ARRAY 11

ARRAY 11 – Antibody

CHEMICAL IMMUNE REACTIVITY SCREEN™

CYREX™ Laboratories

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CLINICAL APPLICATION GUIDE TO CHEMICAL IMMUNE REACTIVITY SCREEN™

OVERVIEW

It takes hundreds of generations for the human body to adapt, modify, and make genetic changes to environmental factors in order to survive, thrive and maintain health. New technology, new industries, new inventions, new chemicals, new foods and diets are constantly and rapidly being introduced in this fast-paced ever-changing world. We are continuously and consistently deluged with environmental toxins. Starting with increasing chemical production in the United States right after World War II, we manufacture 5 trillion pounds of 70,000 different chemicals per year, of which only 1,500 have been studied for toxicity. In the National Health and Nutrition Examination Survey (NHANES) study of 2009,[1] conducted by the Centers for Disease Control and the National Institutes of Health, blood and urine from 2,500 volunteers, none of whom had any occupational or residential risk for toxic exposures, were found to contain an astounding 212 different chemicals. With so many toxic substances in our environment, in what we eat and drink, and in the air we breathe, some of our immune systems can no longer cope with this continued and relentless assault. It is failing, and the effect on all of us, from birth to the elderly, especially in the industrialized world, is our deteriorating health and the rise of autoimmune disorders.

Environmental Toll

The human immune system is responsible for an enormous duty; to recognize and ignore all the cells and tissues within our body (self), and at the same time, attack any and all invaders, such as foreign cells, chemicals, viruses, bacteria, toxins, and fungi (non-self). Something in this delicate balance has changed over the last 50 years and it is pushing our immune systems to its limit: we are at the edge of our immune system's capacity. Interference with this balance by environmental triggers can result in over-activity to harmless antigens, leading to autoimmunity (**see Figure 1**).

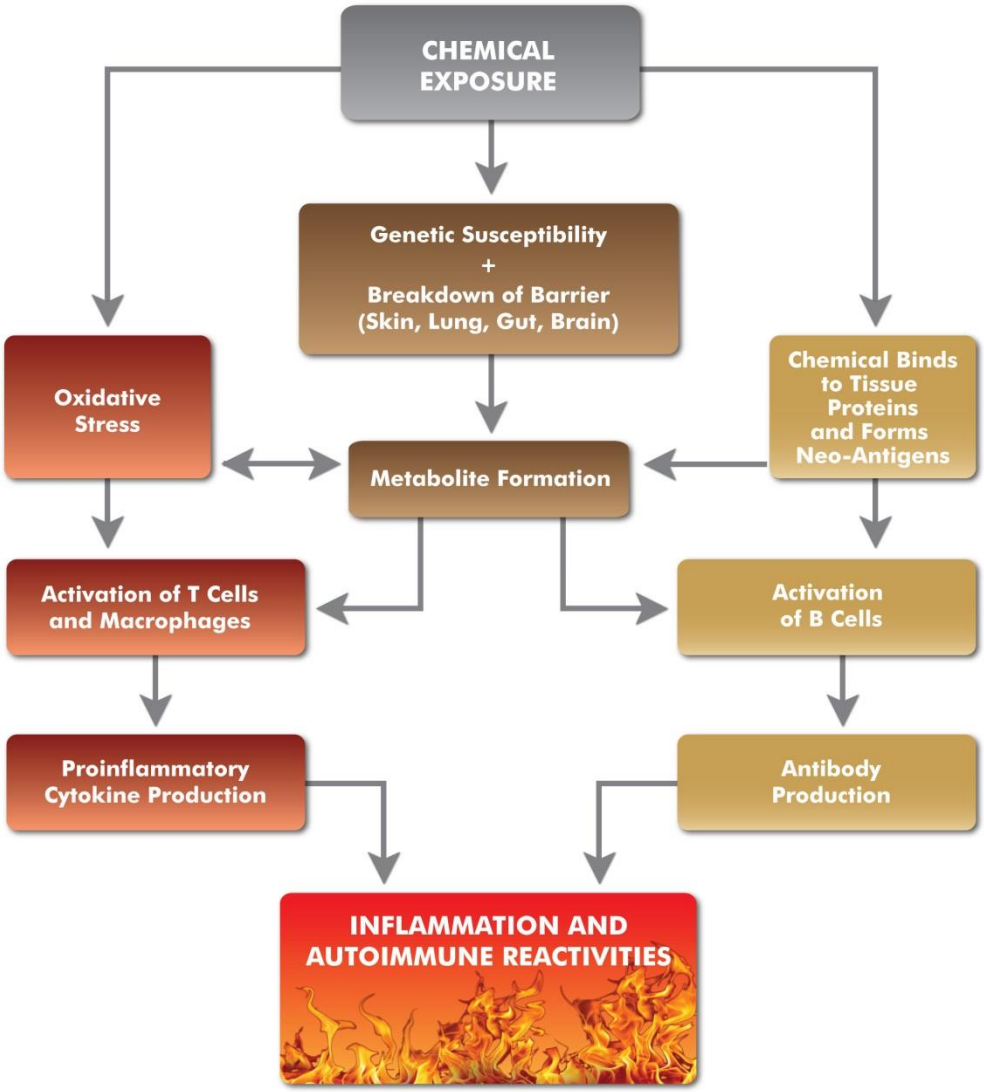


Figure 1. Potential molecular mechanisms in chemical-induced autoimmune reactivity. *Metabolite formation, which activates immune responses, can occur due to oxidative stress, breakdown of a barrier in genetically susceptible individuals, or chemicals bound to human proteins. The heightened immune response may result in damaging inflammation and/or autoimmune reactivity.*

The increase worldwide of autoimmune diseases, such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, multiple sclerosis, scleroderma, myasthenia gravis, and type 1 diabetes is alarming. It is the third most common category of disease in the United States after cancer and heart disease.[2] The National Institutes of Health, in a 2005 report to the U.S. Congress called "Progress in Autoimmune Diseases Research,"[2] showed that over 80 autoimmune diseases affect between 14.7 and 23.5 million Americans. Autoimmune diseases are now the second most common cause of chronic illness in America. Three factors are involved in autoimmunity: genetics, environmental factors (including toxic chemicals and infections)[3] and gut dysbiosis.[4]

With all the above in mind, it has now become vital that we begin the process for prevention of disease, especially autoimmunity. The good news is, this can be done with the laboratory analyses from Cyrex Laboratories followed by the appropriate clinical interventions.

The clinician can now discern if the chemicals to which a patient has been exposed is a body burden and potentially triggering autoimmune reactivity by ordering Array 11. As most of these chemicals cross the blood-brain barrier and cause neurological problems, Array 20 can show if the blood brain-barrier has been compromised and breached. If this has indeed occurred, these environmental toxins can accumulate in the brain and via immune reactivity cause the formation of neuronal autoantibodies, and so the clinician should consider ordering Array 7 or Array 7X. The autoimmune reactivity in neurotoxicity triggered by environmental toxins can give rise to multiple sclerosis, paraneoplastic cerebellar degeneration, brain aging, vascular dementia, Alzheimer's disease, Parkinson's disease, neuromyelitis optica, stiff person syndrome, autism and dyslexia, among others. If the clinician is concerned that the patient may have developed an autoimmune disorder as well as neurotoxicity from exposure to chemicals, Array 5 can help identify which body tissue is targeted.

For a thorough evaluation of the patient adversely affected by environmental toxins as assessed in Array 11, the following outline (**Figure 2**) may guide the clinician in determining the extent of the damage in his/her patient:

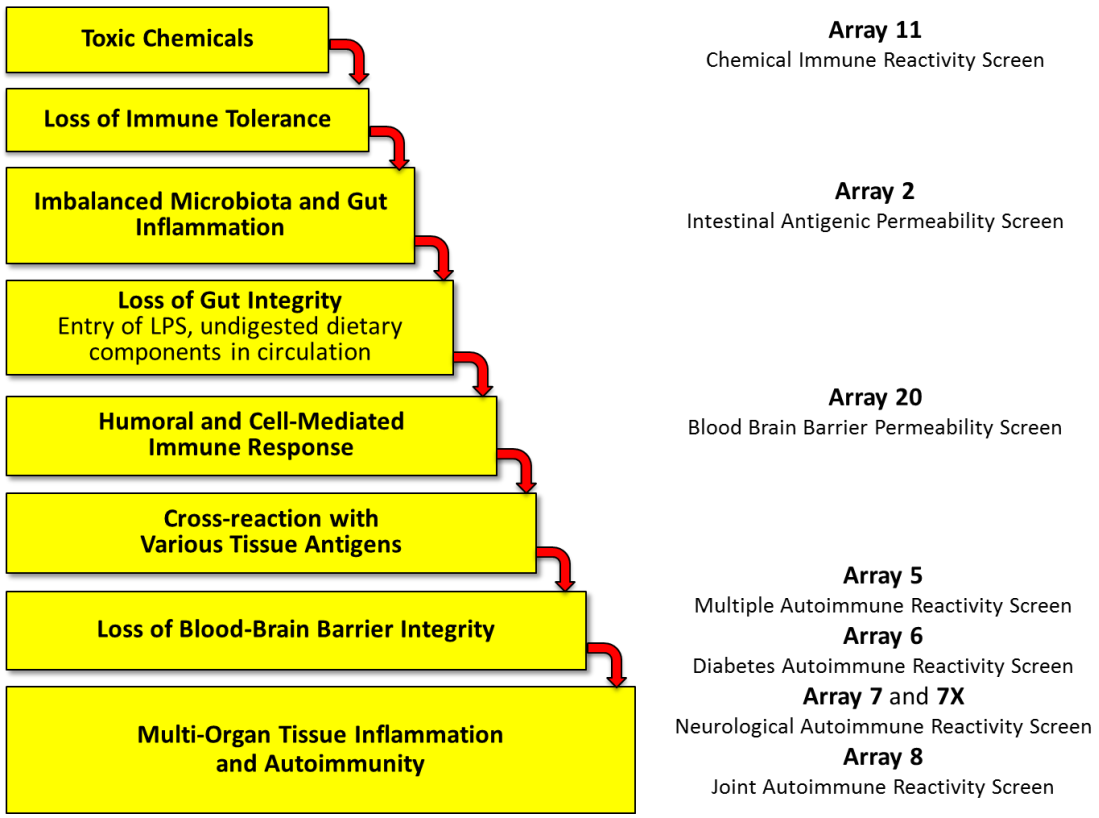


Figure 2. Toxic chemical cascade. *The cascade of events begins with toxic chemicals, causing disturbance in gut flora and a sequence that results potentially in autoimmunity. The Cyrex System™ includes laboratory tools that can assist the practitioner in assessing not only chemical immune burden, but also barrier permeability, release of bacterial endotoxins into the blood stream and autoimmune reactivity to a variety of target tissues.*

Although the precise mechanism by which environmental triggers in particular toxicants, induce autoimmune disorders, are still unknown, it is clear that the breakdown in immunological tolerance and dysfunction of immune homeostasis plus chemical intolerance are major components in the induction of autoimmune reactivities followed by autoimmune diseases.

In a Nutshell

The human immune system has not evolved as fast as humans have created mass amounts of chemicals. Chemical exposures can have adverse effects on some individuals. By using the Cyrex System™ clinicians can assess individual chemical immune burden, as well as barriers permeability and tissue autoimmune reactivity.

The Cyrex Difference

Chemicals are haptens, which are too small to elicit an immune response. Once the chemical infiltrates the body, the chemical, or its metabolites, has the capacity to bind to various tissue antigens, thus initiating immune responses not just against the chemicals bound to human tissue proteins, but against the self-tissue antigens as well. To assess chemical immune burden, Cyrex's Array 11 measures antibodies against chemicals bound to human tissue. This is different from just measuring levels of chemicals in the blood or urine. Chemicals are detected in the blood and urine of more than 90% of the population.

Table 1. A Comparison of antibodies to levels.

Properties of Measurements	Antibodies	Levels
Identify chemical binding to human tissue proteins	✓	
Identify loss of tolerance	✓	
Indicate immune reactivity due to chemical exposure	✓	
Indicate recent exposure to chemical	✓	✓
Indicate exposure to chemical	✓	
Indicate body burden of chemical	✓	
Indicate what is being cleared out of the body		✓
Assumes quantity is the only clinical variable		✓

Clinicians and researchers have used a variety of methods to detect chemicals in the human body. A controversy over the effectiveness of these methods has arisen. Is a person with high levels of mercury in urine, stool or hair at risk for the development of immune dysregulation leading to autoimmune reactivity? Or is the high level really an indication that the person efficiently handles mercury and wastes it out immediately rather than allowing the toxin to infiltrate body tissues? Immunologists are concerned with the mercury bound to human tissue that contributes to the autoimmune cascade.[5] The method for assessing this event is detecting antibodies to the chemical bound to the human tissue. Research that has already been done with assessing the body burden of mercury,[5 6 7 8] so that it is practical to use mercury studies as an example. However, the concepts are appropriate for measuring the body burden of each of the chemical antigens assessed in Array 11. **Table 1** provides a quick comparison of assessing antibodies of chemical bound to human tissue versus chemical levels.

MECHANISMS OF CHEMICAL- INDUCED AUTOIMMUNITY

Chemical Compounds, Breakdown in Immune Tolerance, and Causes of Autoimmune Disorders

A number of clinical reports and experimental studies have shown that autoimmune reactions and/or autoimmune diseases are induced in humans by environmental triggers, as summarized by Bigazzi[9] and by Pollard, *et al*.[10]

The mechanism of chemically-induced autoimmunity is by two different pathways. One is by toxicant initiation of loss of tolerance.[11] This loss of tolerance follows a susceptible person's acute or chronic exposure to various environmental agents. Next is a subsequent triggering of symptoms by extremely small quantities of previously tolerated chemicals, drugs, foods, and food and drug combinations. The aberrant cell death involved in this process makes the hidden cellular material available to antigen presenting cells (APC), which results in antibody production against self-tissue antigens.[12 13] This autoimmune reactivity can lead to full-blown autoimmune disease (**see Figure 3**).

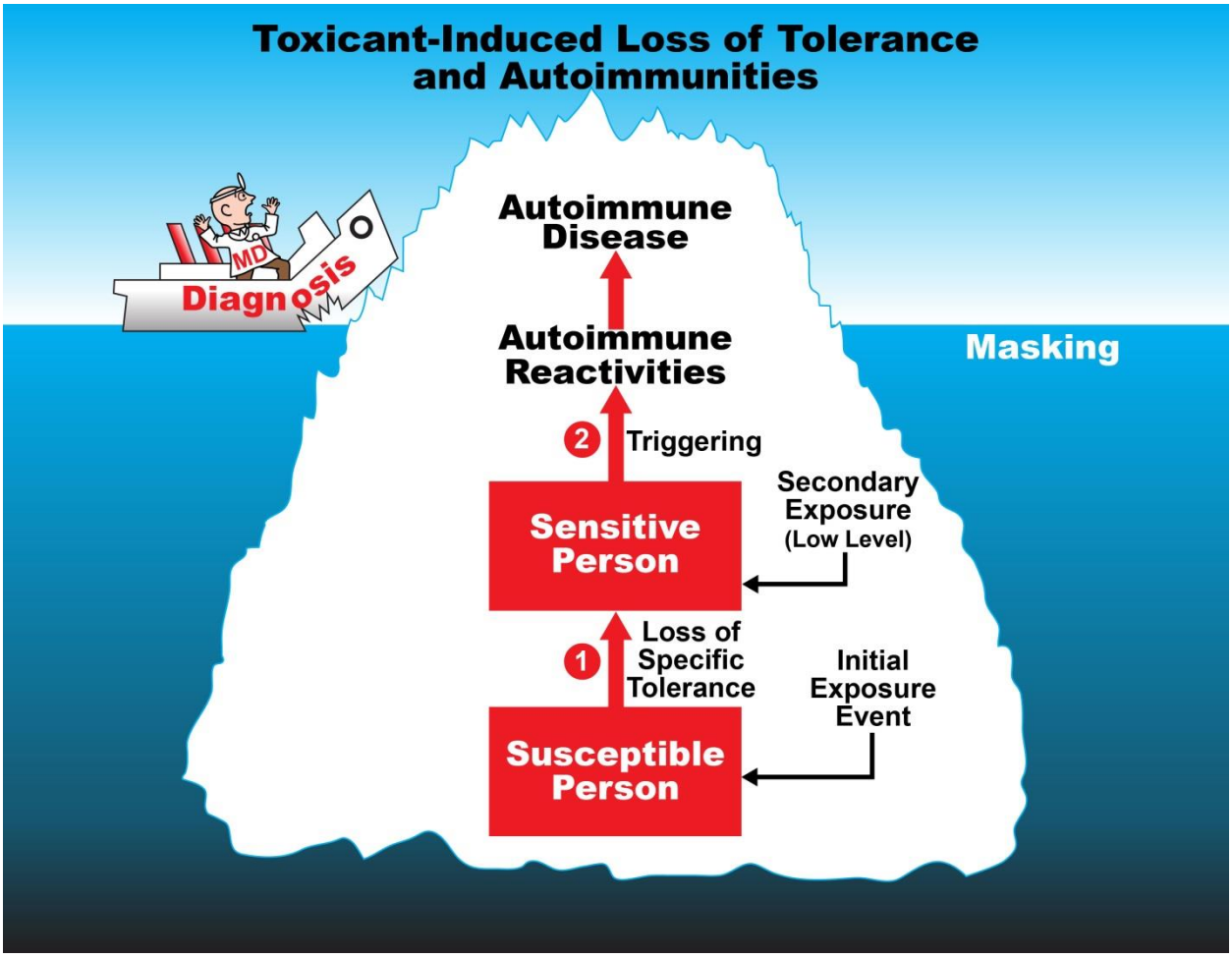


Figure 3. Toxicant-induced loss of tolerance. *Upon loss of immune tolerance, additional chemical exposure/s may trigger an inflammatory cascade that results in autoimmune reactivity.*

The other is by immune reactions to chemical compounds through covalent binding of chemicals or haptens, which are substances capable of reacting with specific antibodies but incapable of inducing the formation of antibodies unless bound to human tissue proteins (carrier proteins). This leads to the subsequent formation of neo-antigens, which also results in the breakdown in immune tolerance and the production of antibody against self[14] (**see Figure 4**). This is due to the fact that reactive organic compounds most often bind covalently; that is, their electrophilic properties enable them to react with protein nucleophilic groups such as thiol, amino and hydroxyl groups. Examples of such reactive, haptenic compounds that frequently lead to intolerance after dermal absorption or inhalation are toluene diisocyanate, trimellitic anhydride, phthalic anhydride, benzoquinone, formaldehyde, ethylene oxide and dinitrochlorobenzene picryl chloride. Sensitizing metal ions react differently with the immune system and induce intolerance by formation of stable protein metal chelate complexes by undergoing multi-point binding with several amino acid side-chains and forming various neoantigens[13] (**see Figure 4**).

During humoral immune responses of the adaptive immune system and contact with the neo-antigen, the neo-antigen is picked up by an antigen-presenting cell (APC). The APC transports the antigen to a T-cell, which introduces the antigen to a B-cell in the lymph node. Clonal expansion of the B-cell begins and triggers antibody production. Immunoglobulin M (IgM) is produced as a primary response in the first days of defense. A secondary response results in IgG production against the neo-antigens (**see Figure 4**). High levels of antibodies indicate a breakdown in immunological tolerance or disruption in immune homeostasis.

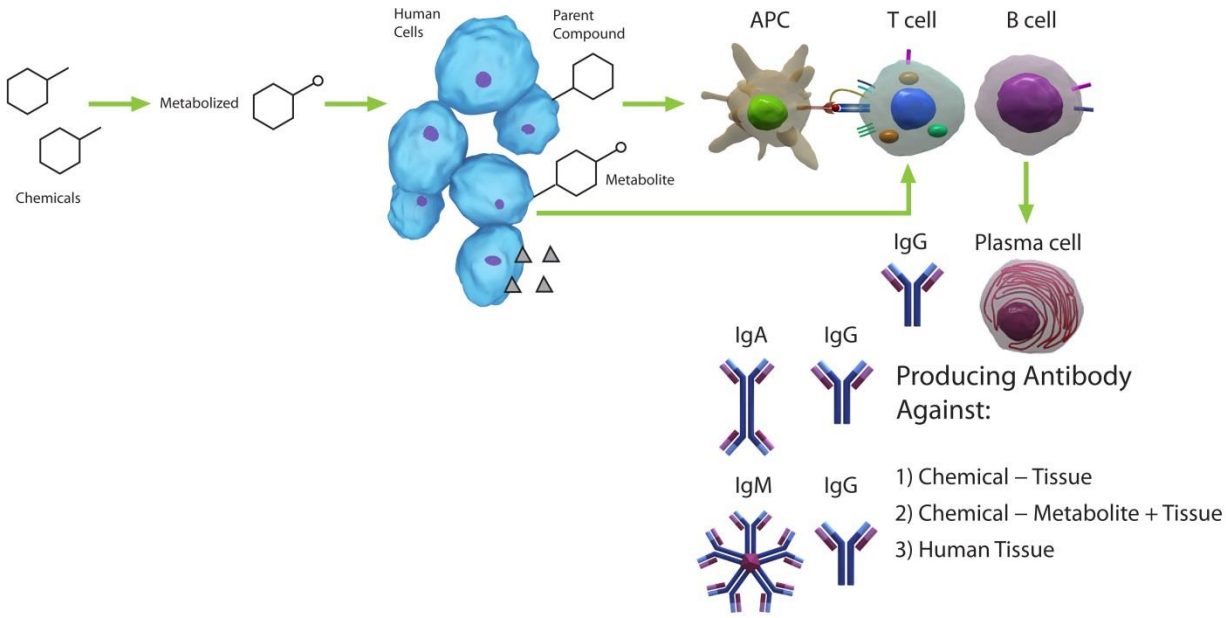


Figure 4. Possible antibodies produced from chemical exposure. *Chemicals and/or their metabolites bind to human tissue proteins forming a neo-antigen. This neo-antigen is picked up by the antigen-presenting cell (APC), which facilitates the production of immunoglobulins. Immunoglobulins may be created to target the chemical bound to human tissue, the chemical/metabolite bound to human tissue and/or human tissue.*

Sensitizing heavy metal chemicals such as mercury, nickel or cobalt react differently from organic compounds, oxidizing proteins and forming protein metal chelate complexes by undergoing multiple binding with several amino acid side chains of a protein.

Different groups of chemicals that elicit adverse immune reactions are unable to bind the proteins when entering the body. However, they can bind to various tissue proteins after conversion to reactive metabolites by the hepatic and extrahepatic tissues or cells. These types of chemicals are considered prohaptens.

The adaptive immune system consists of lymphocytes that recognize a wide selection of foreign antigens, from dietary peptides, which are short chains of amino acids, to chemical compounds, without reacting to self-antigens. However, in cases of intolerance, some auto-reactive lymphocytes escape clonal deletion, lie dormant, and later become activated. Once activated, these auto-reactive lymphocytes may initiate

autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and type I diabetes mellitus.

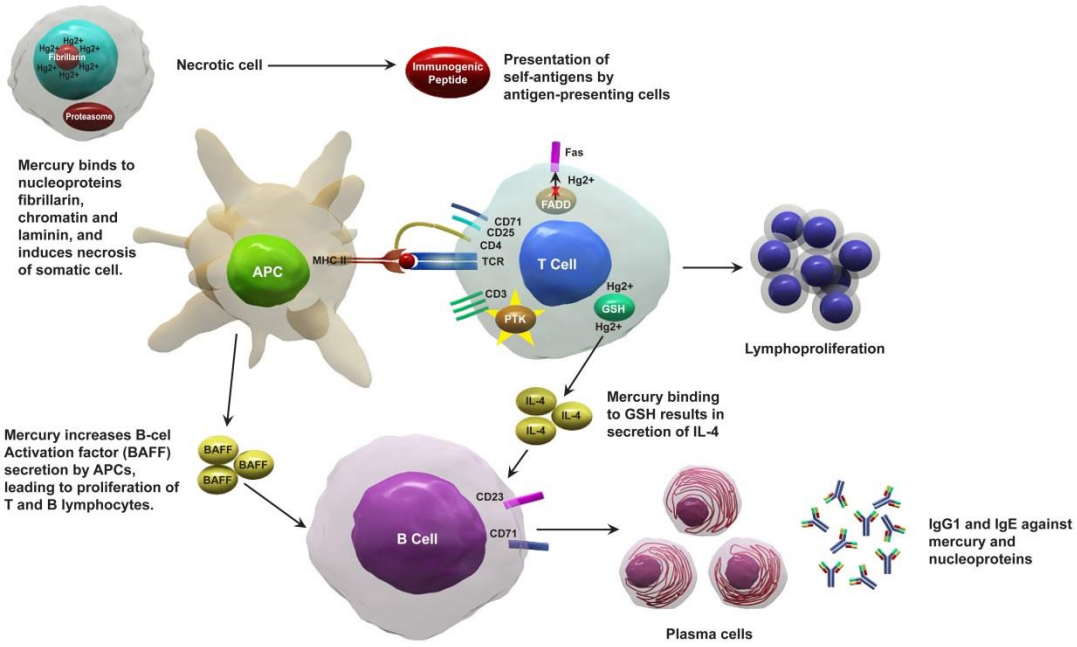


Figure 5. Putative mechanisms for mercury-induced breakdown in immunological tolerance. *Mercury induces necrosis of somatic cells resulting in proteasomal processing of fibrillarin and its subsequent release as immunogenic peptides. These peptides are then processed by antigen presenting cells (APCs) and presented to T cells. Mercury exposure may also result in the clustering of T cell receptors with subsequent protein tyrosine kinase (PTK) activation. The high affinity IL-2 receptor (CD25) and the transferrin receptor (CD71) are also upregulated by mercury. Mercury binds to T cell intracellular proteins, affecting their function. B cells are also polyclonally activated in the presence of mercury with upregulation of CD71 and CD23, differentiation into antibody-secreting plasma cells and consequently elevated serum immunoglobulin levels.*
Modified from Schiraldi and Monestier[15]

As shown in **Figure 5**, in the case of heavy metals, several neo-antigens such as mercury bound to chromatin are formed via oxidative fragmentation. This neo-antigen formation may result in antibody formation against heavy metals, as well as against nuclear and nucleolar materials. Therefore the detection of antibodies against heavy metals, other toxicants, and self-tissue antigens, indicates a breakdown in immunological tolerance and the induction of immune intolerance to environmental triggers. Thus, specific antibody testing against environmental and self-tissue antigens provides a reliable method to assess the body burden of chemicals, which can be used in conjunction with additional laboratory analyses to identify potential autoimmune and/or neuroimmune disorders.

The Metabolism of Chemicals and Induction of Intolerance

It is known that people differ in their responses to environmental triggers because of variations in the body's capacity to clear or detoxify chemicals. Upon overcoming and penetrating the body's barriers, a chemical compound is subjected to metabolism by a large group of enzymes, collectively referred to as xenobiotic-metabolizing enzymes.[16] This selection of enzymes includes cytochrome P450, cytochrome b5, NADPH-cytochrome P450 reductase, and other components.

Variations in genes that encode or carry the instructions for manufacturing cytochrome P450 have been linked to autoimmune disorders. Small differences in gene coding for cytochrome P450 enzymes appear to influence the extent to which additional neo-antigens such as aflatoxins, benzene and carbon tetrachloride adducts are formed. Cytochrome P450 molecules generally neutralize a variety of foreign and internally-produced chemicals. However, during this process, in some patients they can generate intermediate products that are capable of damaging DNA and other cellular components.[16] It is these new intermediates that are involved in the formation of different neo-antigens, which are responsible for the initiation of autoimmune reactions.

A process labeled 'induction' refers to the hepatic synthesis of P450 cytochromes increasing due to the appearance of certain chemicals and other xenobiotic agents. Conversely, chemicals that form a relatively stable complex with a particular cytochrome P450 can inhibit the metabolism of other chemicals that are normally substrates for that cytochrome P450.[17] Allelic variation affecting the catalytic activity of cytochrome P450 will also affect the pharmacological activity of medications[17] and chemical compounds. This mechanism of action produces more neo-antigens and therefore more autoimmune reactivity. One of the best described examples[17] of such action is that of the P450 cytochrome CYP2D6, which was recognized initially in the 5-10% of normal individuals who were noted to be slow to metabolize chemical compounds.[16] This slow metabolizing capacity or prolongation of chemicals in the body could enhance the chance of haptens binding to human tissue proteins and hence give rise to more autoimmunities.

Molecular mimicry. The effect of acute or chronic oxidative stress may induce the release of self-antigens, which, after binding with chemicals, may act as neo-antigens and change the structures of cellular macromolecules and small molecules.[18] Neo-antigens with sufficient homology or likeness to self-proteins can result in a phenomenon known as "molecular mimicry." Because of the great similarity between the sequences of the neo-antigens and the body's own tissues, the host's immune system produces antibodies against the neo-antigens and the host's self-tissues, resulting in an autoimmune reaction.

Bystander effect. It is possible that chronic oxidative stress can result in the generation of many adducted and/or non-adducted molecules acting as neo-antigens. Aldehydic products form adducts with proteins and make them highly immunogenic.[19] During a state of chronic oxidative stress, neo-antigens may cause tissue damage. As tissues are destroyed, self-antigens are released, a process labeled the "bystander effect," leads to the loss of self-tolerance (**see Figure 6**).[18]

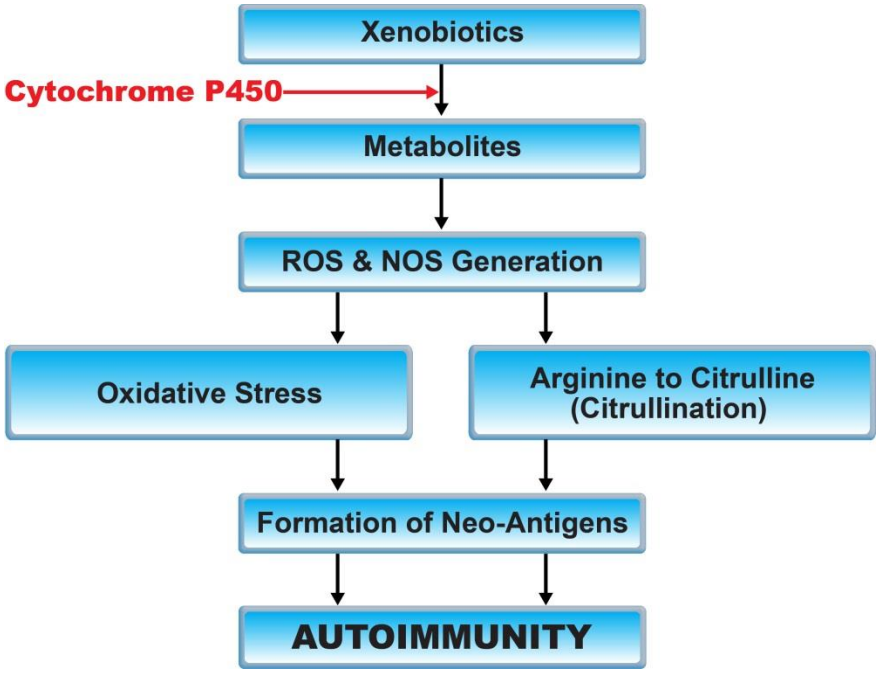


Figure 6. Oxidative stress, citrullination and the induction of autoimmunity. *Xenobiotics are metabolized by Cytochrome-P450. The process can generate reactive oxygen species (ROS) and nitric oxide synthases (NOS). ROS and NOS are involved respectively in the induction of oxidative stress and the citrullination of proteins. Citrullination contributes to the formation of neo-antigens. Immune reaction to these neo-antigens can result first in autoimmune reactivity, and then, autoimmune disease.*

***Citrullination*.** As a chemical compound enters the body, it can be metabolized by cytochrome P450 with the resulting formation of metabolites. The metabolites can generate reaction oxygen species (ROS) and nitric oxide synthases (NOS). ROS such as superoxide anion and hydrogen peroxide may be converted to hydroxyl radicals, which leads to oxidative stress.[18] Oxidative stress has been shown to play a role in the pathogenesis of autoimmunity.[20] NOS plays a role in the conversion of the amino acid arginine in a protein into the amino acid citrulline.[21] This action is called 'citrullination,' which is known to change the structure and function of proteins. The immune system often attacks citrullinated proteins, which can lead to autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.[21]

Glutathione-S-transferases (GSTs), specifically GSTM1, are of a class of detoxifying enzymes that are more beneficial than cytochrome P450.[22] GSTM1 efficiently detoxifies ethylene oxide and styrene. Ethylene oxide is a highly hazardous substance: at room temperature it is a flammable, carcinogenic, mutagenic, irritating, and anesthetic gas with an agreeable aroma; it is used in the production of detergents, thickeners, solvents, plastics, and various organic chemicals, and is a disinfectant used in hospitals and the medical equipment industry to replace steam in the sterilization of medical equipment, such as disposable plastic syringes. Styrene is used in rubber, plastic, insulation, fiberglass, pipes, automobile and boat parts, food containers, and carpet backing. It is considered toxic, mutagenic, and

possibly carcinogenic, and the U.S. Environmental Protection Agency (EPA) has described styrene to be "a suspected toxin to the gastrointestinal tract, kidney, and respiratory system, among others."[22]

Many Caucasians lack the gene for GSTM1.[24] An association between the GSTM1 gene deficiency and an increased tendency to various disorders including autoimmunities has been shown.[22 22 24] Another beneficial enzyme NAT2 (N-acetyltransferase), deactivates chemicals present in certain cooked foods, air pollution, and tobacco smoke.[26] Findings suggest that a slow-acting form of NAT2 contributes to cancer and autoimmunities.[24 26 27 28]

In contrast to haptenic compounds, most chemical compounds eliciting adverse immune reactions are unable to bind to proteins when entering the body; however, they can do so after conversion to reactive metabolites by metabolizing enzymes. These chemical compounds can be considered as prohaptens, which, after being metabolized, manage to bind to human tissue proteins and induce antibody production against both the haptenic chemicals as well as tissue proteins, which is the core of autoimmunities induced by environmental triggers.

An Example of Drug Hepatotoxicity

Individual genetic propensities, immune function, and liver function determine a person's reaction to any given drug. Concentration of a drug is not universal. A drug may be toxic in some individuals at concentrations normally tolerated by most consumers of the drug. This phenomenon is known as idiosyncratic drug toxicity.[16]

Acetaminophen (Tylenol®) is commonly used as a painkiller. Used in therapeutic doses, acetaminophen is conjugated with glucuronic acid or sulfate, after which it is excreted by the kidneys. In overdose conditions, the capacity of these normal pathways is overwhelmed and acetaminophen is then oxidized by liver cytochrome P450 to N-acetyl benzoquinoneimine (NABQI), which can result in a free-radical-mediated peroxidation of membrane lipids leading to hepatocellular damage and liver autoimmunity (**see Figure 7**).[16] Also, the binding of benzoquinoneimine to other tissue proteins can result in the breakdown of immunological tolerance against those tissue proteins, and cause the initiation of autoimmunities against different tissue proteins.

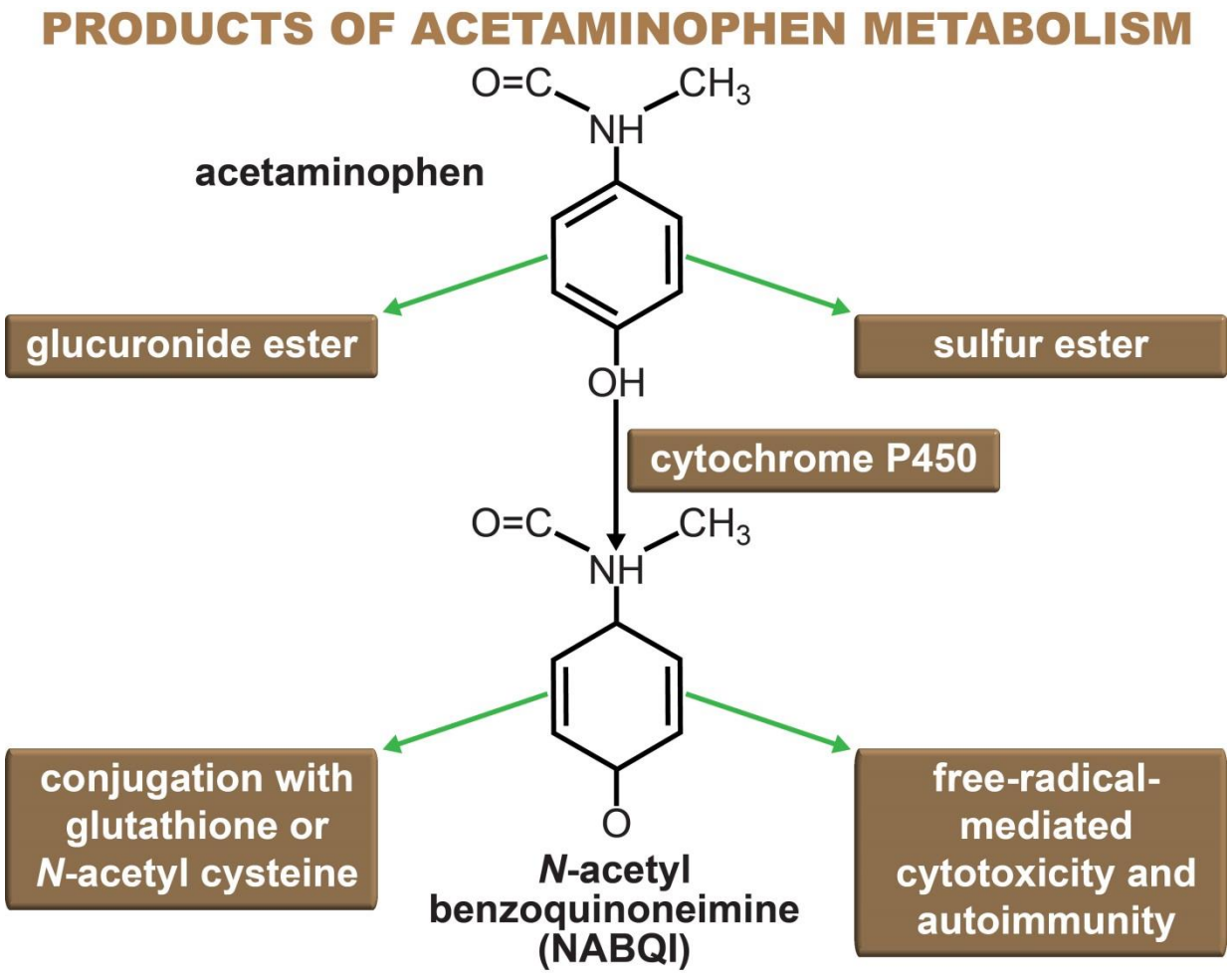


Figure 7. The metabolism of acetaminophen. *Metabolism of acetaminophen by cytochrome P450 and the formation of free radicals is a classic example of how different medicines or their metabolites contribute to tissue cell cytotoxicity and autoimmunity.*

Mechanisms involved in the initiation of a disease process might differ from mechanisms responsible for exacerbation of the established illness. Therefore, one or more of these mechanisms, either individually or jointly, can have strong effects on the development of autoimmune reactions, which may then be followed by autoimmune diseases (**Figure 8**).

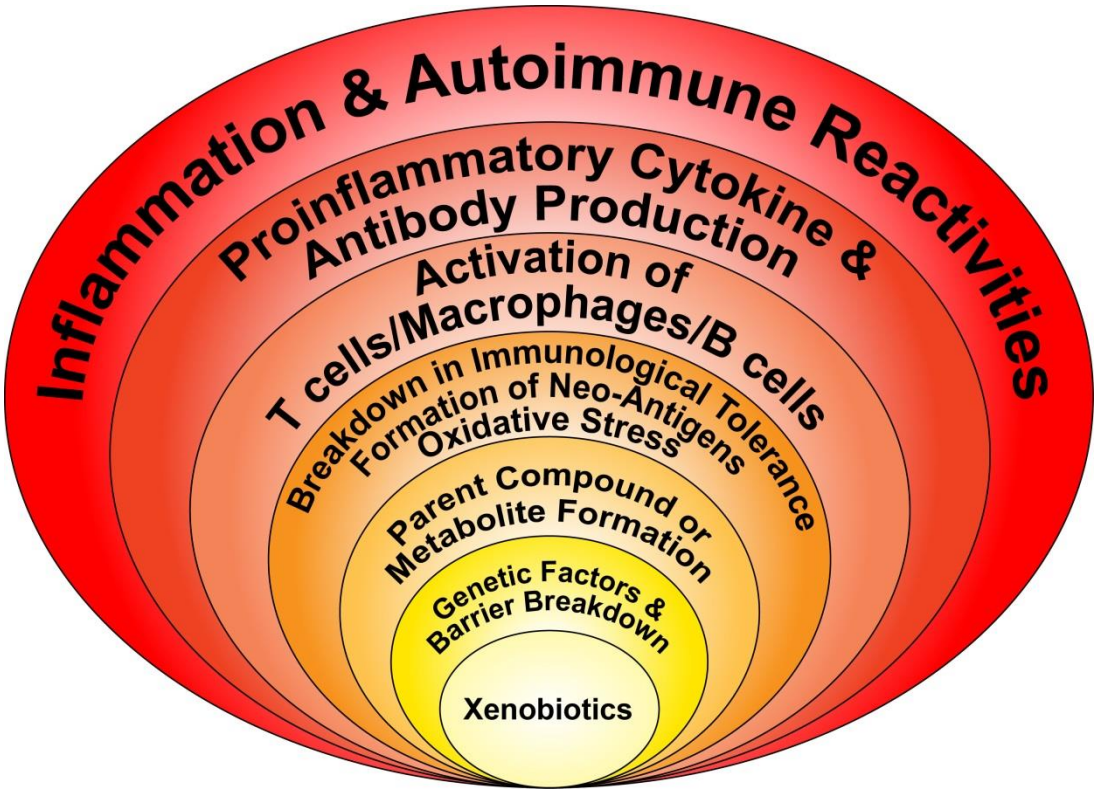


Figure 8. Induction of inflammation and autoimmune reactivities. *A tiny chemical molecule can balloon into a significant clinical condition.*

Causes of Autoimmunity by Chemical Exposure

Chemical infiltration of the body can occur by dermal absorption, inhalation and ingestion. Once through any of these barriers, the chemical may elicit a systemic reaction leading to the production of antibodies. Circulating antibodies can ignite the autoimmune processes described above.

In the case of mercury exposure, the chemical will bind to fibrillarin resulting in aberrant migration of the autoantigen.[14 29 30] Under stressful stimuli, auto-antigens yield intracellular relocalization. For example, the auto-antigen fibrillarin is a substrate of the protease granzyme B, and undergoes modification by mercury via oxidative fragmentation.[29] Chromatin and fibrillarin thus modified during apoptosis, may circulate either in native form or packaged in apoptotic bodies in the serum of patients with systemic autoimmune disorders.[29 31 32] Apoptosis is programmed cell death. It plays a significant role in the deletion of auto-reactive lymphocytes, the removal of virally infected cells and the elimination of cancerous cells.[33] In most tissues, apoptosis, including clearance of cell corpses by neighboring parenchymal cells, transpires in less than one hour. However, in the absence of the first component of complement, the clearance of apoptotic corpses is delayed. Delayed clearance increases immunogenicity. Cell death by exposure of cells to mercury, researchers have shown, may be important in the genesis of autoimmune disorders, most notably, scleroderma.[33]

Clinical Aspects of Array 11

Chemical bound to tissue antibody test results from Cyrex Laboratories can be useful tools in assessing a person's immune response to these environmental triggers, whether the individual's exposure was acute, or in low doses over a long period of time.

- Detection of antibodies against metals or other chemicals in the blood indicates immunological intolerance due to exposure to metals and other chemicals.[29 30 33 34]
- Detection of antibodies against antinuclear (ANA)/antinucleolar antibodies (ANoA) and/or heavy metal binding proteins fibrillarin and chromatin, along with antibodies to metals, indicates autoimmune reaction induced by heavy metals. [31 32 34]
- Detection of antibodies against metals or other chemicals, as well as different brain antigens (neurofilament, tubulin) and receptors, will measure the neurotoxic effect of metals and other chemicals.[14 29 30 33 35]

Thus, specific antibody testing provides reliable evaluations that can be used in conjunction with additional laboratory analysis to identify potential triggers for autoimmune reactivity.

In a Nutshell

Mechanisms by which chemicals may trigger autoimmune reactivity include:

- Molecular mimicry
- Bystander effect
- Citrullination

ARRAY 11 ANTIGEN ROLL CALL

Array 11 – Chemical Immune Reactivity Screen™ includes a variety of everyday chemicals. Humans are exposed to these xenobiotics through normal daily activities. Below is a more detailed look into each of the antigens.

Aflatoxins

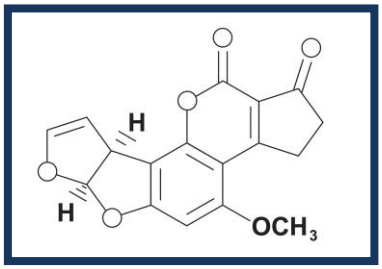
Aflatoxins [C_{17} H_{12} O_6] are organic chemical compounds produced by a polyketide pathway by many strains of the mold, *Aspergillus*. *Aspergillus flavus* is a particular strain common contaminant in

agricultural products such as peanuts, rice, figs and corn. Aflatoxin B1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains.

The Curse of the Mummy has been a popular theme for literature, films and folklore. Egyptian pharaoh Tutankhamen, the Boy King, did not reach from the great beyond to strike down the men who dared to open his tomb. Instead the mysterious and painful deaths of the archeologists, including Americans Arthur Mace and George Jay Gould, who died within 24 hours of entering the tomb, and British adventurer and financier Lord Carnarvon, who passed weeks later, were more likely caused by aflatoxosis.[36] Tutankhamen and other royal counterparts were entombed with baskets of rice. *Aspergillus flavus* is known to grow on rice and the life of this mold and its mycotoxin, Aflatoxin, can thrive in a tomb environment.[37] Lord Carnarvon died after suffering from a high fever, severe pain, pneumonia in both lungs, and eventually heart and respiratory failure; exposure to mycotoxins can cause a form of pneumonia to which immune-compromised individuals, like Lord Carnarvon, are susceptible.[36]

Aflatoxin exposure can occur via airborne spores entering through the lung barrier, ingested food products entering through the intestinal barrier, or contacted spores entering through the skin barrier. The symptoms of aflatoxicosis depend mainly in the amount and duration of the exposure, as well as the age and health of the patient. The severity of aflatoxin harmfulness can be compounded by factors such as vitamin deficiency, caloric deprivation, alcohol abuse, and infectious diseases.[38] Mycotoxins are potent protein synthesis inhibitors, and aflatoxicosis can suppress immune function, including lowering secretory IgA (SIgA) levels.[39] Due to the vital role SIgA plays in host defense, insufficient SIgA levels may cause a heightened vulnerability to microbial diseases, worsen the effects of malnutrition, and interact synergistically with other toxins.[38] [39] Animal studies show that aflatoxin interferes with vitamins A and D, iron, selenium, and zinc nutrition.[40] A review of animal studies[41] maps the effects aflatoxin exposure causes on:

- **Respiratory System**
- **Gastrointestinal System**
- **Immune System**
- **Nervous System**
- **Renal System**
- **Reproductive System**

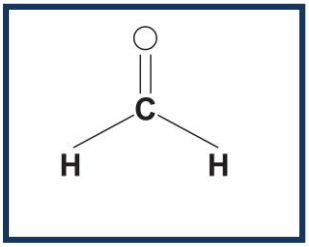


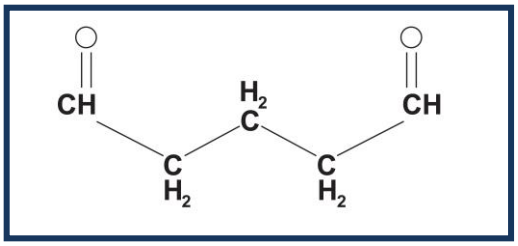
The detection of antibodies to Aflatoxins bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Aflatoxin or its metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to Aflatoxin bound to human protein in serum should avoid exposure to the substance, with special attention taken to clean up the home and work environments.

Quick List Sources of Aflatoxins[146]	
Grains, commonly corn, rice and other cereal grains	Nuts, notably peanuts, almonds, hazelnuts and pistachios
Milk and milk products from animals fed Aflatoxin contaminated feed; eggs	Dried fruits especially figs
Spices	Vegetable oils
Cocoa beans	Moldy living or work environment

Formaldehyde + Glutaraldehyde

Formaldehyde [CH_2O] is an organic compound. Formaldehyde-based materials are common to the manufacture of automobiles. Formaldehyde-containing resins – melamine formaldehyde, urea-formaldehyde, phenolformaldehyde, carbamide formaldehyde – are used as a binder in plywood and particleboard production, home furnishings, household cleaners, paints, textiles, landscape and yard products, medicinal and personal care products, pesticides, fire retardation, increased water repellency, stiffness, carpeting made with synthetic fibers, and wrinkle-resistance in fabric finishing; paper products treated with formaldehyde include paper bags, waxed paper, paper towels, and disposable sanitary products; in the health care industry, formaldehyde is used in disinfectants, preservatives, and embalming fluid. Thus, formaldehyde exposure occurs in multiple home and work environments. Formaldehyde has been classified as a known human carcinogen by the International Agency for Research on Cancer.





Glutaraldehyde [$CH_2(CH_2CHO)_2$] is an organic compound. Glutaraldehyde is commonly used to disinfect medical and dental equipment; it is also used for industrial water treatment and as a preservative. The variety of uses for glutaraldehyde includes:

- **cold sterilizer in the health care industry**
- **tanning agent**
- **biocide in metalworking fluids and in oil and gas pipelines**
- **antimicrobial in water-treatment systems**
- **slimicide in paper manufacturing**
- **preservative in cosmetics**
- **disinfectant in animal housing**

- **tissue fixative in histology and pathology labs**
- **hardening agent in the development of X-rays**
- **embalming solutions**
- **preparation of grafts and prostheses**
- **various clinical applications**

In the healthcare industry, glutaraldehyde is most often used to disinfect equipment that cannot be heat sterilized such as dialysis instruments, surgical instruments, suction bottles, bronchoscopes, endoscopes, and ear, nose, and throat instruments.

The possible routes of exposure to formaldehyde are ingestion, inhalation, dermal absorption and, rarely, blood exchange as in dialysis.[42] Because formaldehyde is so soluble, it is quickly absorbed in the respiratory and the gastrointestinal tracts. [43] Inhalation exposure to formaldehyde has been identified as a potential cause of asthma.[43] Inhalation of formaldehyde leads to the formation of DNA–protein cross-links, DNA single-strand breaks, chromosomal aberrations, sister chromatid exchange and gene mutation in human cells in vitro.[43] This mechanism may be the cause of the carcinogenic, mutagenic and sensitizing action of formaldehyde.

The most common adverse health effects resulting from formaldehyde exposure include:[44]

- **respiratory tract irritation**
- **increased incidence of infectious diseases**
- **sensitization**
- **mutagenic**
- **carcinogenic action**

In March of 1986, a railroad tanker car containing 190,000 pounds of ureaformaldehyde resin underwent uncontrolled venting in Crown Point, Alaska. Following the event, residents of Crown Point showed significantly high IgM and IgG titers to formaldehyde.[45] Symptoms included nasal congestion, sore throat, headache, cough, conjunctivitis, fatigue, rash, dizziness, diarrhea, shortness of breath, nausea, and nose bleeds; additionally, 50% of residents had recurrent, unresolved health complaints approximately 60 days following the spill.[45]

Studies on healthcare workers (laboratory, dental, hospital, veterinary), show that personnel in these industries are among the most affected by formaldehyde exposure.[43] Formaldehyde is a well-known mucous membrane irritant and a primary skin sensitizing agent associated with both contact dermatitis (Type IV allergy), and immediate, anaphylactic reactions (Type I allergy).[43]

Significantly increased sensitization rates common to the healthcare sector were found for the vaccine preservative thimerosal, as well as for glutaraldehyde, formaldehyde and glyoxal.[43] In a 5-year study [46] conducted by the University of Kansas, 468 patients were patch tested to glutaraldehyde. A higher than expected co-reactivity between glutaraldehyde and formaldehyde was noted among healthcare workers, which cannot fully be explained by concomitant exposure.[46] A significantly increased risk for occupational allergic dermatitis was induced most frequently by glutaraldehyde.[47] It is therefore of great

practical importance to evaluate the appearance of concomitant sensitization to glutaraldehyde and formaldehyde, respectively.[48]

In dental practice it is common to have concomitant exposures to formaldehyde and glutaraldehyde.[43] Chronic inhalation of glutaraldehyde caused considerable non-neoplastic lesions in the noses of study rats and mice.[49] Glutaraldehyde disinfectant replacement in the hospital setting can cause a rapid increase in the environmental pollution levels for short periods of time.[50]

The detection of antibodies to Formaldehyde + Glutaraldehyde bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Formaldehyde and Glutaraldehyde can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to Formaldehyde + Glutaraldehyde bound to human protein in serum should avoid exposure to these substances, with special attention taken to clean up the home and work environments.

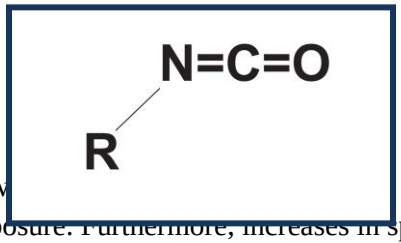
Quick List Sources of Formaldehyde[147]	
Particle board and plywood	Automobile interiors
Disinfectant	Paper product coatings
Permanent press fabrics, synthetic fiber carpeting	Glue
Germicide	Fungicide

Quick List Sources of Glutaraldehyde[148]	
Disinfectants	Cosmetics
Anti-microbial treated water	Tanning agents

Isocyanate

Isocyanates [R–N=C=O] are a family of highly reactive, low molecular weight chemicals. They are widely used in the manufacture of foams, fibers, paints, varnishes and elastomers, and are increasingly used in insulation materials, car seats, surface coatings, furniture, foam mattresses, under-carpet padding, packaging materials, shoes, laminated fabrics, and adhesives, auto body repair, and the automobile industry. Many spray-on polyurethane products contain isocyanates and are used privately and commercially to protect cement, wood, fiberglass, steel and aluminum, and for the protective coatings for truck beds, trailers, boats, foundations, and decks.

Occupational exposure to toluene diisocyanate (TDI) and other isocyanates can cause irritation of the mucous membranes, eyes, upper respiratory tract, and skin. In a study of workers exposed to diisocyanates, IgG antibodies were somewhat more prevalent than IgE; IgG antibodies were detected in 24% of symptomatic workers and 17% of asymptomatic workers.[51]



In a study on apprentice car-painters, a small proportion show diisocyanates-specific IgG and IgE after a few months of exposure. Furthermore, increases in specific IgG and IgG4 appear to have a protective effect on the incidence of work-related lower and upper respiratory symptoms, respectively.[52] These antibodies can remain for years after removal of the chemical from the patient's environment.[53] One study on occupational exposure of TDI showed that serum levels of specific IgG antibodies were significantly higher in subjects showing a positive response in a TDI-bronchoprovocation test (46%) compared with subjects exhibiting a negative TDI-bronchoprovocation test response (7.7%).[54] In a subsequent study, patients with TDI-occupational asthma with high serum IgG to tissue transglutaminase (tTG) had significantly lower methacholine PC20 values; thus TDI exposure may increase tTG activity via reactive oxygen species (ROS) production, leading to persistent airway inflammation in these patients.[55] The detection of serum specific IgG is helpful for diagnosing isocyanate-induced asthma in that the test can be used to document exposure and monitor subclinical conditions.[56]

The detection of antibodies to Isocyanate bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Isocyanate or its metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to Isocyanate bound to human protein in serum should avoid exposure to the substance, with special attention taken to clean up the home and work environments.

Quick List Sources of Isocyanate[149]	
Polyurethane foams, carpet padding	Pesticides
Paints	Automobiles
Protective coatings on cement, wood, fiberglass, steel, aluminum, truck beds, decks, boats, trailers.	Adhesives
Insulation	Plastics

Trimellitic + Phthalic Anhydrides

Trimellitic Anhydride (TMA) [$C_9H_4O_5$] is used mainly in the synthesis of trimellitate esters. These esters are used as plasticizers for polyvinyl chloride found in car interior linings, wire and cable coatings, the wire enamels used to coat magnetic wire, and for other applications where high thermal resistance is required. Trimellitic anhydride is also often used as a binder for glass fibers, sand, and other aggregates.

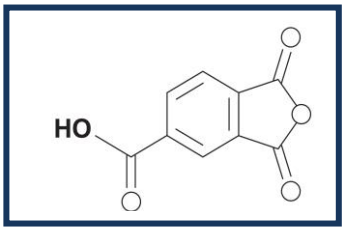
Phthalic Anhydride (PA) [$C_6H_4(CO)_2O$] is a chemical used to provide flexibility to plastics. Phthalates are easily released into the environment because there is no covalent bond between the phthalates and plastics in which they are mixed. Therefore, PAs are released into the air, leach into foodstuff, and are found in house dust. Phthalates are used in:

- **Adhesives**
- **Automotive plastics**
- **Building materials**
- **Cleaning materials**
- **Cosmetics**
- **Detergents**
- **Food packaging**
- **Flooring materials**
- **Fragrances**
- **Garden hoses**
- **Home furnishings**
- **Insecticides**
- **Nail polishes**
- **Nutritional supplements**
- **Pharmaceuticals**
- **Plastic bags**
- **Raincoats**
- **Shampoos**
- **Toys**

TMA and PA are two of the most widely used acid anhydrides in industry. Inhalation and dermal contact are the two most common routes of exposure to acid anhydrides in the workplace.[57]

Adverse health effects of TMA exposure are a result of its direct irritant effects on mucosal surfaces in all exposed humans as well as its ability to cause immunologic sensitization in a small proportion of humans.[58] In immunologically sensitized individuals, re-exposure to TMA can result in immunologic syndromes: asthma-rhinitis, late respiratory systemic syndrome, and very rarely pulmonary disease anemia syndrome.[58]

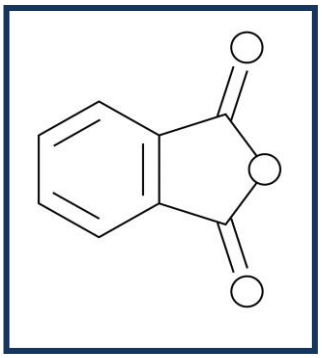
TMA is known to cause occupational asthma characterized by airflow obstruction, airway inflammation and non-specific bronchial hyper-reactivity. The development of this disorder requires sensitization triggered by dermal or respiratory exposure to TMA, followed by its binding to proteins.[57]



The presence of PAs in the air, as well as their use in plastics, makes PA exposure a global issue. A review of studies conducted in different nations[60] confirms there is a relatively large daily exposure to PAs in industrialized countries. In a pediatric study on 198 cases, involving eczema, wheezing, and rhinitis without a cold, and 202 controls, PA exposures were measured in house dust.[60] An association

was found between dust concentrations of specific phthalate esters and asthma, rhinitis, and eczema.[60] It is interesting to note, compared with other types of flooring materials, PVC flooring in the child's bedroom was positively associated with health status.[60] This study demonstrates associations between PA concentrations in dust and selected allergies and asthma.[60] Indeed there has been an increase in allergies and asthma over the last three decades. Many contributing factors are responsible for this phenomenon, and one may be the significant rise of plasticized products made for home, work, and industrial use.

Commonly Used Cosmetic Products	Percent Positive for PAs Tested[61]
Deodorants	71
Fragrances	100
Hair gels	86
Hair mousse	71
Hairsprays	71
Lotions	71
Nail polishes	67



PAs are known to reduce sperm count, cause histological changes in testes, and reduce male fertility as shown in animal studies.[62] Also associated with PAs are preterm deliveries.[63] Reduced sperm motility from PA exposures was found in male partners in infertile couples seeking fertility help.[64] In women, phthalates have been shown to be associated with both BMI and waist circumference, and higher rates of leiomyomas and endometriosis.[65] Phthalate exposure can also lead to increased rates of breast cancer, and even more so for premenopausal females.[66]

Effects of Phthalate Exposure on Humans[67]	
In Utero	Lasting adverse health effects
Children	Inattention Mood disorders Cognitive problems
Males	Reduced Leydig cell function Lower testosterone Hypospadias Infertility
Young Girls	Premature sexual development Early breast development
Adults	Increased weight Insulin resistance Asthma Allergies

	Uterine fibroids Breast cancer

The detection of antibodies to Trimellitic + Phthalic Anhydrides bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. TMA and PA or their metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to Trimellitic + Phthalic Anhydrides bound to human protein in serum should avoid exposure to these substances, with special attention taken to clean up the home and work environments.

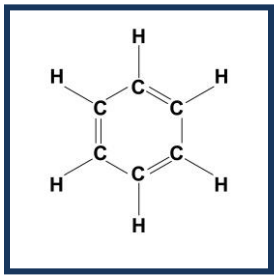
Quick List Sources of Trimellitic Anhydride[150]	
Paints	Plastics
Polyester resins	Epoxies

Quick List Sources of Phthalates[151]	
Health and beauty products	Cleaning products
Building materials	Insecticides
Supplements	Toys
Food packaging	Adhesives

Benzene Ring Compounds (Benzene, Pheno, Toluene, Xylene)

Classified as a hydrocarbon, Benzene [C_6H_6] is an organic chemical compound composed of 6 carbon atoms forming a hexagonal ring arrangement. Benzene is a solvent found in crude oil; because it has a high octane number, it is utilized in gasoline. Benzene is also used as a precursor to heavy chemicals (i.e. ethylbenzene, cumene from which acetone and phenols are derived). Most solvents are neurotoxic and many are carcinogenic.

Acute inhalation exposure to high concentrations of benzene can cause drowsiness, fatigue, nausea, vertigo, narcosis, and other symptoms of central nervous system (CNS) depression; however, the most damaging health effects associated with benzene exposure are chronic effects due to repeated exposure to low concentrations over many years.[68]



Benzene causes bone marrow failure. Many epidemiologic, clinical, and laboratory data link benzene to aplastic anemia, acute leukemia, and bone marrow abnormalities.[69] Specific hematologic malignancies directly related to benzene include: acute myeloid leukemia (AML), aplastic anemia, myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), and chronic myeloid leukemia (CML).[70]

The American Petroleum Institute (API) stated in 1948 that "it is generally considered that the only absolutely safe concentration for benzene is zero."[71]

Due to their lipophilicity, most organic solvents, like benzene ring compounds, tend to accumulate in lipid-rich organs, such as nerve tissues in both the peripheral and the central nervous systems.[72] Studies with adult populations exposed to occupational benzene ring compounds support the finding that changes in the visual function can serve as an early marker of neurotoxic damage, especially that resulting from chronic low-level exposure.[72] [73]

Benzene is known to cross the placenta and has been found in cord blood at levels equal to or higher than maternal blood;[74] furthermore, an association between maternal airborne benzene exposure and birth defects has been noted.[75] Texas ranks number one in the United States for benzene levels in ambient air and accounts for 48% of all benzene emissions in the nation.[76] In a study conducted in Texas, it was shown that the risk of having a spina bifida-affected infant more than doubled for mothers living in census tracts with estimated benzene levels of ≥ 3 µg/m3.[76] Benzene can lead to genetic toxicity by covalently binding to DNA and forming DNA adducts, which, if not repaired, disrupt the microenvironment of the cell, leading to inhibition of important enzymes, cell death, and alteration of other cells.[74] If this occurs during the critical window of fetal development, the complex cellular processes involved in neurulation (*e.g.*, folate metabolism, cell proliferation, cellular adhesion, and vascular development) may be disturbed.[76] The end result could be neural tube defects.

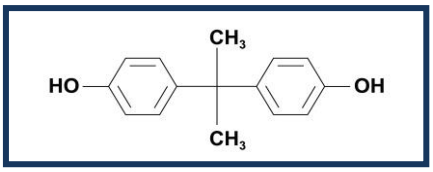
The detection of antibodies to Benzene Ring Compounds bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Benzene or its metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to Benzene Ring Compounds bound to human protein in serum should avoid exposure to these substances, with special attention taken to clean up the home and work environments.

Quick List Sources of Benzene Ring Compounds[152]	
Tobacco Smoke	Industrial emissions
Auto exhaust	Glues
Paints	Furniture wax

Detergents	Air around gas stations

Bisphenol A

Bisphenol A (BPA) is an organic compound [$(CH_3)_2C(C_6H_4OH)_2$] used to make polycarbonate polymers and epoxy resins, along with other materials used to make plastics. Paper products, most notably thermal receipt paper, contain BPA, as do compact discs, impact-resistant safety equipment, medical devices, the coating in food and beverage cans, bottle tops, water supply pipes, and many forms of food and drink packaging, e.g., water and infant bottles. Some dental sealants and composites may also contribute to BPA exposure.



A published cross-sectional analysis[77] of BPA concentrations and health status in the general adult population of the United States, using data from the National Health and Nutrition Examination Survey 2010, (NHANES) conducted by the Centers for Disease Control (CDC) and Prevention's National Center for Health Statistics, 2500 adults were assessed. Higher BPA concentrations were associated with diagnoses of cardiovascular disease and diabetes, as well as subclinically abnormal concentrations of liver enzymes GGT, alkaline phosphatase, and lactate dehydrogenase.[77] This information suggests that exposure among the general US population is likely to exceed the 50-µg/kg per day reference dose currently recommended by the US Environmental Protection Agency.[77 78] These daily exposures are most likely through continuous, multi-route exposure, principally diet, but also through transdermal exposure and inhalation of airborne dust.[77 78]

BPA functions as a xenoestrogen by binding strongly to estrogen-related receptor γ (ERR-γ).[78 79] BPA has been shown to antagonize thyroid hormone T3 action at the transcriptional level.[80] Therefore, BPA may disrupt the function of various types of hormone receptors and their cofactors to disturb the hormonal environment. In adults, BPA is eliminated from the body through a detoxification process in the liver; however, in infants and children, the pathway is not fully developed, which prevents them from clearing BPA from their systems.[81] In a rat study, significant *in vitro* inhibition of BPA detoxification was found with nine drugs including naproxen, salicylic acid, carbamazepine and mefenamic acid.[82] Lin and colleagues reported that exposure to BPA decreased rat insulinoma (INS-1) cells' viability, disrupted cell function, and increased cell apoptosis in a dose-dependent manner, which may explain the role BPA can play in the increased incidence of diabetes mellitus and increased damage to β-cell function.[83]

BPA may also have neurotoxic effects and may promote neurodegenerative disorders. In vivo and in vitro studies have shown the endocrine disruptor effects of BPA, including the induction of hyperprolactinemia.[84 85] Prolactin is known for its role in lactation and is also a critical player in modulating immune and inflammatory responses via immune signaling pathways. This can lead to autoimmunity via the increase of immunostimulatory response to prolactin. Furthermore, BPA and T3

have such a degree of molecular structure similarity that it can potentially lead to autoimmune cross-reactivity with antigen-antibody complexes.[86]

The detection of antibodies to Bisphenol A bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. BPA or its metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to BPA bound to human protein in serum should avoid exposure to the substance, with special attention taken to clean up the home and work environments.

Quick List Sources of Bisphenol A[153]	
Plastic food/drink packaging	Compact discs
Epoxies	Lacquer coating of food cans, bottle caps, water supply pipes
Some dental sealants and composites	Thermal receipt paper

BPA-Binding Protein

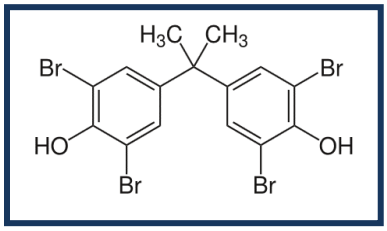
Protein Disulfide Isomerase (PDI) is a multifunctional enzyme mainly found in the endoplasmic reticulum of eukaryotes where its main function is to catalyze the rearrangement (isomerization) of disulfide bridges during folding of membrane and secreted proteins. PDI can act as a high capacity reservoir for various ligands including hormones such as estradiol (E2) and thyroxine (T3). Because PDI is a target for bisphenol A (BPA) binding, it is also called BPA-Binding Protein.[87]

The binding of BPA to BPA-Binding Protein results in the disruption of PDI actions, which may adversely affect many cellular processes.[87] BPA has been shown to exhibit negative (estrogens, indomethacin) or positive (progestogen, androgen) effects on PDI reductase activity, suggesting that BPA-binding to PDI could affect endocrine target cells through nuclear receptors, but also all cells through PDI and consequently by affecting folding of proteins in the endoplasmic reticulum.[88] [89] Defective protein folding has been described as a basis for human disease.[90] Furthermore, BPA's inhibitory action on PDI, may cause an interference in the protein synthesis required for synapse formation, which results in the inhibition of synaptogenesis,[91] which may explain why PDI involvement is implicated in brain impairment disorders such as ischemia, Parkinson's disease, Alzheimer disease, and Creutzfeldt-Jakob disease.[90] [91]

The detection of antibodies to BPA-Binding Protein in serum indicates a breakdown in immunological tolerance against self-tissue. This could be due to BPA binding to PDI and the formation of neo-antigens. These new antigens are comprised of the haptenic BPA plus the tissue enzyme PDI. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue.

Tetrabromobisphenol A

Tetrabromobisphenol A (TBBP-A) [$C_{15}H_{12}Br_4O_2$] is a chemical derivative of bisphenol A. TBBP-A is commonly used as a flame retardant in building materials, paints, synthetic textiles, and plastic products, including epoxy resin electronic circuit boards and other electronic equipment.



Human milk samples were taken from mothers in the greater Boston, Massachusetts's area; levels of TBBP-A were detected in 35% of the analyzed samples.[92] Self-reported demographic, dietary and behavioral data were examined as predictors of levels. There was a significantly positive association with the number of stereo and video electronics in the home and reduction in participants who regularly chose organic foods compared to those who did not.[92] These results indicate that lifestyle factors play a role in body burdens of TBBP-A and that home electronics may be an important source of TBBP-A exposure in the indoor environment.[92]

Among phenolic compounds, TBBP-A showed the most potent inhibitory effect on the thyroxine (T_3)-binding activity of protein disulfide isomerase.[91 92] Thus, it is suspected of being a hormone function disruptor. Additionally, TBBP-A is a potent inhibitor of sarcoplasmic reticulum Ca2+ ATPase pumps in skeletal muscle and testicular cells.[93] In another study, TBBP-A was shown to induce cell death, at least in part, by apoptosis through activation of caspases.[94] TBBP-A also increased intracellular [Ca2+] levels and reactive-oxygen-species (ROS) within neuronal cells.[94]

The high usage of TBBP-A, and limited water solubility of the chemical, may lead to persistence in the environment and possibly accumulation in biological systems. Two studies found concentrations of TBBP-A in river sediment and downstream industrial sewage sludge in Osaka, Japan and Sweden respectively.[95 96] Occupational exposures in the electronic recycling industry showed that workers were exposed to high levels of TBBP-A; the authors concluded that studies need to be done on the health effects of these exposures.[97]

The detection of antibodies to Tetrabromobisphenol A bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. TBBP-A or its metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to TBBP-A bound to human protein in serum should

avoid exposure to the substance, with special attention taken to clean up the home and work environments.

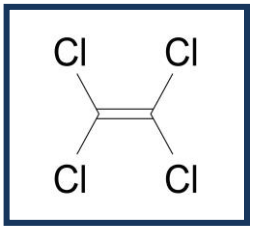
Quick List Sources of Tetrabromobisphenol A[154 155]	
Flame retardants	Electronic circuit boards
Epoxies	Automobiles
Lighting fixtures	Simulated marble floor tiles
Furniture parts	Glass-reinforced panels

Tetrachloroethylene

Tetrachloroethylene (PCE) [$Cl_2C=CCl_2$] is an occupational chemical used in very commonly in dry cleaning of clothes and fabrics and metal degreasing, and is prevalent as a drinking water contaminant.

In 1980 New England government officials discovered that PCE was leaching into public drinking water supplies from the inner vinyl lining of asbestos cement water distribution pipes. A Cape Cod study of 1,658 children whose mothers were exposed to the PCE-contaminated drinking water and a comparable group of 2,999 children of unexposed mothers was conducted.[98] The results of this study suggest that the risk of certain congenital anomalies is increased among the offspring of women who were exposed to PCE-contaminated drinking water around the time of conception.[98] These anomalies reviewed[98] include:

- **increases in the risk of gastrointestinal defects (particularly oral clefts 2.2 - 3.5 fold increase)**
- **neural tube defects (particularly anencephaly 2.5 fold increase)**
- **modest increases in cardiac anomalies (3 times higher)**



Furthermore, analysis of females in the Cape Cod study showed an increased risk of breast cancer after high exposure to PCE.[99 100] Because PCE is lipid-soluble and women typically have a higher proportion of body fat than men, it is anticipated that women would retain PCE longer than their male counterparts.

A study by the EPA showed that developmental exposure to PCE is common: 100% of samples of breast milk from four U.S. urban areas had detectable levels of PCE.[101] Tetrachloroethylene health effects include immunotoxicity, notably the development of autoimmunity in chronic PCE exposure in adults, both environmental and occupational, including the development of systemic lupus erythematosus, scleroderma, diabetes, and hepatitis.[102]

Effects resulting from acute inhalation exposure of humans to tetrachloroethylene include irritation of the upper respiratory tract and eyes, kidney dysfunction, and neurological effects such as reversible mood and behavioral changes, impairment of coordination, dizziness, headache, sleepiness, and unconsciousness.[103] The primary effects from chronic inhalation exposure are neurological, including impaired cognitive and motor neurobehavioral performance.[104] The three primary neurological effects most consistently associated with subchronic or chronic PCE exposure in human studies are vision, visuospatial memory, and neuropsychological function (e.g., reaction time). Occupational and residential exposure studies showed an association of visual deficits after chronic PCE exposure as well as deficits in color vision.[98]

PCE exposure may also cause adverse effects in the kidney, liver, immune system and hematologic system, and on development and reproduction. It is considered a carcinogen.[99]

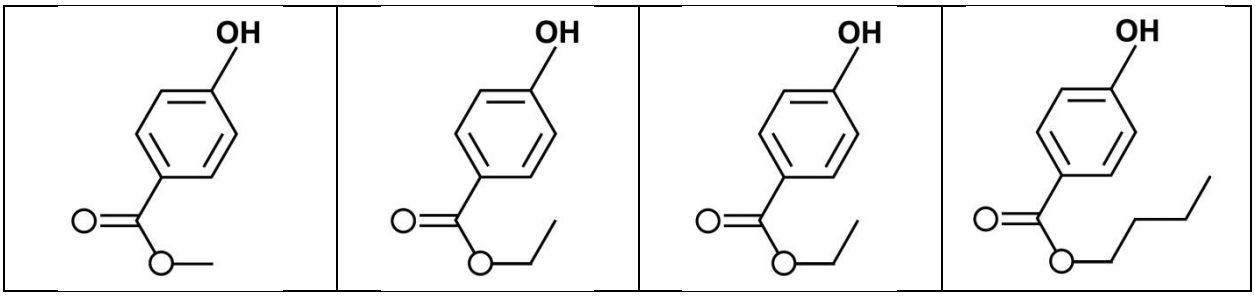
The detection of antibodies to tetracholorethylene bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Tetracholorethylene or its metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to Tetracholorethylene bound to human protein in serum should avoid exposure to the substance, with special attention taken to clean up the home and work environments.

Quick List Sources of Tetrachloroethylene[156 157 158]	
Spot removers	Leather products
Paint removers	Adhesives
Water repellents	Paper coatings
Dry-cleaned clothes	Chlorinated water

Parabens

Parabens are used as preservatives and have bactericidal and fungicidal properties. They are used in many personal care and beauty products, pharmaceuticals, food and beverages. They can be found in shampoos, commercial moisturizers, shaving gels, personal lubricants, topical and parenteral pharmaceuticals, spray tanning solution, makeup, and toothpaste. They are also used as food additives.

METHYL PARABEN	ETHYL PARABEN	PROPYL PARABEN	BUTYL PARABEN
$C_8H_8O_3$	$C_9H_{10}O_3$	$C_{10}H_{12}O_3$	$C_{11}H_{14}O_3$



In rat studies, parabens have been shown to produce male reproductive disorders with a dose-dependent decrease of absolute and relative weights of epididymis and ventral prostates, as well as reduction in sperm reserve and daily production.[105] Researchers postulate that parabens may have some adverse effects on the male endocrine system resulting in the lowering of circulating androgen action, which impedes androgen signaling.[106] Research on androgen actions of parabens is still being debated.

Parabens can indirectly affect estrogen levels via inhibition of sulfotransferase activity inside the cytosol of skin cells; the blocked sulfotransferases cause estrogen levels to remain higher than normal.[67 107] Parabens are able to penetrate human skin intact, without breakdown by esterases, and are thus absorbed into the blood stream.[108] An increasing number of breast cancers are occurring in the upper outer quadrant of the breast, where paraben-containing antiperspirants are used;[67] interestingly, methyl paraben was the most commonly found paraben (18/20) and was detected at the highest level in human breast tumors.[108]

Methyl- and propyl parabens are the two most commonly found in humans and are also strong inhibitors of mitochondrial function.[107 109] Anyone with mitochondrial dysfunction-related disorders should take special care to avoid these chemical compounds. The effect of parabens on mitochondrial function has been speculated as playing a role in male infertility.[110]

The detection of antibodies to Parabens bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Parabens or their metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens may result in autoimmune reactivity. Persons with antibodies to Parabens bound to human protein in serum should avoid exposure to the substance, with special attention taken to clean up the home and work environments.

Quick List Sources of Parabens[159]	
Healthy and beauty products	Pharmaceuticals
Food products with known high levels of Parabens include:	
• Red wine • Iced tea	• Fish and shellfish • Dairy products

- Pancake syrup
- Grains
- Turkey meat products
- Fruit products

Mercury Compounds

Mercury (Hg) is a heavy chemical element that is liquid at standard temperature and pressure. Mercury has been used in thermometers, barometers, float valves, mercury switches, and other devices. It remains in use in amalgam material in dentistry, in energy-efficient light bulbs, in scientific research applications, and in gold mining.

Humans are constantly exposed to low, sub-toxic levels of mercury. Mercury poisoning can result from exposure to water-soluble forms of mercury (such as mercuric chloride or methyl mercury), inhalation of mercury vapor, or ingestion of fish/seafood or high fructose corn syrup contaminated with mercury. Mercury is neuro-, nephro-, and immunotoxic.[111] In a healthy individual with adequate nutrition, metallothionein is expressed and mercury is eliminated from the body. If the individual consumes an unhealthy diet leading to mineral imbalances, especially low zinc and high copper, then it is possible that either there will not be enough metallothionein to eliminate the mercury or the metallothionein may not function properly, which will lead to oxidative stress.[112] Exposure to mercury can have an immunosuppressive effect.[113]

The debate over mercury-containing dental amalgams continues. Pigatto and Guzzi[114] have pointed to an overlooked, yet important, factor that is related to prolonged exposure to mercury derived from dental amalgam, namely lichenoid contact stomatitis (a.k.a. oral lichen planus or oral lichenoid lesion). Lichenoid contact stomatitis is a mucositis, possibly autoimmune in origin, leading to visible oral lesions inside the mouth, and mercury-containing dental amalgam has long been implicated in the pathogenesis of lichenoid contact stomatitis.[114] The precise role is one of inducing a contact delayed-type hypersensitivity reaction and promoting allergic events in susceptible individuals.[114] In persons having mercury-containing dental amalgams, mercury is constantly emitted from the amalgam surfaces especially during mastication. Persons who grind their teeth while sleeping create even more release of mercury from their dental amalgams. In Pigatto and Guzzi's careful review of the literature, they conclude that amalgam removal and replacement with a non-mercury alternative is associated with a high rate of complete remission in patients with amalgam-related lichenoid lesions.[114] Additionally, these authors found that parallels also exist between mercury amalgams and the inflammatory autoimmune skin disorder lichen planus.[114]

Autoimmune patients referred to the Institute of Dental Research in Prague were included in a study [115] on the health benefits of metal-containing dental amalgams. To participate in the study, patients had to have at least one metal dental amalgam and have a diagnosed autoimmune disease. Disorders included systemic lupus erythematosus, autoimmune thyroiditis, multiple sclerosis, and atopic eczema. Between 3 and 16 dental amalgams were removed from each study participant. Of the multiple sclerosis patients, all saw symptom improvement after removal of the metal-containing dental amalgams.[115] Researchers observed a decrease of lymphocyte reactivity to inorganic mercury half a year after amalgam removal in patients with health improvement.[115] In a similar study specifically for autoimmune thyroiditis, where the

patients had positive antibodies to thyroid peroxidase/thyroglobulin as well as metal dental amalgams, the patients who improved after amalgam removal also has a decrease in antibody production to thyroid markers.[116] Other researchers found that women with higher mercury levels showed greater odds for thyroglobulin autoantibodies.[117] In the dental amalgam autoimmune study,[115] there were 8 out of a total 35 patients who did not realize symptom improvement, or got worse after removal of dental amalgams; each of these persons smoked cigarettes, which are known to contain mercury, nickel, cadmium and lead.[115 118]

Dental patients receiving mercury-containing amalgams are not the only ones at risk for the development of disorders. Dentists and dental assistants, whether implanting or extracting metal amalgams, are exposed to mercury at varying levels. Chronic low-level exposure, as seen in dental offices, is associated with mood disorders.[119]

Organic mercury compounds are primarily toxic to the central nervous system. Methyl mercury damages DNA and impairs mitosis and it is especially toxic during all developmental phases, while inorganic mercury is primarily nephrotoxic.[6] The development of arrhythmias and cardiomyopathy has been linked to toxic levels of mercury. The reviewed results of mercury intoxication[6] include:

- **cerebral palsy**
- **seizures**
- **mental retardation**
- **deafness**
- **blindness**
- **ultimately, death.**

Mercury can profoundly affect the immune system at concentrations well below that required to damage the central nervous system and the kidneys.[6] Toxic doses of mercury (especially the organic species) induce cell death in the immune system, resulting in immunosuppression.[6 35] This immune dysfunction has been overshadowed by the more life-threatening neurotoxic effects.

A study supporting the concept of mercury-induced immune suppression was conducted in Brazil. It focused on three population groups, one that is directly involved in gold mining, one living in the watershed area of the river several 100 miles downstream from the first group, and another river community neither directly, nor indirectly involve in gold mining, and east of the other two river communities.[35] Researchers found a correlation between mercury exposure and incidence of malaria, which leads them to believe mercury-induced immune suppression can result in vulnerability to biological infections.[35]

The detection of antibodies to Mercury bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Mercury or its metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue (**see Figure 5**). Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity. Persons with antibodies to mercury bound to human protein in serum should avoid exposure to the substance, with special attention taken to clean up the home and work environments.

Quick List Sources of Mercury[160]

Dental amalgams	Shellfish and fish (especially large fish such as tuna, marlin and shark)
Energy-efficient light bulbs	Pesticides and fungicides
Thermometers	Some medical devices
Mercurial skin creams	Paints (especially marine)

Mixed Heavy Metals (Nickel, Cobalt, Cadmium, Lead, Arsenic)

Nickel (Ni) and Cadmium (Cd) compounds are currently mainly used in re-chargeable nickel–cadmium batteries, and cadmium can be found in cigarettes. Cobalt (Co) is used in the preparation of magnetic, wear-resistant and high-strength alloys, and for its distinctive blue, forms of cobalt are used to color glass, ceramics, inks, paints and varnishes. Lead (Pb) is used in gasoline, paints, and some food containers. Arsenic (As) can be found in apples, almonds and water.

Emissions of heavy metals to the environment occur via a range of processes and pathways, including into the air (e.g. during combustion, extraction and processing), into surface waters (via runoff and releases from storage and transport) and into the soil (and hence into ground waters and crops). In a study[120] of heavy metal toxicity due to the consumption of vegetables grown in soil of an old mining community, researchers found unsafe levels of heavy metals in parsley and carrot roots, lettuce and cabbage.[120] Body burdens of heavy metals were higher in females than in males.[120] The agriculture industry involves the handling of heavy metals in the form of pesticides and fertilizers. Monitoring commercial farmers for occupational heavy metal toxicity showed significantly elevated levels of lead and arsenic in farm workers compared to a non-farming population.[121] Ninety-five percent of these study participants reported using personal protective equipment when handling the pesticides and fertilizers.[121]

Of the many occupations and professions allowed to conduct business in residential areas, the auto body refinishing industry is one of environmental and health concern. The painting and scrapping processes are often done without any safety measures.[122] The results of an industry study showed that the soil around these auto body refinishing businesses were contaminated by higher levels of lead, cobalt, nickel, cadmium and other chemicals than control samples.[122] Toxic organic compounds from the exhausts of auto body refinishing locations reacts with atmospheric nitrogen oxides in the presence of sunlight and form a ground level ozone layer, which is also a major threat to the environment.[123] Auto body refinishing shops have serious concerns relating to health issues as well. According to the Federal Register, chemicals in the auto body refinishing industry may play a role in cancer, asthma, kidney diseases and disordered central nervous system.[124]

Research results on the health effects of individual heavy metals are listed below:

- **Exposures to nickel can enhance metabolic disorders, mitochondrial dysfunction, and the monocytic cell infiltration into lung and adipose tissue.[125]**
- **Researchers have shown the impairment of lung function, after allowing for smoking habits, showed a dose-response relation with the intensity of the cobalt**

chromium dust exposure in terms of the number of hours exposed per week and self-estimated exposure level.[126]

- **Both cadmium and lead have been shown to skew immune responses towards Th2 isotype.**[127]
- **Long-term, low-level lead exposure in children may lead to diminished intellectual capacity.**[128] **Acute exposure to lead is known to cause proximal renal tubular damage.**[129] **Long-term lead exposure may also give rise to kidney damage**.[128]
- **The intake of large quantities of arsenic leads to gastrointestinal symptoms, severe disturbances of the cardiovascular and central nervous systems, and eventually death. In survivors, bone marrow depression, hemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy may be observed.**[128]

The detection of antibodies to Mixed Heavy Metals (Nickel, Cobalt, Cadmium, Lead, Arsenic) bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance. Heavy Metals or their metabolites can bind to human tissue proteins and form neo-antigens. These new antigens are comprised of the haptenic chemical plus the tissue antigen. The formation of neo-antigens initiates an immune response, which may result in antibody production against the chemical and the human tissue. Continued exposure to the chemical and the subsequent production of antibodies against various tissue antigens, may result in autoimmune reactivity.

Quick List Sources of Heavy Metals[161 162 163 164 165]	
Cigarette smoke	Automobile emissions
Batteries	Paints, inks and varnishes
Cosmetics	Blue or red colored porcelain
Crystal food and beverage containers	Other food sources

TOXICOLOGY

Antibodies Versus Levels: Measuring Body Burden of Chemical Exposure

Mercury is a neurotoxin, which at high levels can kill nerve cells, resulting in blurred vision, lack of coordination, slurred speech, tremors, cognitive function problems, and in some cases, death. Prenatal and early postnatal exposure may cause slow development, blindness, cerebral palsy and other defects. Exposure to large amounts of methylmercury and/or inorganic mercury, which can result in

immunosuppression, is not the only concern for health care providers and patients. Chronic low doses usually associated with regular fish consumption, working in dental offices and metal dental fillings, also can have neurotoxic and immunotoxic implications.

Neurologically, chronic low doses of mercury can affect brain-derived neurotrophic factor, a protein that regulates neuronal growth and differentiation in both the peripheral and central nervous systems.[14] The effects produce a variety of symptoms, including confusion, anxiety, stomach upset, headache, fatigue, anger, depression, and skin and lung complaints.[119] Immunologically, this kind of heavy metal exposure decreases resistance to some infectious agents, and may progress into the development of autoimmunity.[14] Mercury-induced autoimmunity includes T-cell development, polyclonal B-cell activation, a rise in serum IgG antibodies, the formation of renal immune complex deposits and the production of ANA autoantibodies (ANoIA).[31] Mercury-induced ANAoIA share similarities to the binding of fibrillarin and chromatin, components of the nucleoproteins to which mercury and other heavy metals bind.[31 32 34]

Health officials of the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have established threshold values to try to prevent health problems associated with toxicity. The FDA estimates that mercury exposure at no more than 1 part per million (1 ppm), which is equal to 0.5 micrograms per kilogram of body weight per day is safe, however, the EPA suggests a much stricter limit at 0.1 mg/kg/day.[34]

Using the FDA and EPA guidelines for toxicity levels, scientists have conducted studies in all hemispheres of the globe. Researchers for the well-publicized biomonitoring study on New York City adults,[130] which concluded that a quarter of NYC adults had higher than national blood mercury levels, found a lack of clinical complaints to correlate with the above abnormal results. Authors of the study stated, "Laboratory methods for determining chemical exposures have become increasingly sensitive, so the detection of lead, mercury or cadmium in the blood of an adult, does not necessarily imply a health risk."[130]

On the other side of the globe, University of Rochester scientists[131] completed a nine-year study in the Republic of Seychelles. On this Indian Ocean island nation, people subsist on an average of twelve fish meals per week and have mercury levels ten times higher than those found in US citizens.[131] After performing a battery of nearly three dozen developmental and neurological tests on children at 6, 19, 29 and 66 months of age, the results of this longitudinal study consistently show no ill effects from a high-fish diet resulting in elevated mercury levels.[131]

Again, in the US, the Baltimore Memory Study[132] also determined that blood mercury levels are not associated with worse neurobehavioral performance in this population of 50 – 70 year-old urban adults. Authors of the study deduced, "In evaluating whether toxicants have adverse effects on central nervous system function, it is important to consider whether exposure was recent or cumulative, whether effects are acute or chronic, and whether the biomarker is adequate to assess differing dose patterns."[132] They also question the validity of using a single blood draw to estimate cumulative dose.[132]

The question arises: *How can the medical community judge toxicity when research shows high levels of mercury do not equal clinical complaints, and worse yet, low levels considered to be safe can wreak havoc on the nervous and immune systems?* The answer lies within each individual. Each person's immune system is unique and different and has a threshold value of toxicity, which can fluctuate during

his/her lifetime. Some people possess a strong natural chelation process and thus can handle large doses, or chronic low doses of heavy metals or other toxicants without health risk, while others have an immune system that overreacts to invading elements, even when the level of bioaccumulation is not considered harmful by governmental standards. Still others have a poorly functioning immune system and dysfunctional chelating capabilities.

A variety of laboratory analyses are available. There are limits associated with each specimen type and methodology. Only after removal of mercury laden dental work will *fecal* analysis reflect the body's burden of accumulated mercury and the body's ability to chelate mercury naturally.[133] Ninety percent of mercury is eliminated through the fecal process. *Urine* testing is scrutinized for its biological variability as well as whether or not the sample is challenged.[134 135] Less than 10% of mercury is naturally eliminated via urine.[135] High levels of mercury in urine indicate that an excessive exposure (but not chronic, low-level exposure) has occurred, but does not indicate form, or quantity of mercury.[136] Urinary analysis is useful to find a historical average of exposure to metallic and inorganic mercury only. Methylmercury found in fish cannot be detected through urinary analysis.[134 135] To measure methylmercury specifically, blood level and hair testing is used. In *blood*, the level of mercury decreases by one half every three days as mercury infiltrates other organs.[134] A high blood mercury level suggesting a relatively recent exposure, like urinary analysis, indicates that an excessive dosage has occurred, but does not indicate the form or quantity of mercury.[135] A review of the literature found that whole blood collection tubes with EDTA anticoagulant is the most appropriate specimen collection method; the use of other tubes and anticoagulants require additional, specific measures to be taken for accurate results.[134] *Hair* analysis is criticized for its difficulty in performance and the use of a sample that has been exposed to additional toxicants through shampoo, dyes and air pollution.[137]

The above methodologies, while providing evidence of exposure, fail in determining a person's health risk.[34 130 131 132 134] Where is the individual's immune system tested to discover what implications toxic exposure, both high and low dose, have on the body? Humoral immunity, the system in which antibodies are produced to fight/recognize invading elements, is tested for a variety of clinical purposes from allergies to viral infections. This same trusted testing methodology can be used to detect antibodies to tissue-bound mercury and other toxicants, as is done by Cyrex Laboratories.

Influencing Factors

The development of chemical antibodies depends not only on the amount and duration of chemical compound exposure, but also on the genetic background of the person exposed.[32] **Figure 9** illustrates how three people exposed to the same amount of chemicals have unique responses to the exposure. How is it that one person develops cancer, one develops an autoimmune disorder and another remains healthy? Genetics should be considered.

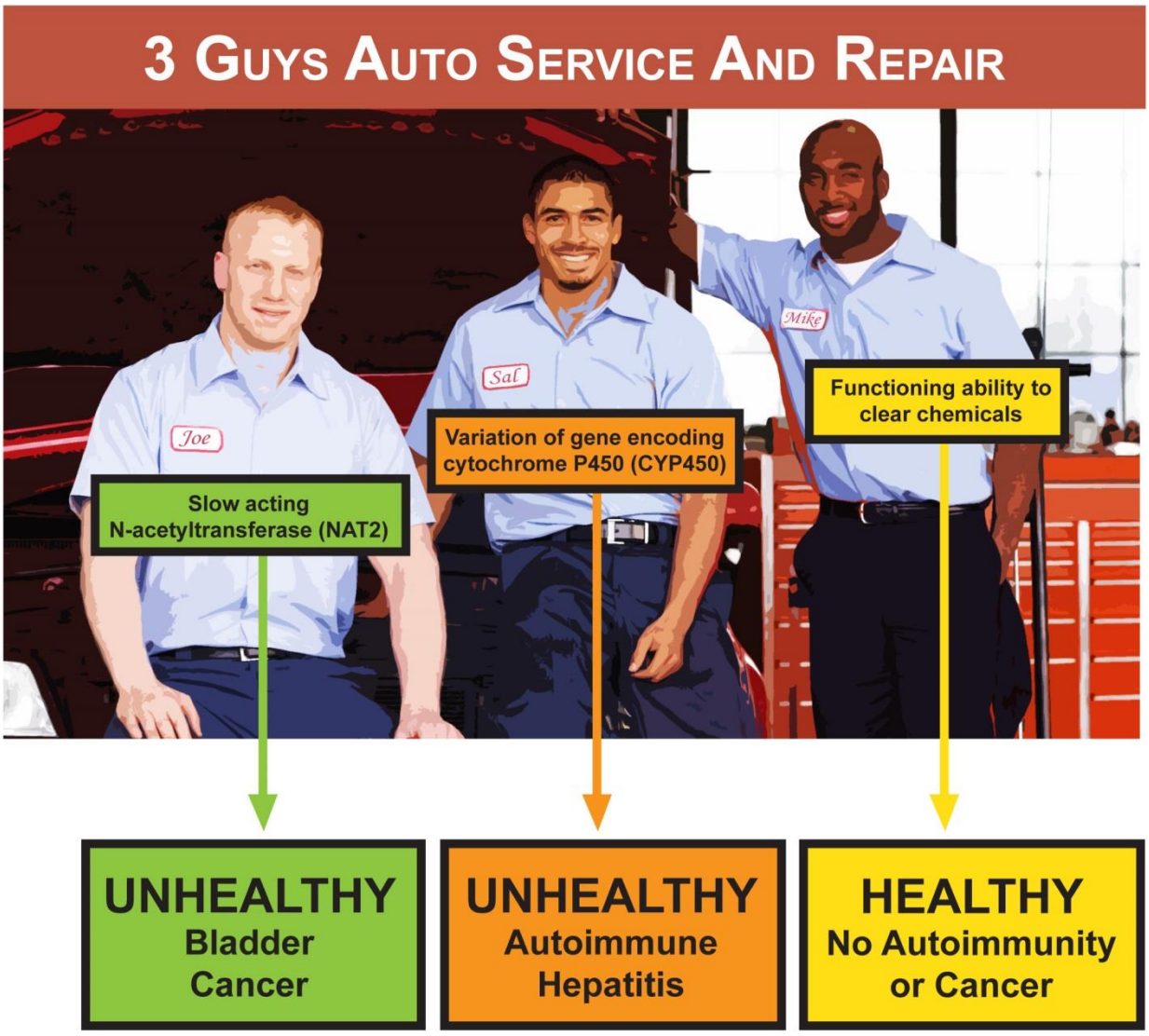


Figure 9. Same exposures: different outcomes. *Joe, Sal and Mike opened 3 Guys Auto Service and Repair. Each works the same number of hours per week, and each performs the same tasks of pumping gas, engine repair and cashiering. They are exposed to the same chemicals. Mike inherited the ability to clear chemicals from his body. Joe and Sal developed diseases due to genetic factors.*

Genetic

Although genetic and environmental factors both play a central role in autoimmunity, many times it is not clear which one is the main link to the heterogeneity of autoimmune prevalence. The importance of genes in autoimmunity was emphasized when it was noticed that the risk of autoimmunity is increased in twins and siblings of affected individuals.[138] Thereafter, gene analysis studies have confirmed the genetic relevance and suggested different methods for predicting the development of autoimmune conditions such as SLE, RA, DM1, and MS on an individual basis.[139 140 141 142] Predisposition to the adverse effects of

environmental agents may be influenced by genetic variations that affect both susceptibility and resistance. In a mouse study, Kono and colleagues[32] identified a locus, Hmr1, that appears to contribute to the resistance of DBA/2 mice to mercury autoantibodies and that maps to a region on chromosome 1 implicated in susceptibility to spontaneous lupus.[32] When it comes to malignancies, inheritance probably explains no more than 5% of all cancers in the United States.[143] The genetic disruptions that transform a normal cell into a cancerous one typically arise from complex interactions between carcinogens and the body's toxin clearing system.[143]

Environmental Exposures

The development of an autoimmune disease may be influenced by the genes a person inherits together with the way the person's immune system responds to certain environmental triggers, such as toxic chemicals and infectious agents. The role of environmental factors can be better understood when one considers that 1) only 24-50% of identical twins develop the same autoimmune disease and 2) the fact that major histocompatibility complex (MHC) differences are not the only factor contributing to the susceptibility to autoimmunity, but lifestyle, the toxin's metabolism, and exposure rates are important factors that cause individuals to react differently to the same chemicals.

This concept is based on the fact that some autoimmune diseases are known to be more common in polluted environments or worsen by additional triggers such as bacteria or viruses.

CLINICAL APPLICATION OF CHEMICAL IMMUNE REACTIVITY TESTING

Systemic Immune Effects

Depending on the person's genetic ability to handle toxins, amount and duration of exposure, and body burden of accumulated insults, the resulting immunotoxicity causes:

- **Immune suppression**
- **Immune stimulation**
- **Cancer**

Immune suppression leaves the patient more susceptible to infections. Combinations of chemical and stealth organism exposures have been shown to contribute to illness. In a Brazilian study, subjects with elevated mercury exposure were more susceptible to contracting malaria.[34] Cancer can also occur with this combination of exposures. Subjects exposed to aflatoxins were four times more likely to develop liver cancer than unexposed controls, while the subject group infected with hepatitis B virus and exposed to aflatoxins was sixty times more likely to develop liver cancer.[143]

Immune stimulation may result in allergic reactions or autoimmunity. Autoimmune responses can be induced by environmental chemicals through a variety of effects including cellular, biochemical and

molecular.[9] The chemical's toxic effects can indirectly damage the structural integrity or function of organs or tissues (**see Figure 4**).[9] As a chemical enters the body it is metabolized. The parent compound and the metabolite may attach to human tissue. The toxic effect of the chemical and its metabolite can cause tissue damage resulting in the release of tissue antigens into circulation. At this point the APC can pick up the parent compound bound to tissue, the metabolite bound to tissue or the tissue antigen. The APC hands the antigen/s to the T-cell, which begins the process of antibody production. The potential immune responses are:

- **Antibodies to the chemical bound to human tissue**
- **Antibodies to the chemical's metabolite bound to human tissue**
- **Autoantibodies to human tissue.**

Chemicals can directly influence cytokine production or inhibition of certain lymphocyte subsets.[9] Polycyclic aromatic hydrocarbons (PAHs) are known to influence TH17 cells, which produce IL-17A and inflammatory cytokine implicated in autoimmunity.[144]

Clinical Issues in Children

A child's body absorbs environmental toxins transplacentally during fetal development, or via the ingestion of house dust, breast milk, and other dietary sources during early childhood. The critical period in the development of the central nervous system of a child is from fetus through the first 3 years of life.[145] A study from Department of Environmental Health, Harvard School of Public Health discussed neurodevelopmental disabilities, including autism, attention-deficit hyperactivity disorder, dyslexia, and other cognitive impairments, that affect millions of children worldwide.[85] The prevalence of these is increasing and chemicals that damage the developing brain are among the known causes. In 2006, the authors of this study did a systematic review and identified five industrial chemicals as developmental neurotoxicants: lead, methylmercury, polychlorinated biphenyls, arsenic, and toluene.[85] Since then, epidemiological studies have documented six additional developmental neurotoxicants – manganese, fluoride, chlorpyrifos, dichlorodiphenyltrichloroethane, tetrachloroethylene, and the polybrominated diphenyl ethers.[85]

***Clinical manifestations of chemical exposures can present at any age*.** It is important to consider:

A) The patient's environment and any exposures:

- **Work**
- **Home**
- **School**
- **Sport or workout facility**
- **Hobby**

B) To what toxins is the person exposed on a daily basis? Toxic exposures include:

- **Nail salons**
- **Dental offices**
- **Hospitals**
- **Janitorial services**
- **Electronic recycling centers**
- **Cashiering**
- **Bank teller**
- **Food**
    - **Charred meats**
    - **Moldy nuts or grains**
    - **Mercury in fish and seafood**
- **Urban areas Industrial/Manufacturing workplaces**
- **Mobile/Manufactured homes**
- **Auto body repair shops**
- **Construction workers, including painters, welders, etc.**
- **Anyone applying pesticides**
- **Certain hobbies that use glue, welding, paint, etc.**
- **Agricultural areas near where pesticides are used**
- **Cigarette smoking**

Array 11 can be used to:

- Identify the loss of immune tolerance associated with xenobiotics exposures, which may lead to autoimmune reactivity.
- Assist in setting guidelines for the avoidance of specific chemicals to reduce the risk of igniting the autoimmune process.
- Monitor the effectiveness of the clinical treatment and management of patients.

Array 10 is recommended for:

- Increased chemical sensitivities/intolerance.
- Loss of immune tolerance and/or abnormal immune function.
- Autoimmune disease and/or a family history of autoimmune disease.

CLINICAL INTERPRETATION FOR ANTIBODY ARRAY 11 – CHEMICAL IMMUNE REACTIVITY SCREEN

Elevated antibodies to chemicals bound to human tissue indicate immune reactivity.

- Educate the patient on everyday sources of elevated chemicals.
- Help the patient clean up his/her home and work environments as well as possible.
- Support the patient's ability to clear the toxins from his/her body in a safe way.

Array 11 test results are not diagnostic for any clinical condition or disease. These reports may be used in conjunction with other pertinent clinical data for the purposes of diagnosis.

Table 2A. Array 11 Antigens, Associated Conditions and References.

CHEMICAL	EXPOSURE ASSOCIATED WITH	REFERENCE
Aflatoxins	Aflatoxicoses[1] Immune suppression[4] Liver tumors[2,3,4] Reye's syndrome[5] Upper respiratory tumors[2,5]	1. Bennett and Klich. *Clin Microbiol Rev*, 2003; 16(3):497-516. 2. Coulombe. In, The toxicology of aflatoxins: human health, veterinary and agricultural significance. Academic Press. 1993 pp. 89-101. 3. Liu and Wu. *Environ Health Perspect*, 2010; 118:818–824. 4. Qazi and Fayyaz. *Mycopath*, 2006; 4(2):27-34. 5. Robens and Richard. *Rev Environ Contamination Toxicol*, 1992; 127:69-94.
Formaldehyde + Glutaraldehyde	Allergic contact dermatitis[1] Formaldehyde asthma[2,3] Formaldehyde hypersensitivity[1] Rhinitis[2,3]	1. Lyapina *et al*. *J IMAB*, 2012, 18(4):255-262. 2. Thrasher *et al*. *Arch Environ Health*, 1987; 42(6):347-350. 3. Thrasher *et al*. *Am J Indust Med*, 1988; 14:479-488.
Isocyanate	Occupational asthma[1,2,3,4,5]	1. Hur *et al*. *World Allergy Org J*, 2008; 15-18. 2. Hur *et al*. *J Clin Immunol*, 2009; 29(6):786-794. 3. Palikhe *et al*. *Allergy Asthma Immunol Res*, 2011; 3(1):21-26. 4. Park *et al*. *Korean J Intern Med*, 2002; 17(4):249-251. 5. Orloff *et al*. *Environ Health Perspect*, 1998; 106:665-666.

Table 2B. Array 11 Antigens, Associated Conditions and References.

CHEMICAL	EXPOSURE ASSOCIATED WITH	REFERENCE
Trimellitic + Phthalic Anhydrides	Asthma[1,3,4] Diabetes[1] Eczema[2] Endocrine disruption[2] Neurobehavioral problems[2] Obesity[1] Phthalate allergy[5] Rhinitis[2,3]	1. Bornehag *et al. Environ Health Perspect*, 2004; 112:1393-1397. 2. Crinnion. *Altern Med Rev*, 2010; 15(3):190-196. 3. Farraj *et al. Toxicologic Sci*, 2006; 92(1):321–328. 4. Kuper *et al. Toxicologic Pathol*, 2008; 36:985-998. 5. Pakarinen *et al. Allergy*, 2002; 57(10):894-899.
Benzene Ring Compounds	Acquired dyschromatopsia[2] Aplastic anemia[3] Cognitive function problems[1] Hand-eye coordination problems[1] Leukemia[3] Memory problems[1] Speed stimulation of motor nerve[1] Vision problems[1]	1. Ithnin *et al. Am J Environ Sci*, 2011; 7(3):248-253. 2. Lee *et al. Neuro Toxicology*, 2007; 28:356–363. 3. NIOSH. Health Hazard Evaluation Report No. 93-802-2338, 1993.
BPA Binding Protein	Moderate to severe alcoholic liver disease[1] Hepatoma[1] Liver cirrhosis[1] Systemic lupus erythmatosus[1]	1. Nagayama *et al. J Toxicol Sci*, 1994; 19(3):163-169.
Bisphenol A	Diabetes[1,3] Elevated liver enzymes[3] Endocrine dysruption[2] Heart disease[3] Immune dysfunction[1] Thyroid dysfunction[4]	1. Lin *et al. Cell Death Dis*, 2013; 4:e460. doi:10.1038/cddis.2012.206. 2. Matsushima *et al. J Biochem*, 2007; 142(4):517-524. 3. Melzer *et al. PLoS ONE*, 2010; 5(1): e8673. doi:10.1371/journal.pone.0008673. 4. Moriyama *et al. Clin Endocrinol Metab*, 2002; 87:5185–5190.
Tetrabromobisphenol A	Thyroid dysfunction[2,3] Immune dysregulation[1,3] Neuronal dysregulation[1]	1. Al-Mousa and Michelangeli. *PLoS ONE*, 2012; 7(4):e33059. doi:10.1371/journal.pone.0033059. 2. Kitamura *et al. Biochem Biophys Res Communications*, 2002; 293:554–559. 3. Ogunbayo and Michelangeli. *Biochem J*, 2007; 408:407–415.
Tetrachloroethylene	Increased risk of breast cancer[2,3] Neural tube defects[1] Oral clefts in offspring of exposed mothers[1]	1. Aschengrau *et al. Environmental Health*, 2009; 8:44. 2. Gallagher *et al. Environmental Health*, 2011; 10:47. 3. Vieira *et al. Environmental Health: A Global Access Science Source*, 2005; 4:3.

Table 2C. Array 11 Antigens, Associated Conditions and References.

CHEMICAL	EXPOSURE ASSOCIATED WITH	REFERENCE
Parabens	Breast cancer[2,3,4] Male infertility[1,4] Malignant melanoma[4]	1. Chen et al. Toxicol Applied Pharmacol, 2007; 221:278–284. 2. Crinnion. Altern Med Rev, 2010; 15(3):190-196. 3. Darbre et al. J Appl Toxicol, 2004; 24:5-13. 4. Darbre and Harvey. J Applied Toxicol, 2008; 28(5):561-578.
Mercury Compounds	Amalgam-related lichenoid lesions[6] Anxiety[5] Depression[5] Immune suppression[4] Increase risk for thyroid disorders[3] Memory loss[5] Neuronal plasticity dysfunction[2]	1. Dufault et al. Behavioral and Brain Functions, 2009, 5:44 doi:10.1186/1744-9081-5-44. 2. Gallagher and Meliker. Environ Internl, 2013; 40:39–43. 3. Havarinasab and Hultman. Autoimmun Rev, 2005; 4:270– 275. 4. Heyer et al. Toxicologic Sci, 2004; 81:354–363. 5. Pigatto and Guzzi. Trends Immunol, 2009; 31(2):48-49.
Mixed Heavy Metals (Nickel, Cobalt, Cadmium, Lead, Arsenic)	Encephalopathy[3] Metal hypersensitivity[1,2,5] Increased risk of skin or lung cancer[3] Itai-itai disease[3] Lead encephalopathy[3] Metabolic disorders[7] Pneumoconiosis[4] Polyneuropathy[3] Proximal renal tubular damage[6]	1. Büdinger and Hertl. Allergy, 2000; 55:108-115. 2. Carey et al. Toxicologic Sci, 2006; 91(1):113–122. 3. Järup L. Br Med Bulletin, 2003; 68:167–182. 4. Seldén et al. Thorax, 1995; 50:769-772. 5. Thierse et al. J Immunology, 2004; 172:1926–1934. 6. WHO. Lead. Environmental Health Criteria, vol. 165. Geneva: World Health Organization,1995. 7. Xu et al. Particle Fibre Toxicol, 2012; 9:40.

Array 11 is one of the Environmental Triggers assessments available through the Cyrex System™. Depending on the result of Array 11, it may be logical to identify Increased Barrier Permeability (Arrays 2 and 20), or to assess Biomarkers of Autoimmune Reactivity (Arrays 5, 6, 7/7X). **Table 3** is a guide to potential follow up testing after Array 11.

Table 3. Based on Array 11 results and additional pertinent clinical data, these are suggested follow up testing for Array 11.

Positive Result	Consider Follow Up Array					
	2	3	5	6	7/7X	20
Aflatoxins	x	x	x		x	x
Formaldehyde + Glutaraldehyde	x		x		x	x
Isocyanate	x	x	x		x	x
Trimellitic + Phthalic Anhydrides	x		x		x	x
Benzene Ring Compounds	x	x	x		x	x
BPA Binding Protein	x		x		x	x
Bisphenol A	x		x	x	x	x
Tetrabromobisphenol A	x		x		x	x
Tetrachloroethylene	x		x		x	x
Parabens	x		x		x	x
Mercury Compounds	x		x		x	x
Mixed Heavy Metals	x		x		x	x

SPECIMEN REQUIREMENT

2 mL serum
Ambient

RELATED TESTING

- **Antibody Array 2 - Intestinal Antigenic Permeability Screen**
- **Antibody Array 3 – Wheat/Gluten Proteome Reactivity and Autoimmunity**
- **Antibody Array 5 – Systemic Autoimmune Reactivity Screen**
- **Antibody Array 6 - Diabetes Autoimmune Reactivity Screen**
- **Antibody Array 7/7X – Neurological Autoimmunity Reactivity Screen**
- **Antibody Array 20 – Blood Brain Barrier Permeability**

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157. http://www.epa.gov/airtoxics/hlthef/tet-ethy.html
158. http://www.health.ny.gov/environmental/chemicals/tetrachloroethene/
159. http://www.cdc.gov/biomonitoring/pdf/Parabens_FactSheet.pdf
160. http://www.epa.gov/ttnatw01/hlthef/mercury.html
161. http://www.epa.gov/airtoxics/hlthef/nickel.html
162. http://www.epa.gov/airtoxics/hlthef/cobalt.html

163. http://www.epa.gov/airtoxics/hlthef/cadmium.html
164. http://www.epa.gov/airtoxics/hlthef/lead.html
165. http://www.epa.gov/airtoxics/hlthef/arsenic.html

SUPPLEMENTAL INFORMATION:

As with many lab tests, prescription and OTC medications may interfere with the results of Array 11.

Many medications are hapten chemicals[10] themselves and therefore may cause interference with the hapten chemicals bound to tissue assessed in Array 11. Common medications that have been shown to interfere with Array 11, based on *in vitro* inhibition tests performed at Cyrex labs, are as follows:

- Tylenol® (acetaminophen)
- Aspirin (acetylsalicylic acid)
- Prozac® (fluoxetine)

These commonly consumed medications were assessed *in vitro*; Cyrex cannot know whether or not a specific patient's *in vivo* handling of medication will produce the same result as the *in vitro* study. Cyrex has not tested the exhaustive list of medications currently on the market for *in vitro* interference. **Consider the medications the patient currently uses, before drawing specimens for Array 11. As with all other Cyrex antibody arrays, antibody immunosuppressant medications, including corticosteroids, also interfere with test results.**

Medication Half-Life Information

Tylenol® (Acetaminophen)

Half-life elimination:

- Neonates: 7 hours (range: 4-10 hours)
- Infants: ~4 hours (range: 1-7 hours)
- Children: 3 hours (range: 2-5 hours)
- Adolescents: ~3 hours (range: 2-4 hours)
- Adults: ~2 hours (range: 2-3 hours); may be slightly prolonged in severe renal insufficiency (Cl_{cr}<30 mL/minute): 2-5.3 hours

Please note that this can be prolonged following toxic doses or individuals with certain backgrounds.

Time to peak, serum: Oral: Immediate release: 10-60 minutes (may be delayed in acute overdoses); I.V.: 15 minutes

Excretion: Urine (<5% unchanged; 60% to 80% as glucuronide metabolites; 20% to 30% as sulphate metabolites; ~8% cysteine and mercapturic acid metabolites

Aspirin (Acetylsalicylic Acid)

Half-life elimination:

- Parent drug: 15-20 minutes; Salicylates (dose dependent): 3 hours at lower doses (300-600 mg), 5-6 hours (after 1 g), 10 hours with higher doses

Time to peak, serum: ~1-2 hours

Excretion: Urine (75% as salicyluric acid, 10% as salicylic acid)

Prozac® (Fluoxetine)

Half-life elimination:

- Adults:
  Parent drug: 1-3 days (acute), 4-6 days (chronic), 7.6 days (cirrhosis)
  Metabolite (norfluoxetine): 9.3 days (range: 4-16 days), 12 days (cirrhosis)

Time to peak, serum: 6-8 hours

Excretion: Urine (10% as norfluoxetine, 2.5% to 5% as fluoxetine)

Array 11 – Recommended Clearance Schedule		
Medication	**Age**	**Collect Specimen After**
Tylenol® Acetaminophen	Neonates	20 hours
	Infants	14 hours
	Children	10 hours
	Adolescents	8 hours
	Adults	6-11 hours*
Aspirin Acetylsalicylic Acid	All	12-20 hours*
Prozac® Fluoxetine	Adults	6-24 days*

*see half-life specifics above

Using your favorite internet website (Drugs.com, RXlist.com, etc) or simply searching for the drug name along with "half-life," locate the specific medication's reported half-life. Pay attention to any additional caveats that can affect half-life. If additional caveats are not involved, double the half-life posted. This is the medication clearance schedule for collecting specimens. If additional caveats are involved, based on the information provided, determine the optimal time to collect your patient's specimen.