



Analysis of research into the role of toxic agents in the etiology of multiple sclerosis

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Although genetic factors are believed to play an important role in the etiology of multiple sclerosis, evidence also suggests the involvement of environmental (non-genetic) factors. One group of environmental factors that may play a role in triggering MS is toxic agents such as organic solvents, heavy metals, and radiation. Toxic agents have been determined to cause or contribute to a variety of neurological diseases; for example, chronic lead poisoning can result in encephalopathy. Therefore, it is feasible that one or more toxic agents are involved in the development of multiple sclerosis. However, no toxic agent to date has been unequivocally implicated as a risk factor for the disease.

This Phase II document of the Accelerated Cure Project's Cure Map summarizes the published research on the involvement of toxic agents in MS, documenting what is known and what is not yet known about the role toxic agents play in this disease. It uses the general framework of categories outlined in our Phase I document on toxic agents and disease ("A framework for describing diseases caused by toxic agents"). Each Phase 1 category is discussed below in its own section.

Supporting material for this document can be found in "Phase II: Toxic Agents Supplement – MS Studies," which is a listing of the studies investigating the possible involvement of individual toxic agents in MS. Another document, "Analysis of specific toxic agents as possible triggers of MS," reviews and assesses the research conducted to date on the six toxic agents that have been most thoroughly studied for a role in MS: cigarette smoke, ionizing radiation, lead, mercury, organic solvents, and radon. Each of these documents can be downloaded from the Accelerated Cure Project web site at www.acceleratedcure.org/downloads/.

Composition of toxic agent

The composition of a toxic agent helps determine its disposition and activity in the body. Therefore, if one or more toxic agents are involved in triggering MS, clues to their nature may be derived from what is known about the pathophysiology of MS. There are two categories of toxic agents that should be considered potential risk factors for MS: toxic substances and radiation.

Substances

Two characteristics of substances (elements and compounds) that greatly affect their potential to be transported throughout the body and cause harm are lipophilicity and size. In MS, a disease of the central nervous system, these two characteristics become important when considering substances that can penetrate the blood-brain barrier (BBB), a tight layer of endothelial cells that protect the brain from harmful cells and molecules.

Toxic agents that can pass through this protective wall may be able to cause or contribute to focal demyelinating lesions or more diffuse forms of damage seen in MS. Generally, although there are exceptions, the ability of a substance to permeate the BBB depends on its size and lipophilicity – the smaller and more hydrophobic a molecule is, the more readily it can enter the brain¹. Of the individual substances that have been explored for a possible etiological role in MS, several meet the requirements of small size and lipophilicity. For instance, organic solvents (50-150 kDA) and cyanide (26.03 kDA, a component of cigarette smoke) both meet these criteria. However, none yet has been confirmed as a definite risk factor for MS. In addition, many other substances are known to meet these requirements but have not yet been rigorously assessed for involvement in MS.

Besides directly infiltrating the BBB, toxic substances may also contribute to the development of MS in other ways, such as by making the BBB more permeable to other substances, causing genetic defects that make a person more susceptible to MS, or altering immune system functionality. Toxicity in these cases may not depend on characteristics such as size or hydrophobicity and therefore it is hard to characterize *a priori* the composition of substances that could have these effects.

Radiation

Exposure to radiation may result in damage to DNA which may initiate tumor formation and other disease processes. Two types of radiation, ionizing radiation (X-rays, alpha particles, and gamma rays) and radon (a radioactive element), have been explored as risk factors for MS, but only a few studies on each type have been performed. Indirect support for the involvement of radiation in MS comes from research suggesting that MS subjects have increased chromosomal breakage, which may be a result of radiation exposure but may be due to other causes as well. (See our Phase 2 Genetics document “Analysis of chromosomal abnormalities as a possible cause of Multiple Sclerosis” for more information.) Interestingly, some researchers hypothesize that ultraviolet radiation may in fact protect people from multiple sclerosis, based on the tendency for the incidence of MS to be lower at lower latitudes. Overall, it has not yet been determined whether and how radiation may contribute to (or guard against) the development of MS.

Origin of toxic agent

Identifying toxic sources associated with MS may be helpful in understanding why certain geographical locations have higher risks of multiple sclerosis than others. To date, no large scale studies have been conducted to find sources of toxic agents that are more prevalent in areas of high vs. low MS incidence. Most of our knowledge about possible sources associated with MS therefore comes from studies of individual toxic agents and our knowledge about their origins.

Toxins

Toxins are specific substances released by biological entities either as a defense mechanism or as an aid in the capture and digestion of food.

- **Infectious agents** – Toxins produced by infectious agents will be addressed in the Pathogens track of the Cure Map.

- **Animal venoms/plant toxins** – No study has directly assessed whether there is a link between MS and history of venomous bites or stings or contact with plant toxins. Because distributions of animal and plant species vary throughout the world, it may be informative to compare geographical prevalence of MS with the presence of toxic species and/or frequency of human exposure to these species.

Endogenous

The endogenous (internal) production of toxic agents may play a role in the pathogenesis of MS. For example, overproduction of nitric oxide (NO), an endogenously produced signaling molecule, has been studied as a factor in the pathogenesis of multiple sclerosis². However, production of endogenous toxic agents generally occurs as a secondary response to other factors such as infections, diet, or genetic factors. Because this document is concerned with finding the primary causes or triggers of MS, we will not discuss endogenous agents in depth.

Toxicants

Toxicants are products or by-products of anthropogenic (human) activities. Most of the individual toxic agents studied in relation to multiple sclerosis fall under this category. Cigarette smoking is a prime example of an anthropogenic activity that may increase the risk of MS. X-rays and ionizing radiation are used in health care, organic solvents are used for industrial purposes, and mercury is an essential part of dental amalgam. Until recently, lead was commonly used in gasoline, paint and pipes. So far, no toxicant or toxicant-producing activity is a clear risk factor for multiple sclerosis; nor has any been studied extensively enough to be ruled out as a trigger of multiple sclerosis. (See “Analysis of specific toxic agents as possible triggers of MS” for discussions of each of these individual factors.)

Earth naturals

Very few studies have attempted to associate the presence of naturally occurring substances with multiple sclerosis. Although the geographical distribution of multiple sclerosis has been studied extensively, no common geological factors have been identified in regions with elevated rates of MS. A comprehensive large scale comparison between the prevalence of earth naturals and the prevalence of MS in multiple geographic areas would be useful but could prove difficult because of the number of factors that would need to be included.

Therefore, MS studies focusing on earth naturals for the most part have analyzed the levels of specific toxic agents in specific regions. Mercury, radon and lead are examples of earth natural substances that have been studied as risk factors for MS. Scientists have searched for correlations between MS clusters and areas of unusually high amounts of various elements, but so far no firm conclusions can be drawn from the evidence³⁻⁸. Lead has been studied the most extensively, but not all areas with a high prevalence of multiple sclerosis have high lead content. Other trace metals have also been identified in MS clusters, including molybdenum, zinc, and chromium, but whether these have any effect on the risk of MS has not been fully explored.

Vector

No common path of transmission of a toxic agent has been determined to be associated with multiple sclerosis. As with the origin of a toxic agent, it is difficult to identify a common vector without first identifying a specific toxic agent that may be a risk factor. The fact that it is impossible to pinpoint exactly when MS begins to develop in a given person also makes it difficult to determine which vectors may be relevant to the disease. Certain migration studies indicate that the risk of developing multiple sclerosis is dependent on childhood circumstances (although an overview of all migration studies prior to 1995 sheds doubt on this hypothesis, citing small sample sizes and confounding factors⁹). If exposure to MS triggers does occur in childhood, an MS vector would be particularly difficult to identify due to the lapse of time between the initial trigger and clinical onset which typically occurs in adulthood.

Food and drink

Because foods or beverages can harbor toxic agents either as components or contaminants, it is conceivable that an agent found in food or drink may contribute to the development of MS. Studies of nutritional risk factors in MS have assessed a number of food categories, such as dairy products and products containing saturated fat. So far no specific food or beverage has been conclusively tied to MS, but if future research does strongly implicate one, it may be worth asking whether its involvement is due to the presence of toxic substances it contains rather than nutritional elements.

A number of ecological studies have taken the alternative approach of analyzing soil and water contents in areas with high rates of multiple sclerosis, in search for toxic agents that may end up being ingested (see “Earth Naturals” section above). However, it should be noted that abnormal levels of metals in soil do not immediately correspond to high levels in food as the metallic content in plants is dependent on a variety of factors including climate and weather¹⁰.

Medication

Ionizing radiation, a suggested risk factor for MS, is used in radiological treatments which can be a major source of radiation exposure for humans. Two studies have found that MS patients are more likely than control subjects to have received radiological treatment, but it is unclear whether treatment is a risk factor for MS or whether having MS increases the chance that a person will have radiological treatments^{11, 12}.

Epidemiological studies have also examined whether vaccines for other diseases appear to increase the risk of MS. The hepatitis B vaccine has undergone the most intensive scrutiny, with some studies finding no evidence for its involvement in MS and others finding support for a role, although possibly a small one (see Hernan et al¹³). In addition to a hepatitis B antigen, the vaccine contains an adjuvant (aluminum hydroxyphosphate sulfate) and yeast proteins; in the past it also contained thimerosal, which is a mercury-based preservative. Whether any of these components could increase the susceptibility to MS and how this would occur have not yet been determined.

Finally, a few studies have also looked at whether oral contraceptives affect the risk of MS, but none has found a positive correlation¹⁴⁻¹⁶.

Substance abuse

A few studies have found that people with MS are more likely than non-MS controls to have been smokers, and that higher levels of smoking can result in increased risk, although not every study on this topic has detected an association^{11, 12, 16-21}. One study explored whether alcohol, drug, tobacco, or medication abuse affect the risk of multiple sclerosis. In a case-control study of 108 MS patients, subjects completed a questionnaire on history of substance abuse. Only drug abuse was associated with a significantly increased risk of MS, but no specific drug was identified¹⁷. A similar case-control study found a higher frequency of past hard liquor consumption in MS cases compared with controls, but this association was not significant after adjustment for smoking history²¹. Finally, the abuse of toluene in the form of glue-sniffing has also been reported as a possible cause of MS-like symptoms. For example, one paper describes a 31-year-old man with a history of toluene abuse who reported recurring symptoms such as “progressive incoordination, lower limb weakness, right hemifacial tingling and paraesthesia in the hands.” However, abnormal white matter diffusivity indicated that he did not suffer from clinical multiple sclerosis²².

Domestic uses

Little research has been performed on whether exposures to toxic agents used in domestic settings increase the risk of MS. Two studies examined the correlation between MS and the use of herbicides and pesticides but no link was found. It is also unclear whether the exposure was due to occupational or domestic use of these products^{12, 23}.

Occupational exposure

Specific occupations that have been assessed for a possible higher incidence of multiple sclerosis are listed in Appendix A. Some, such as metalworking, have been linked with an increased risk of MS. However, these studies do not tend to investigate any specific toxic agents that are used in these respective industries and that could affect risk of MS through occupational exposure.

Other studies have investigated the effect on risk of MS of occupational exposure to a specific agent, namely organic solvents. Organic solvents have been linked to multiple sclerosis in several studies although most of them do not assess actual exposure but likelihood of exposure (see Landtblom et al²⁴ and Landtblom²⁵ for a meta-analysis and discussion of the research findings on this topic). Organic solvent exposure has been documented in various occupations, including automobile assembly, printing, typography, carpentry, cabinetmaking, shoe and leather production, and painting.

Finally, one study documented 18 people with MS-like symptoms who had all worked at a New York manufacturing plant. The authors performed a case-control analysis of plant employees and found a higher risk for these symptoms in workers who were exposed to die casting and/or to organophosphates (substances known to cause axonopathy)²⁶.

Environmental exposure

A multitude of toxic substances are released into the environment through vehicle exhaust, industrial waste, and other human activities such as cigarette smoking. Some investigations of potential MS clusters have focused on specific sources of environmental pollution, such as smelters and oil refineries²⁷. Several^{28, 29} of these have reported an excess of MS cases around these sources. However, to date these investigations have not revealed any consistent patterns of exposures linked to MS, nor have they pinpointed particular toxic substances as risk factors for MS.

A few individual toxic agents that may be encountered through environmental exposure have been studied in MS. For example, researchers have studied lead levels in areas with high prevalence of MS; however, these studies typically have concentrated on soil levels as opposed to airborne lead. Again, no concrete evidence exists that indicates environmental exposure to one or more specific agents is a significant risk factor for MS.

An alternative approach involves determining whether people with MS, prior to onset, were more likely to be living in environments with a generally high level of toxicants. In other diseases such as asthma, exposure to high levels of toxicants such as air pollutants has been found to increase a person's susceptibility. Some researchers have examined whether living in an urban vs. a rural setting may be associated with MS (people in urban settings are presumably more likely to come in contact with certain types of toxicants such as vehicle exhaust than those in rural areas). So far no definite relationship has been determined between multiple sclerosis and urban life; studies have found positive correlations between the risk of multiple sclerosis and both urban and rural settings³⁰⁻³³. For example, in one study of Southern Hesse, Lauer, et al, found that people with MS were more likely to have lived in rural areas as children than would be expected based on population distributions³⁴. However, other studies have linked MS with urban environments, such as a study of US veterans which associated urban residence at the time of entrance into the military with a higher risk of MS for white males.³⁵

Animal bites

This has not been assessed. As mentioned above, no studies have explored whether there is a connection between MS and venomous animal bites. Numerous studies looking into infectious triggers of MS have assessed whether exposure to household pets is associated with risk of MS, with inconclusive results overall. Even if domestic animal exposure is a risk factor for MS, it has not been proposed that this could occur through transmission of toxic agents via bites.

From nature

Whether people who develop MS have had greater exposure overall to toxic agents found in nature has not been assessed. Specific agents produced by natural sources have been studied, such as radon which is present throughout the earth in varying concentrations and is the main source of radiation exposure for most people. Mercury and lead are also present in nature in varying amounts but in quantities lower than toxic levels. As stated before, no specific toxic agent including these has yet been strongly linked with risk of MS.

Entrance

Toxic agents can be introduced into the body in a variety of ways, and these modes of entrance can greatly influence their level of toxicity. No specific studies have tried to characterize specific entry mechanisms as risk factors for MS. Rather, any inferences that can be made about entry site(s) that may be involved in MS are based on investigations of specific toxic agents and their known entrance routes.

For some of the toxic agents that have been studied with respect to MS, entrance into the body is well-defined. For example, cigarette smoke is inhaled through the lungs. On the other hand, there are some toxic agents, such as organic solvents, that could enter the body through multiple paths. In these cases, researchers often do not attempt to identify the mode of entrance, but rather only consider exposure levels.

The following are sites of entry that toxic agents may take into the body:

Skin

No studies have assessed whether the skin (intact or broken) is a key entrance site for toxic agents that increase susceptibility to MS. In terms of specific toxic agents, dermal entry is a likely pathway for organic solvents to enter the body. However, MS studies that analyze organic solvent exposure do not report whether or not subjects were in direct contact with the substance. Since the skin is exposed to innumerable substances every day, identifying entrance through this path might be difficult.

Lungs (inhalation)

This is a mode of entrance for several of the toxic agents that have been investigated in MS. Cigarette smoke and vapors from organic solvents are absorbed through the lungs. Also, before being banned from gasoline, high levels of lead were present in the air in heavily populated areas. Mercury is a volatile substance and may be inhaled, for instance through accidental or occupational exposure. Radon, a radioactive gas, also enters the body through inhalation.

GI tract (ingestion)

Food or beverages containing lead, mercury or other toxic agents not yet studied in MS may be ingested and enter the body through the GI tract. It is possible that studies of dietary patterns in people with multiple sclerosis may one day identify toxic risk factors that enter through this route.

Eyes

This mode of entry has not been assessed. Toxic agents could enter through this route via eye drops, contact lens solution, and eye ointments, but no studies have correlated any of these substances with an increased risk of MS. Studies on past eye irritation or inflammation in multiple sclerosis patients could provide information on whether or not this route was an entrance for a toxic agent.

Suppositories

This mode of entry also has not been assessed.

Intravenous or other injections

This has not been assessed directly. As mentioned previously, vaccines (which are usually injected) have been investigated as possible risk factors for MS but their involvement has not yet been determined. As for other types of injections, medical records with information on injections or histories of drug abuse may clarify whether or not multiple sclerosis subjects had been exposed to toxic agents through this route.

Implants

Because of their high mercury content, dental amalgams are the only implants that have been studied repeatedly for an effect on risk of MS. Implants may be more feasible to study in relation to MS than other modes of entrance since they are generally recorded

in medical files and are often easily detectable.

A number of studies have assessed whether people with MS are more likely to have dental amalgams than non-MS subjects (see for example Bangsi et al³⁶ and Casetta et al²³). So far no conclusion can be reached on whether or not dental amalgams play a role in the pathogenesis of multiple sclerosis. (See the section on mercury in the document “Analysis of specific toxic agents as possible triggers of MS” for more information.)

Radiation

Ionizing radiation can enter the body without direct contact. Only a few studies have assessed exposure to radiation as a risk factor for MS, but each of these reported evidence for a link between radiation and MS.^{5, 6, 11, 12}

Pharmacokinetics – disposition of toxic agent

Pharmacokinetics refers to how the body handles the toxic agent to which it has been exposed, including how it absorbs, distributes, transforms, stores and excretes the agent. This section reviews the evidence present in multiple sclerosis that may reflect processing of a toxic agent.

Local toxicity

Local toxicity covers a range of effects that are produced at the site of entry of a toxic agent into the body. Some of these effects (such as hair loss and corrosion) are acute in nature and/or easily identifiable and therefore potentially feasible to research. However, to date no studies have been published that explore the correlation of local toxicity effects with multiple sclerosis. The potentially long separation in time between the initial triggers of MS and the development of MS would complicate any efforts to associate local toxicity events with MS. In addition, not all toxic exposures cause noticeable local effects.

Absorption

The main sites of absorption are the GI tract, lungs and skin. No evidence that associates absorption through these sites with development of MS has been found independent of specific substances previously mentioned, which may tend to be absorbed in particular sites.

Distribution

The distribution of toxic agents throughout the body, including to the brain, occurs primarily through the blood. However, many substances do not remain in the blood stream for extensive lengths of time, except in the case of ongoing chronic exposures. Instead, they are stored or excreted, so implicating a toxic agent as a cause of MS by testing the blood of MS patients may prove difficult. One small study investigating the potential for lead poisoning in MS did analyze blood samples from five MS subjects; each were found to have normal lead levels³⁷. Two other studies that assessed blood or serum levels of a number of chemical elements found similar or lower levels of lead and mercury in MS cases compared with controls^{38, 39}.

Systemic toxicity

The central nervous system and the immune system both exhibit damage or alteration of function in MS and thus are candidate targets for systemic effects of toxic agents. Possible mechanisms by which various toxic agents could affect the immune system or central nervous system (CNS) are described below under “Pharmacodynamics.” Actual evidence linking any particular toxic agent with systemic effects in MS is sparse, however. Several toxic agents that have been studied in MS have been shown to be capable of entering the CNS and causing damage. For example, lead has a high affinity for cerebral endothelial cells and can accumulate in high amounts in the brain. Mercury also accumulates in CNS cells such as microglial cells, neurons, and epithelial cells¹. However, no analyses of CNS tissue have shown conclusive evidence of systemic toxicity – for instance, post-mortem brain analysis of multiple sclerosis patients revealed no significant increases in either lead or mercury compared with control subjects⁴⁰.

In addition to the CNS and immune system, other organs or systems are often impaired during the course of MS but this impairment is generally secondary to the loss of nervous system function. However, it is possible that a toxic agent that is involved in triggering MS could also be distributed to and cause harmful effects in other organs or tissues, which could be detected. For example, if an agent that increases the risk of MS also impairs reproductivity, then loss of fertility would likely be documented in MS subjects. Therefore, recording and analyzing other conditions experienced by MS subjects either before or after MS onset may reveal common patterns that would point toward a toxic trigger.

Modification

Modification of toxic agents has not been extensively studied in multiple sclerosis patients because no correlation between a toxic agent and the disease has been strong enough to warrant further investigation.

- **Biotransformation** – The biotransformation of toxic agents has not been studied in relation to multiple sclerosis, although this may be an important area to research. It is possible that multiple sclerosis patients are unable to transform certain toxic agents into less harmful metabolites, making them more susceptible to toxic effects. This impairment could be a result of a genetic defect, for instance. (A discussion of genetic factors involved in metabolism of toxic agents is included below in the “Epidemiology” section.)
- **Interaction** – Evidence suggests that cigarette smoke and indoor radon have a synergistic effect on lung cancer rates when encountered in combination. Since both substances are also hypothesized to be risk factors for multiple sclerosis, it is possible that their interaction may further increase the risk of MS. However, no specific studies on the synergistic, additive, potentiative, or antagonistic effects of toxic agents in relation to multiple sclerosis have been published.

Storage

The main storage sites of toxic agents include plasma, adipose tissue, bone, and the liver and kidneys. Very few studies have analyzed these tissues in MS subjects to assess the presence of toxic agents. As mentioned above, Westerman, et al, studied the lead levels in the bones and blood of five MS subjects, but found no significant differences from controls³⁷. Interestingly, fat can sequester lipophilic toxic agents and

therefore concentrations of those agents in other tissues can decrease as a person's amount of body fat increases. Likewise, these agents can be released into the bloodstream and made available to other tissues as fat is mobilized. It may therefore be useful to investigate whether there is any correlation between body weight changes and risk of MS.

Excretion

A common method of determining exposure to toxic agents is to analyze bodily excretions for high concentrations of substances of concern. However, because the body attempts to excrete toxic agents as quickly as possible, it may not be possible to detect transient exposure to a toxic agent that triggers a disease like MS in which onset may occur years after exposure. Nevertheless, a few attempts have been made to measure levels of toxic agents in the excretions of MS subjects and controls.

In one study (Perry, et al), scientists studied urine samples from 12 multiple sclerosis subjects and 12 control subjects. The controls were chosen from the same households as the MS subjects to eliminate environmental and dietary factors as confounders. Researchers found no significant difference between MS subjects and controls in excretion of trace metals, including lead, tin, nickel, silver, copper, iron, molybdenum, and zinc⁴¹. Westerman, et al, also studied the urinary lead content of MS subjects and found normal levels compared with control subjects³⁷. In a study of dental amalgams, researchers found that multiple sclerosis patients had significantly higher hair mercury levels compared to control subjects. However, it is unclear how many of the cases and controls had dental amalgams in place at the time of the study⁴².

Excretion of substances through other routes such as in feces or via exhalation has not yet been assessed in MS subjects. Cerebrospinal fluid, which removes toxic agents from the CNS, has been assessed in numerous studies of MS, but not for the presence of toxic agents.

Pharmacodynamics

Toxic agents exert their effects on the human body through numerous types of biochemical or physiological mechanisms. This document focuses on three potential mechanisms by which toxic agents could contribute to the development of MS: increasing permeability of the blood-brain barrier, alteration of the immune system, and demyelination. To date, no comprehensive list of all toxic agents known to cause these physiological changes has been compiled together with an analysis of their potential relationship to multiple sclerosis. However, some of the agents that have been analyzed for a role in MS are known or suspected to be capable of exerting these types of changes. The available evidence regarding the role of toxic agents in these aspects of MS pathogenesis is discussed below.

Alteration of the immune system

Multiple sclerosis is often labeled an autoimmune disease because of the presence of inflammatory cells (T and B lymphocytes, monocytes and macrophages) as well as cytokines, antibodies, and complement components in MS lesions. Both immune system stimulators and suppressors have been hypothesized to play a role in the

pathogenesis of MS, although how they cause or exacerbate the disease remains to be determined.

- **Lead** – There is no direct evidence that lead alters the immune system in a way that can increase the risk of MS. However, researchers have found that lead may stimulate the immune response in mice by enhancing the immunogenicity of molecules of neural proteins. For example, in one study, myelin basic protein and glial fibrillary acidic protein were incubated with lead before being injected into mice⁴³. Mice receiving the lead-altered protein generated significantly more autoantibodies against the neural proteins than mice receiving only native protein. Researchers hypothesized that lead could exacerbate neurological diseases such as MS by increasing the levels of antibodies against myelin protein. Another study found that adding lead to the diet of mice with lead enhanced their T-cell response to certain mitogens such as concanavalin A⁴⁴.
- **Mercury** – Mercury may have immunomodulatory or immunotoxic effects on humans as shown primarily through experiments on animals. For instance, in certain animals, mercury has been shown to induce an autoimmune response⁴⁵. Its effects on MS subjects have not been extensively researched, although one study analyzed blood samples from multiple sclerosis subjects who had amalgams at the time of the study with MS subjects who had their amalgams removed. The study found that CD8+ T cell levels were increased in the subjects who had their amalgams removed compared with those who still had the implants, indicating that mercury may suppress these regulatory cells⁴².
- **Nicotine** – Cigarette smoke has immunosuppressive effects, and recent research suggests nicotine may be the major component responsible for this change. For example, studies have shown that T-cell and antibody responses are inhibited in rats chronically exposed to nicotine^{46, 47}; another study has shown that exposure to nicotine alters the differentiation of human dendritic cells⁴⁸. More information can be found in Sopori and Kozak's 1998 review on the immunomodulatory effects of cigarette smoke⁴⁹.
- **Tobacco glycoprotein** – This substance, present in cigarette smoke, has been shown to have stimulatory effects on the immune system, for instance by causing the proliferation of T cells as demonstrated in an in vitro study of human lymphocytes⁵⁰.

Demyelination

During the course of the disease in MS, myelin in the central nervous system is damaged or destroyed. There is no direct evidence that demyelination in MS is caused by toxic agents; however, the following toxic agents have been shown to cause demyelination in other circumstances:

- **Cyanide** - Demyelination of the nervous systems of animals given large doses of cyanide has been well-documented⁵¹. However, modest demyelination has also been demonstrated with smaller exposures. For example, in one experiment, rats given small doses of cyanide or thiocyanate in conjunction with a restricted diet showed signs of myelin degeneration in their spinal cords⁵².

- **Lead** – Accidental lead poisoning has been shown to cause demyelination as seen in the postmortem analysis of brain tissue from four nonhuman primates⁵³. However, another study to test the results of direct exposure to lead and other metals found that lead pellets implanted in the brains of rats failed to cause significant necrosis after 21 days⁵⁴.
- **Mercury** – Mercury poisoning has numerous effects on the nervous system (both central and peripheral) which include demyelination, neuronal loss, brain atrophy, and astrocytosis. Chang 1977⁵⁵ and Atchinson and Hare 1994⁵⁶ both review the range of neurotoxic effects due to mercury poisoning.
- **Organic solvents** – A few studies have documented changes in the white matter of brains of people exposed to organic solvents, indicating alteration of the myelin sheath^{22, 57, 58}. Organic solvents may not be directly responsible for these changes, but instead may allow other toxic or demyelinating factors to enter the brain by altering the blood-brain barrier (see below).
- **Radiation** – One study suggests that ionizing radiation may aggravate lesions in the brain and increase the rate at which demyelination occurs. This study describes a sharp deterioration in the clinical status in four of five patients with various demyelinating neurological diseases after receiving radiation treatment⁵⁹. Another study found evidence of myelin fragmentation and loss of oligodendrocyte precursor cells following administration of radiation in humans (therapeutic) and in rats (experimental)⁶⁰.

Increasing permeability of the blood-brain barrier

The blood-brain barrier is a dense wall of endothelial cells which prevents macromolecules from crossing from the bloodstream into the brain. Normally only small molecules such as oxygen and carbon dioxide can pass through. However, in multiple sclerosis, permeability of the blood-brain barrier is enhanced, allowing T-lymphocytes and other blood cells to pass through more readily to the brain. It is hypothesized that the breakdown of the blood-brain barrier is an early step in the development of multiple sclerosis lesions.

Several elements and compounds have been shown to disrupt the blood-brain barrier, including aluminum, nitrobenzenes, and pyridostigmine bromide (a nerve gas antidote)^{1, 61}. Of the toxic agents specifically investigated for a role in MS, the following are known or suspected to disrupt the blood-brain barrier:

- **Lead** – The blood-brain barrier is a target for lead toxicity. Studies have shown that lead interferes with many different functions of the barrier, including transport and metabolic processes. More information can be found in Zheng's review on the blood-brain barrier and metal toxicity¹.
- **Mercury** – Numerous studies have shown that mercury alters the blood-brain barrier in animals, although most of them involved the administration of relatively high doses. In 1977, Chang summarized the findings in this field in a paper on the neurotoxic effects of mercury⁵⁵. A more recent overview cited additional studies demonstrating the alteration of blood-brain barriers by inorganic mercuric chloride¹, but does not discuss the implications this research has for multiple

sclerosis.

- **Nicotine** – Researchers have demonstrated that nicotine increases microvascular blood flow within certain areas of the brain, including visual-auditory, sensorimotor-cortical, and interpenduncular systems⁶². This may result in greater influxes of toxic agents or other factors across the blood-brain barrier.
- **Organic solvents** – The small size and hydrophobic nature of organic solvent molecules allow them to penetrate the blood-brain barrier with relative ease; they may also affect and impair the function of the blood-brain barrier. At least two studies have found increased concentrations of macromolecules (e.g., albumin) in the cerebral spinal fluid of people exposed to organic solvents compared with controls, indicating that this exposure may have increased the permeability of the blood-brain barrier^{63, 64}. One study did not find significant overall differences in CSF albumin levels in solvent-exposed subjects compared with controls, but cited evidence of slight blood-CSF barrier damage in a subset of the subjects with recent occupational exposure⁶⁵.
- **Radiation** – Among the effects that radiation can have on the central nervous system is increased permeability of the blood-brain barrier, which may be mediated by cell death in endothelial cells and altered gene expression in resident CNS cells⁶⁶.

Other

A few studies have proposed more indirect methods through which toxic agents could play a role in the development of multiple sclerosis:

- **Genetic** – Both radiation and chemical agents may cause genetic mutations that could lead to multiple sclerosis in susceptible individuals. Please refer to our Phase 2 Genetics document “Analysis of spontaneous genetic mutations as a possible cause of Multiple Sclerosis” for more information.
- **Increased nitric oxide levels** – Recent evidence has suggested that nitric oxide may play a significant role in the progression of multiple sclerosis. Nitric oxide is an endogenous signaling molecule with a variety of functions and may be involved with inflammation in MS². Research has shown that cigarette smoke may increase blood levels of nitric oxide in the body⁶⁷⁻⁷⁰, but not all data concurs with this hypothesis⁷¹.
- **Increased vulnerability to disease** – One study found that the risk of current, past, or chronic *Chlamydia pneumoniae* infection (another potential MS risk factor) was positively associated with cigarette smoking⁷².

Epidemiological effects

The following factors can influence a person's exposure to toxic agents or the effects produced by these agents. As a result, they can increase or decrease susceptibility to

the diseases associated with toxic agents:

- **Age** – MS typically manifests itself in adulthood, although it can also be diagnosed in children and adolescents. However, since subclinical development of the disease may precede clinical symptoms by a substantial period of time, it is impossible to know exactly when MS disease processes are initiated in an individual. This makes it difficult to determine what specific age groups are most likely to be exposed to or affected by any environmental factors involved in MS. Some studies of migration of people between areas of lower and higher MS prevalence suggest that childhood or adolescence may be times when people are exposed to risk factors for MS, although these studies do not explore which risk factors may be involved. No information is available on exposure of people with MS to toxic agents before birth, which may also be important because the placenta is permeable to certain toxic agents and therefore the diet and habits of the mother could be risk factors for the baby.
- **Gender** – Multiple sclerosis affects roughly twice as many females than males. While some of the toxic agents studied in relation to multiple sclerosis may affect women differently than men, no such differential effects have not been demonstrated to play a role in MS. It may also be that due to societal and occupational factors, females may have greater exposure to certain toxic agents. For example, in many societies, females spend a majority of time inside the home, increasing their potential exposure to radon⁶.
- **Genetics** – Certain genetic polymorphisms may affect a person's ability to neutralize toxic agents. It is possible, although this has not been extensively researched, that some individuals with MS are more susceptible to intoxication because of genetic defects. Landtblom, et al, studied two enzyme systems (GSTM1 and CYP2D6) that metabolize organic solvents, but found no differences in the genetic predisposition of MS subjects who had been exposed to organic solvents and MS subjects who had not been exposed⁷³. Agundez, et al, likewise found no correlation between polymorphisms in the CYP2D6 gene and risk of MS when comparing 118 MS subjects to 200 controls⁷⁴, and Stavropoulou, et al., found no association with MS for GSTT1 or GSTM1 (although a greater frequency of the GSTM1 null genotype was found in female vs. male MS cases)⁷⁵.
- **Socioeconomic status** – Several studies have investigated possible associations between MS and socioeconomic status, which may affect exposure to certain toxic agents such as medication. In a review of the social epidemiology of multiple sclerosis, Lowis reported that studies provide mixed results on whether MS is linked with socioeconomic status⁷⁶. There is no direct evidence to support the hypothesis that an increased risk of MS for higher income classes, if there is one, is due to increased exposure to toxic agents.
- **Diet** – Because food and drink are vectors for toxic agents, diet could play an important role in exposure to toxic risk factors for MS. For example, mercury levels in water are amplified in fish, lead and other metals present in soil can build up in vegetation, and drinking water can contain a multitude of toxic agents. This area has not been researched extensively and the research that has been completed is unclear. Our Phase 2 Nutrition documents analyze the research

that has been published on dietary characteristics that may be associated with MS.

- **Health status** – Weakened health status may make a person more susceptible to the effects of toxic agents. Documenting the health status and medical history of people with MS prior to onset may therefore provide clues about the involvement of toxic risk factors in MS.
- **Occupation** – Several case-control or cohort studies have been conducted attempting to find correlations between particular occupations and the risk of multiple sclerosis. Certain industries such as metal working and chemical industries have been linked with an increased risk of MS, but the evidence implicating them generally comes from only a few studies. Furthermore, little information can be found on specific toxic agents for which exposure is increased in any given occupation. A listing of occupations studied in MS can be found in Appendix A.
- **Environment and geographical location**– As mentioned previously, several scientists have characterized the soil, air and water properties of areas with high prevalence of multiple sclerosis, but no toxic agents have been consistently present in MS cluster sites. Most of the studies have focused on specific toxic agents, but no overall consensus has emerged, i.e., no one toxic agent has been highlighted across studies of multiple clusters. In 2002, Pugliatti, et al, published a review that maps the prevalence of MS in different areas of the earth⁷⁷. A global study of various environmental factors such as geology and trace metal prevalence may help identify correlations between high prevalence MS clusters and corresponding environmental concentrations, but so far none has been published.

Appendix A: Table of occupations/industries studied in relation to MS

Occupation/Industry	Study	Risk of MS	Region
Agriculture	Souberbielle BE, 1990 ⁷⁸	=*	Paris, France
	Landtblom AM, 1993 ¹²	=	Sweden
Chemical	Casetta I, 1994 ⁷⁹	-	Ferrara, Italy
	Lauer K, 1985 ⁸⁰	=	Southern Hesse, Germany
	Lauer K, 1985	+	Southern Hesse, Germany
	Lauer K, 1989	+	Denmark (insignificant in Norway, Switzerland, and Sweden)
Commerce	Lauer K, 1990	+	France
	Casetta I, 1994	=	Ferrara, Italy
Commerce and administration	Lauer K, 1985	+	Southern Hesse, Germany
Construction	Lauer K, 1985	(female only)	Southern Hesse, Germany
		(male only)	
Domestic work and restaurant trade	Lauer K, 1985	-	Southern Hesse, Germany
Driving	Lauer K, 1985	=	Southern Hesse, Germany
Education	Lauer K, 1985	=	Southern Hesse, Germany
Electrical work	Souberbielle BE, 1990	=	Paris, France
	Lauer K, 1985	+	Southern Hesse, Germany
Food	Lauer K, 1985 ⁸¹	(male only)	Norway, Switzerland, Denmark, Sweden
Hairdressers	Lauer K, 1990 ⁸²	=	France
	Souberbielle BE, 1990	+	Paris, France
Health work	Lauer K, 1985	+	Southern Hesse, Germany
	Dean, 1990 ⁸³	(female only)	Great Britain
Industry	Souberbielle BE, 1990	=	Paris, France
	Casetta I, 1994	=	Ferrara, Italy
	Casetta I, 1994	=	Ferrara, Italy
	Lauer K, 1989	+	Switzerland, Norway (insignificant in Denmark, Sweden)
Metal work	Landtblom AM, 1993	=	Sweden
	Lauer K, 1985	+	Southern Hesse, Germany
	Lauer K, 1989	+	Switzerland (insignificant in Denmark, Norway, Sweden)
Mining	Lauer K, 1990	+	France
	Lauer K, 1989	=	Denmark, Norway, Sweden, Switzerland
Nurse anaesthetists	Flodin U, 2003 ⁸⁴	+	Sweden
	Stenager E, 2003 ⁸⁵	=	Denmark
	Landtblom A-M, 2006 ⁸⁶	+	Sweden
Paper manufacturing	Lauer K, 1989	+	Norway, Switzerland, Denmark (insignificant in Sweden)
Printing and publishing	Lauer K, 1990	=	France
	Lauer K, 1989	+	Switzerland, Denmark (insignificant in Norway, Sweden)
Public administration	Lauer K, 1990	=	France
	Casetta I, 1994	+	Ferrara, Italy
Quarrying	Lauer K, 1989	=	Denmark, Norway, Sweden, Switzerland
Radiology	Landtblom AM, 1993	+	Sweden
Skilled work	Casetta I, 1994	=	Ferrara, Italy
Textile and clothing	Lauer K, 1989	+	Denmark (insignificant in Switzerland, Norway, Sweden)
	Souberbielle BE, 1990	=	Paris, France
Textile and leather manufacturing	Lauer K, 1990	+	France
	Lauer K, 1985	=	Southern Hesse, Germany
Unemployed	Souberbielle BE, 1990	=	Paris, France
	Casetta I, 1994	=	Ferrara, Italy
Utility workers	Johansen CM, 1999 ⁸⁷	=	Denmark
Welding	Landtblom AM, 1993	=	Sweden
Wood processing	Lauer K, 1989	+	Norway, Switzerland (insignificant in Denmark and Sweden)
	Lauer K, 1990	=	France

* - negative correlation found, + positive correlation found, = no correlation found

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