased NMDA receptor stimulation, as well as stimulating other glutamate receptors (diagrammed in Fig. 1). There are a large number of studies showing that toxic effects of organophosphorus toxicants in mammals can be greatly lowered by using NMDA antagonists (see, for example Dekundy et al, 2007; Lallement et al, 1998; Martin and Kapur, 2008) showing that such increased NMDA activity has an substantial role in producing the response to these toxicants.

What about other pesticides and other groups of implicated chemicals? Let us take the different classes of chemicals one at a time. The organochlorine

pesticides, chlordane, lindane, dieldrin and aldrin have all been shown to lower GABAa receptor activity (Gant et al, 1987; Corrigan, 1994; Cassidy et al, 1994; Brannen et al, 1998; Narahashi et al, 1995) and this, in turn is well known to produce elevated NMDA activity (Blaszczak and Turski, 1998; Watanabe et al, 1995; Tusell et al, 1992), see Fig 1. In fact these same citations show that seizure activity produced by these GABAa antagonists, including these pesticides, is lowered or blocked by NMDA antagonists, showing that the elevated NMDA activity produced by such toxicants has a key causal role in the mechanism of seizure generation. Because MCS involves the action of short-term stressors producing chronic illness, it may be of special interest that this pathway produces chronic changes in brain function that can be blocked by short-term interruption of the pathway (Kaindl et al, 2008).

Pyrethroid pesticides which also initiate cases of MCS act to produce long term sodium channel opening (Narahashi et al, 1995; Valentine, 1990; Wu and Liu, 2003; Bradberry et al, 2005; Proudfoot, 2005). This in turn, produces increased NMDA stimulation (Wu and Liu, 2003; Yu, 2006; Doble, 1996), see Fig. 1. Type II pyrethroids also act as GABAa antagonists (Valentine, 1990) and may be expected, therefore, to also act along the same pathway impacted by the organochlorine pesticides, and thus can lead to increased NMDA activity along that pathway, as well.

Organic solvents, TRP receptors and NMDA stimulation

Clearly the greatest puzzle of chemical activity in MCS is how does the huge family of organic solvents act to initiate cases of MCS or elicit sensitivity symptoms in those who have become sensitive? These chemicals are the predominant set of chemicals that trigger reactions on a day to day basis in MCS patients. They have also been referred to as volatile organic chemicals (VOCs) and yet it is clear that nonvolatile chemicals ingested or absorbed through the skin can produce reactions, so the volatility is important due to the most common mode of exposure, inhalation, rather than being an essential part of the mechanism of sensitivity. I will refer to this extremely large group of chemicals as organic solvents even though that does not cover this entire spectrum of chemicals.

Pall and Anderson (2004) argued that the probable target for such organic solvents in MCS is the vanilloid (TRPV1) receptor, and presented 12 distinct types of evidence arguing for such a TRPV1 role in MCS. That paper was extensively documented with 222 citations and while specific references are provided some of this discussion, for the rest the reader is referred back to that paper. One type of evidence that we presented is that some solvents well known to be involved in MCS such as formaldehyde and other aldehydes were quite active TRPV1 agonists. And a variety of alcohols are vanilloid agonists and may be converted into still more active aldehydes via alcohol dehydrogenases in the body. It is known that capsaicin, the classic TRPV1 agonist, requires both hydrophobic regions and a hydrogen bonding group in

order to act as an agonist, suggesting that strictly hydrophobic solvents might require cytochrome P450 metabolism in order to act as a vanilloid agonist or might act synergistically with a solvent that does have a hydrogen bonding group. There is evidence from animal models of MCS that are also animal models of Gulf War illness for such synergistic interactions of organic solvents and related compounds (Binns et al, 2004): Fully 28 studies of synergistically acting stressors, most but not all of which were organic compounds, were reviewed in that document.

Some mycotoxins are known TRPV1 agonists, so it is possible that the role of molds in MCS may be explained through the role of the TRPV1 receptor. Chemical sensitizers including toluene diisocyanate (TDI) and eugenol, which produce local sensitivity to a wide range of chemicals, are known TRPV1 agonists. MCS patients often report sensitivity to chlorine gas from swimming pools or from drinking water and chlorine acts as a TRPV1 agonist *in vivo* (Morris et al, 2005), producing an irritant response. TRPV1 stimulation produces neurogenic inflammation and also reactive airways disease (Geppeti et al, 2008; Jia and Lee, 2007; Planells-Cases et al, 2005; Costa et al, 2008), often called reactive airways dysfunction syndrome or RADS, a form of asthma showing reaction to a spectrum of chemicals similar or identical to those involved in MCS. Both RADS and neurogenic inflammation are often aspects of MCS cases (Meggs, 1994 and 1997).

Millqvist and her colleagues have published a series of papers showing that MCS patients are hypersensitive to capsaicin, the classic TRPV1 agonist, again providing support for a TRPV1 role in MCS (Johansson et al, 2002; Millqvist, 2000;Ternesten-Hasséus et al, 2002; Millqvist et al, 2005 and 2008). Many studies have shown that capsaicin treatment leads the TRPV1-stimulated cells in several regions of the body to release glutamate neurotransmitter, leading in turn to NMDA stimulation (10 such studies are cited in Pall and Anderson, 2004). These studies provide further support for the contention that each class of chemicals involved in MCS lead to increased NMDA stimulation.

There is an additional parallel between MCS and TRPV1 stimulation. MCS patients have a phenomenon known as desensitization or masking such that low level chronic or repeated chemical exposure leads to decreased reactivity to chemical exposure (Ashford and Miller, 1998). This may be the basis of using low level chemical exposure to treat MCS patients (Weaver, 1996; Rea, 1997). Low level chronic or repeated exposure to many TRPV1 agonists leads to lowered TRPV1 activity through a complex series of changes involving increased intracellular calcium levels, complex protein phosphorylation control and probably receptor internalization (Szallasi and Blumberg, 1999; Itagaki et al, 2004). Thus the desensitization/masking phenomenon found in MCS may be produced, to part or in whole, by this lowered TRPV1 activity.

While there are many properties suggesting a TRPV1 role in MCS, it is clear now that some of the interpretations given by Pall and Anderson (2004) to some of the relevant data were too narrow. It was argued, for example, that TRPV1 was primarily responsible for the sensory irritation (SI) response, a response elicited by chemicals including alkanes, alkyl benzenes, halogenated benzenes, halogenated alkylbenzenes, alcohols, ketones, ethers, aldehydes, formaldehyde, isocyanates, and chlorine (Nielsen, 1991; Alarie, Y. et al, 1998; Inoue and Bryant, 2005; Cometto-Muñiz and Abraham, 2008), a broad range of chemicals also implicated in MCS. It is now clear that this SI response involves as major players, other members of the transfer receptor potential (TRP) family of receptors, not just TRPV1. Specifically Bíró et al (2007) discuss evidence for a role of TRPA1, TRPM8 and TRPV2, 3 and 4 receptors in this response, as well as TRPV1. Bautista et al (2006) implicated specifically the TRPA1 receptor in the response to several environmental irritants. Many of the TRP receptors have roles in responding to xenobiotics (Nilius, 2007) and while our knowledge of such roles has been expanding rapidly in recent years, it is still, no doubt, incomplete. Neurogenic inflammation and reactive airways disease aspects of MCS, discussed above and below, are produced not only through TRPV1 stimulation but also through the action of other TRP receptors (Geppeti et al, 2008; Jia and Lee, 2007). Whereas some chemical sensitizers act as TRPV1 agonists, sensitizers can also act as TRPV3 agonists (Xu et al, 2006).

Others have argued for a central role for the sensory irritation response and the receptors involved in that response in MCS (Skov and Valbjorn, 1987; Meggs, 1993 and 1997; Anderson and Anderson, 1999a and b, 2003; Millqvist et al, 1999;Millqvist, 2000 and 2008; Nordin et al, 2005).

In Pall and Anderson (2004), we used the desensitization response produced by low level chronic exposure to capsaicin or other bona fide TRPV1 agonists to assess whether some solvents that had never been tested as possible TRPV1 agonists might have such activity. The reasoning was that if responses to a chemical was reported to be substantially reduced after low level capsaicin treatment, that chemical should be labeled as a probable TRPV1 agonist because the response to it was lowered along with TRPV1 desensitization. It is clear now, that desensitization of one TRP receptor is often accompanied by desensitization of others. For example, TRPV1 and TRPA1 can undergo cross-desensitization (Rohacs et al, 2008; Ruparel et al, 2008) and TRPM8 and TRPA1 desensitization can also be produced in parallel (Zanotto et al, 2008). In another study, a series of TRPC receptors were desensitized together by a receptor internalization process (Itagaki et al, 2004). It seems likely, therefore, that some organic solvents that were argued to be probable TRPV1 agonists, as suggested earlier in this paragraph, may well be agonists of other TRP family receptors.

Of the other TRP family receptors, the one most likely to have a substantial role in MCS, based on current evidence, is TRPA1. TRPA1 is responsible for the activity of a number of different sensory irritants (Bautista et al, 2006;

Gerhold and Bautista, 2008), with TRPV1 being responsible for others. For a number of such irritants, the chemicals react by reversible covalent modification with the TRPA1 receptor (Hinman et al, 2006). Among the TRPA1 agonists are certain aldehydes including acrolein and aldehydic components of cigarette smoke (Andre et al, 2008; Simon and Liedtke, 2007) and MCS patients are known to commonly be sensitive to cigarette smoke. Formaldehyde which is commonly involved in initiating cases of MCS was shown in a recent study to act via the TRPA1 receptor in a model of inflammatory pain, rather than acting via the TRPV1 receptor (McNamara et al, 2007).

Activation of the TRPA1 receptor has been reported to lead to the release of the neurotransmitter glutamate, leading in turn, to increased NMDA activity (Kosugi et al, 2007; Ding et al, 2008). Given that such increased NMDA activity is also produced by TRPV1 receptor stimulation, as discussed above, it should not be surprising that organic solvent-produced changes in the nervous system can, in many cases, be blocked or lowered by using NMDA antagonists. For example, there are a number of responses to formaldehyde exposure that have been shown to be greatly lowered by NMDA antagonists (see for example, Coderre and Melzack, 1992; McMahon et al, 1993; Wiertelak et al, 1994; Wang et al, 1999).

In conclusion, there are compelling similarities between the diverse organic solvents and related chemicals involved in MCS and the diverse organic chemicals involved in the sensory irritation response. It seems likely that the transfer receptor potential (TRP) receptors are involved in both, with the two most likely members of this receptor family to be involved in chemical responses in MCS and in SI, based on current evidence, being the TRPV1 and TRPA1 receptors, both of which can produce an increase in glutamate release and consequent NMDA stimulation. These various data suggest, therefore, that the proposed pattern of chemical involvement in MCS acting through increased NMDA activity is likely to be sustained for the organic solvent group of chemicals.

Before leaving this issue of the apparent roles of TRP receptors in MCS, I need to discuss the TRPM2 receptor that may have a role in amplifying responses in MCS. The TRPM2 receptor is known to be stimulated by oxidants including hydrogen peroxide, with much of the stimulation being produced by ADP-ribose, a signalling molecule whose levels can be greatly increased by oxidants (Kühn et al, 2005; Fonfria et al, 2004; Wilkinson et al, 2008; Naziroglu, 2007; Buelow et al, 2008; Lange et al, 2008). The pathway of synthesis of poly(ADP)-ribose is as follows: Oxidants produce nicks in DNA strands in the nucleus of cells which can lead, in turn to a massive stimulation of poly(ADP)-ribose polymerase activity, producing poly(ADP)-ribosylation of chromosomal proteins. When this poly(ADP)-ribose becomes subsequently hydrolyzed, it produces free ADP-ribose which acts as a signaling molecule. One oxidant that is very active in this process is peroxynitrite (Pacher and Szabo, 2008), a molecule that the author has

argued (see below) has a key role in MCS and related illnesses and whose synthesis is greatly increased by NMDA stimulation (reviewed in Pall, 2002; Moncada and Bolaños, 2006; Brown and Bal-Price, 2003). Consequently, TRPM2 activity is predicted to be elevated in MCS and to be stimulated by chemical exposure in MCS. TRPM2 may both directly and indirectly leading to increases in nitric oxide and peroxynitrite production, thus amplifying the already elevated levels of these compounds (see Yamamoto et al, 2008 for discussion). There is some evidence that another TRP receptor, TRPM7, may also have a role in this process (Miller, 2006). The role of TRPM2 and possibly 7 may be one of several interacting mechanisms that may lead to the extraordinary chemical sensitivity reported in MCS patients.

There is evidence that other TRP receptors are elevated in to response oxidants and products of oxidative stress biochemistry, including TRPV1 and TRPA1 (Taylor-Clark et al, 2008; Bessac et al, 2008; Andersson et al, 2008; Trevisani et al, 2007; Puntambekar et al 2005; Schultz and Ustinova, 1998; Ustinova and Schultz, 1994) but these effects may be more modest than those on TRPM2. The effects on TRPV1 receptors makes them more susceptible to stimulation by their effectors whereas with TRPM2, oxidative stress acts to open the receptor channel independently of any effector and so may produce a greater physiological response under many circumstances.

Other apparent initiators and summary of NMDA role

Three other apparent initiators of cases of MCS were discussed above, carbon monoxide, hydrogen sulfide and mercury. Do any of these act to increase NMDA activity?

Carbon monoxide has been reported to produce such increased NMDA activity and NMDA antagonists block or lower the toxic responses to carbon monoxide exposure (Thom et al, 2004; Liu and Fechter, 1995; Penney and Chen, 1996; Ishimaru et al, 1992). Hydrogen sulfide also can produce increased NMDA activity and again its toxic effects are lowered by NMDA antagonists (Cheung et al, 2007; Qu et al, 2008; Kamoun, 2004). Mercury, acting through its metabolic product methylmercury, also acts to produce increases in NMDA activity, and again methylmercury toxicity is lowered by NMDA antagonists (Juárez et al, 2005; Allen et al, 2002; Faro et al, 2002; Miyamoto et al, 2001; Zhang et al, 2003; Rossi et al, 1997). Methylmercury acts to produce such increased NMDA activity, at least in part, by lowering the transport of the glutamate, the most important physiological NMDA agonist (Juárez et al, 2005; Allen et al, 2002).

In summary, then, we have evidence that all seven classes of compounds reported to initiate cases of MCS can each act to increase NMDA activity (Fig 1). At least for some members of each class under some conditions, NMDA antagonists can lower the toxic responses to each of them. While evidence linking any one of these to increased NMDA activity may be coincidental, the pattern of evidence for all seven strengthens the argument that increased

NMDA activity is not likely to be coincidental. When coupled to the six types of additional evidence, discussed at the beginning of this section, on the apparent NMDA role in MCS, one can argue that there is very substantial evidence not only that increased NMDA activity has a role in MCS but also that chemicals are likely to act indirectly by increasing such NMDA activity.

There is extensive evidence that increased NMDA activity produces increases in nitric oxide and also its oxidant product peroxynitrite (reviewed in Pall, 2002; Moncada and Bolaños, 2006; Brown and Bal-Price, 2003), and it will be argued below that all three of these, NMDA activity, nitric oxide and peroxynitrite, are likely to have key roles in MCS.