

Detoxification Profile

Overview One of the body's primary self-defense mechanisms is the conversion and neutralization of metabolic products and toxins into soluble and safe by-products which can then be eliminated. Many challenges to this system — a leaky gut, repeated exposure to food-borne toxic chemicals, environmental pollutants, bacterial endotoxins, and other substances — can increase the detoxification burden. This overload can lead to greater production of free radicals and damage to many body systems.

Assessing multiple path-ways with challenge substances provides clinical information about individuals with imbalanced detoxification.

The innovative Detoxification Profile from Genova Diagnostics assesses the body's capacity to carry out detoxification through functional challenges—caffeine, acetaminophen, and salicylate—which evaluate specific aspects of the detoxification process and free radical damage. These functional assessments provide a comprehensive profile of the body's detoxification capacity and potential susceptibility to oxidative damage.

Clinical relationships Long term exposure to environmental pollutants and continued affronts to the detoxifying systems may lead to oxidative stress, high levels of P-450 activity, and reduced capacity for Phase II conjugation reactions. This can result in accumulation of the toxic compounds, damage to essential fatty acids, impairment of oxidative phosphorylation, and reduced energy production.

Patients suffering from toxic burdens may experience a wide range of symptoms, among them fatigue and poor tolerance for exercise. These processes have been postulated to be a central factor in the development of Chronic Fatigue Syndrome (CFS). A recent study reported that many CFS patients had disordered liver detoxification ability and showed signs of increased toxic exposure.¹ Buist suggests that CFS may be a result of xenobiotic or toxin exposure.²

Bland recently reported the relationship between impaired detoxification capability, mitochondrial dysfunction and chronic fatigue. His results suggest that oxidative damage to mitochondria and the detoxification process itself is a fundamental mechanism in the development of CFS.³

Detoxification processes All ingested and microbially-produced toxins are presented to the first-pass clearance system. First-pass clearance involves the biotransformation and clearance of a chemical from the body before it reaches the systemic circulation. This clearance may take place in several organ tissues including the intestinal mucosal wall and the liver.

The liver is the body's primary detoxifying organ. Here, detoxification is carried out in two related processes known as Phase I and Phase II. Phase I serves to biotransform substances through oxidation, reduction or hydrolysis, using the cytochrome P450 mixed-function oxidase enzymes. This process increases the solubility of molecules and prepares them for Phase II reactions which will further increase their solubility.⁴⁻⁸

The Phase I reactions are necessary for detoxification, but the resulting production of reactive oxygen species can at times be very damaging. Thus, the liver needs to be able to generate oxidation capacity when needed, yet at the same time generate no more than what is needed. Perhaps this is why Phase I systems are inducible by different compounds.

In Phase II, conjugation reactions add a polar hydrophilic molecule to the metabolite or toxin, converting lipophilic substances to water-soluble forms for excretion and elimination.

What this test does:

Performs sensitive assessments of hepatic detoxification.

Physicians can determine capacities of specific pathways to develop more precise treatment.

Turn-around Time 7 days

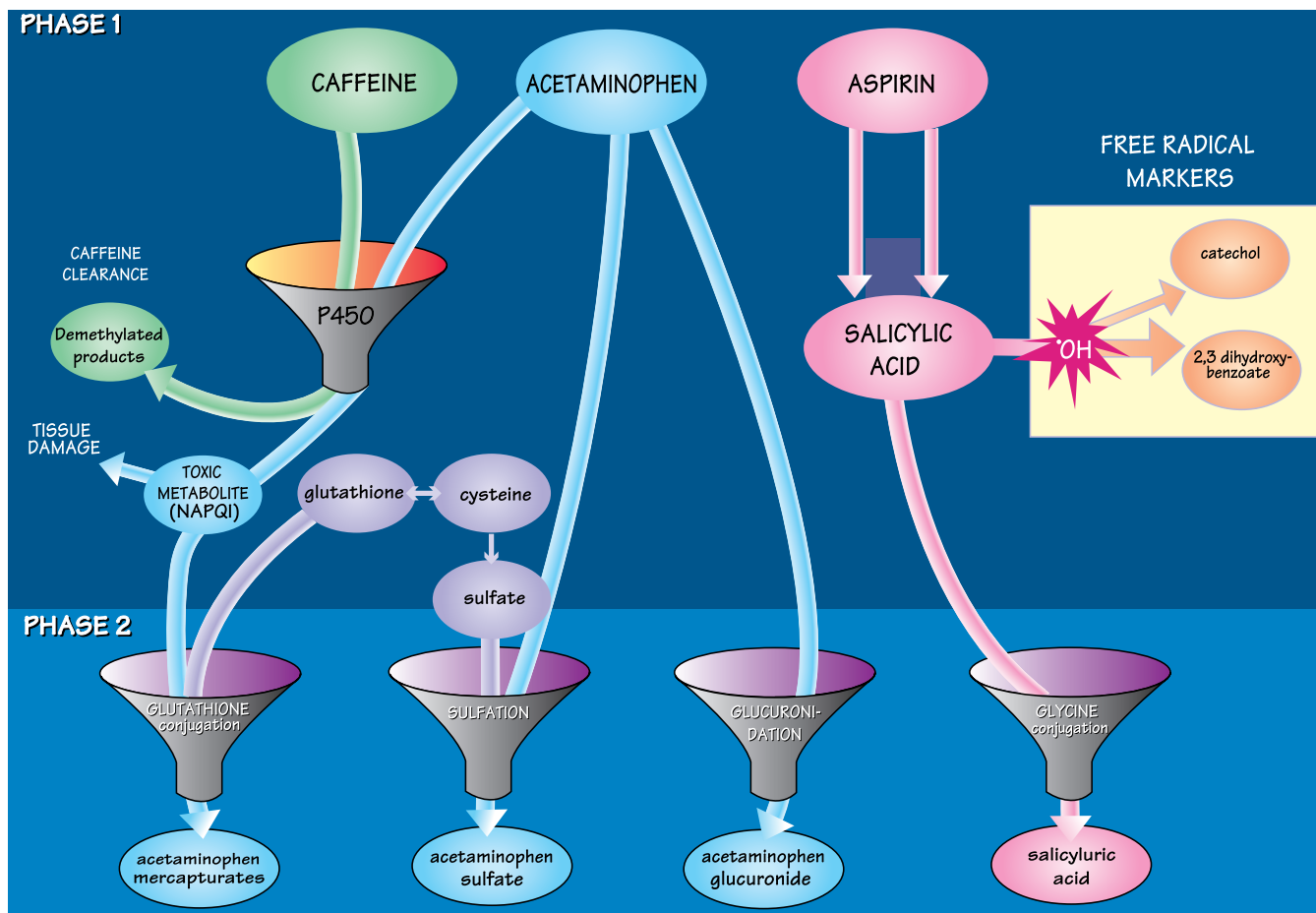


Figure 1

Phase II reactions may follow Phase I for some molecules or act directly on the toxin or metabolite. Major Phase II pathways include glutathione, sulfate, glycine, and glucuronide conjugations.⁹ Individual xenobiotics and metabolites usually follow one or two distinct pathways.

While the modification of Phase I and II enzyme activities has its basis in the research setting, there is growing appreciation of the clinical applications of such strategies. Although exhaustive clinical studies have yet to be performed, we have the biochemical and logical basis upon which to recommend interventions in order to help patients with evidence of chemical sensitivity or high exposures to toxic compounds.¹⁰⁻¹²

It should be noted, however, that nutritional modification of the P-450 and/or conjugation pathways has strong potential to change drug metabolism. Due to this potential metabolic impact, practitioners should use caution and awareness when recommending such strategies in patients taking prescription medications.¹³

Assessment of the metabolic status of these major detoxification processes assists with our understanding of the body's capacity to detoxify foreign substances. (See Figure 1)⁴⁻⁸

P-450 Phase I oxidation

The family of P-450 enzyme systems is quite diverse, with specific enzyme systems being inducible by particular drugs or metabolites.⁹ Caffeine is a substance capable of testing a number of P-450 systems simultaneously.¹⁴

Measurement of salivary caffeine clearance provides a noninvasive procedure for quantifying hepatic microsomal function, as caffeine is almost completely absorbed by the intestine and is metabolized in the liver by P-450 enzymes.¹⁵ Levels are affected only slightly by the presence of liver disease, although they are substantially

Substrates of cytochrome P-450 enzymes

CYP1A2
Theophylline, caffeine, phenacetin, acetaminophen

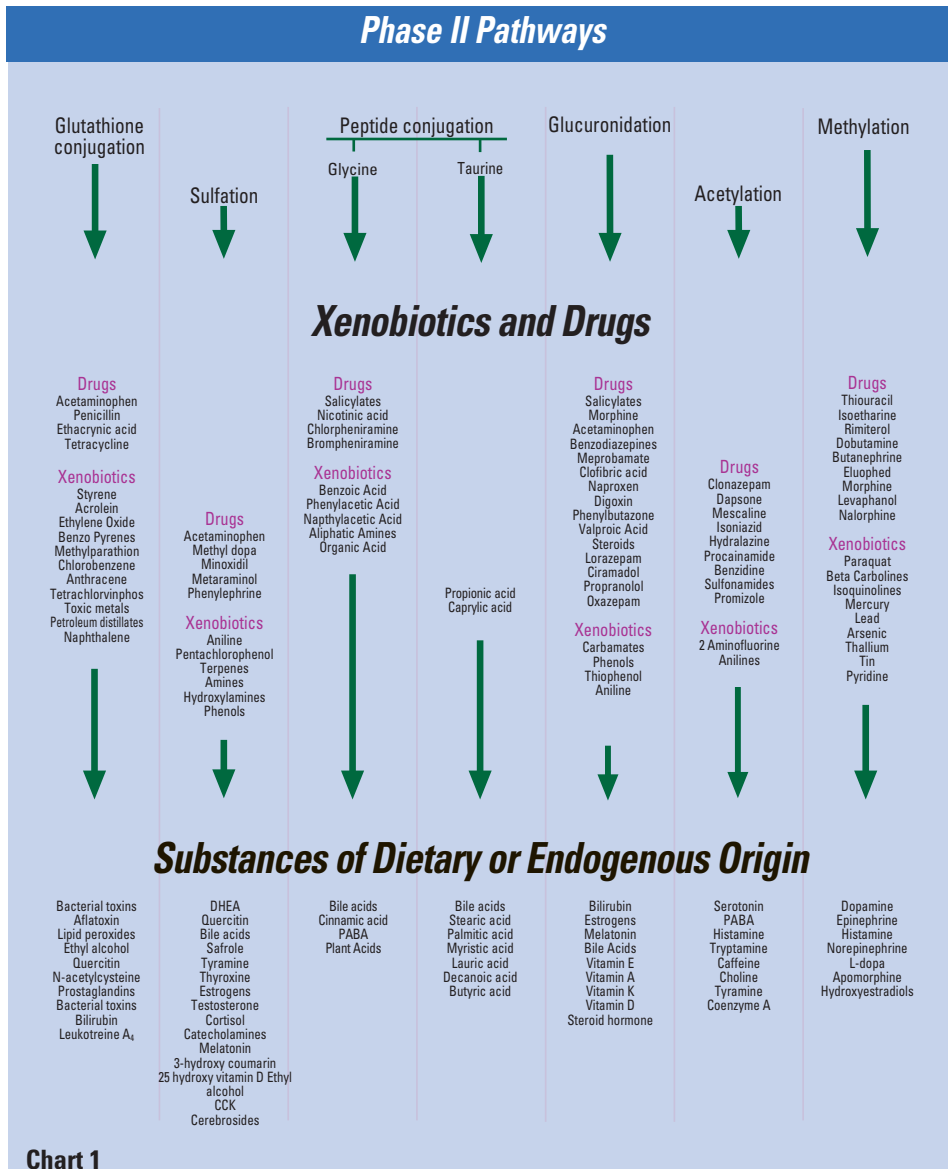
CYP2D6
Cardiology: Alprenolol, bopindolol, carvedilol, metoprolol, propranolol
Psychiatry: Amitriptyline, clomipramine, desipramine, nortriptyline
Others: Codeine, dextromethorphan, ethylmorphine, 4-methoxyamphetamine

CYP2C Family
Phenytoin, ibuprofen, naproxen, oxycam drugs, S-warfarin, Diazepam, hexobarbitone, imipramine, omeprazole

CYP2E1
Acetaminophen, caffeine, alcohol, chlorzoxazone, enflurane

CYP3A
Lidocaine, erythromycin, cyclosporin, ketoconazole, testosterone, estradiol, cortisone

Table 1



reduced in cirrhotic patients.^{15,16} P-450 activity and caffeine clearance is reported to be upregulated by smoking.^{16,17} A variety of drugs and xenobiotics are oxidized by the P-450 system (Table 1). Thus, this system plays a crucial role in the detoxification and removal of many potentially toxic substances.

Phase II pathways

Salicylates and benzoate are detoxified primarily through glycation. Many other substances are detoxified as well via the glycine conjugation pathway. Patients suffering from xenobiotic overloads and environmental toxicity may not have sufficient glycine reserves for the metabolic load.

Benzoate is present in many food substances and is widely used as a food preservative. It has been employed in studies of glycation, but one of the difficulties in assessing benzoate conjugation is the presence of endogenous levels of benzoate from food and gut sources.¹⁸

Aspirin is readily degraded into salicylic acid and is potentially a better indicator molecule. Although salicylate is conjugated with glycine, similar to benzoate, it also is converted to glucuronide derivatives and three oxidized derivatives (Chart 1). The slower glucuronidation pathway relative to glycine conjugation makes aspirin a more sensitive challenge to the glycine conjugation capability, while the oxidized derivatives allow unique insights into free radical status.¹⁹

Sulfate conjugation

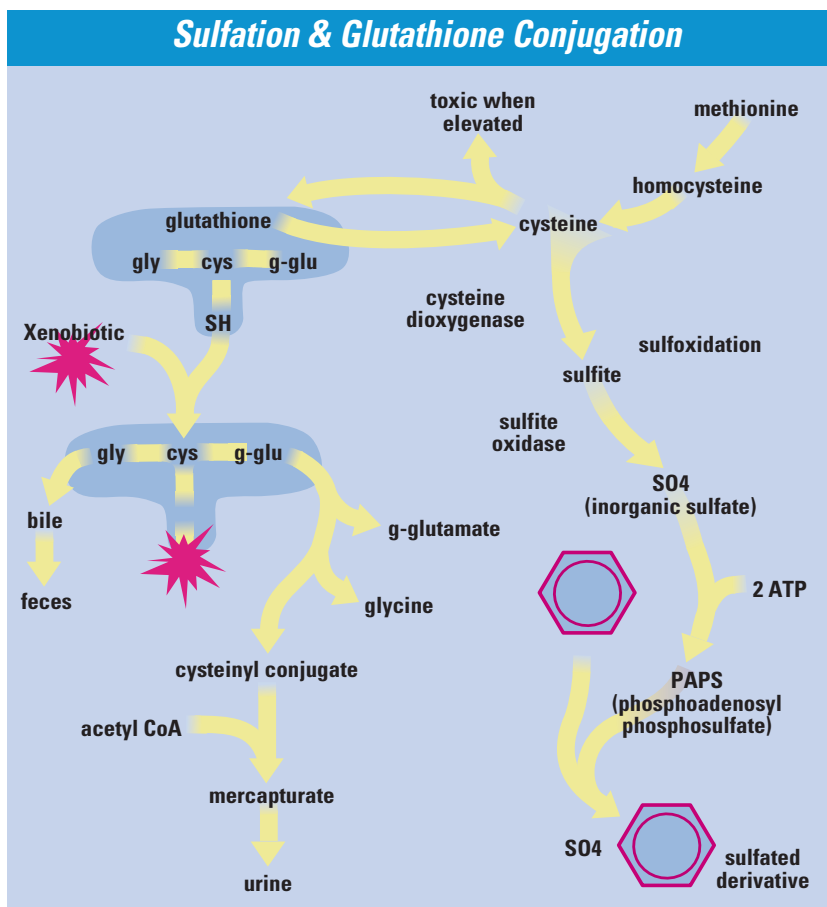
Neurotransmitters, steroid hormones, certain drugs, and many xenobiotic and phenolic compounds employ sulfation as their primary route of detoxification.²⁰ Acetaminophen (also known as paracetamol) is an example of a molecule detoxified primarily through the sulfation pathway. Because acetaminophen is widely available, intensely researched, and safe under most conditions, Genova Diagnostics employs it to evaluate the sulfate conjugation pathway.

Steventon identified many poor sulfoxidizers with diminished acetaminophen sulfate conjugation and reported that these individuals are most susceptible to environmental illness and problems of the nervous system, including Parkinson's disease and motor neuron disease.^{21,22} Acetaminophen sulfate conjugation has also been shown to be reduced in patients with rheumatoid arthritis.²³

Inorganic sulfate for the sulfation reactions may come directly from dietary sources or indirectly through sulfoxidation reactions (Figure 2). Significant amounts of sulfate precursors may be provided by direct absorption from ingested foods. Methionine and cysteine are probably the most important sources of inorganic sulfate in mammals.²⁴

Glutathione is a reservoir of cysteine, so it also may be an important source of inorganic sulfate. Inorganic sulfate is activated with two ATP molecules, producing phosphoadenosyl phosphosulfate (PAPS). It is this PAPS molecule that then reacts with exotoxins to produce sulfated derivatives. The availability of free sulfate may be the rate-limiting step for these reactions.

Some individuals accumulate cysteine and have low sulfate levels. In patients with neurologic disorders such as Alzheimer's disease, Parkinson's disease, and motor neuron defect, it appears that the enzyme responsible for sulfoxidation of cysteine to sulfate is often deficient, leading to an elevated plasma cysteine/sulfate ratio.²⁵



The clinical significance of this is profound. Most people with deficient sulfate and xenobiotic loads will benefit from cysteine and glutathione supplementation. Individuals with elevated plasma cysteine/sulfate ratios, however, may worsen with cysteine supplementation and require inorganic sulfate as a supplement, in order to bypass this sulfoxidation step.

Molybdenum is a cofactor for sulfite oxidase, one of the enzymes involved in sulfoxidation. Studies indicate that insufficient molybdenum may be a rate-limiting nutrient for this reaction, and supplementation may be indicated.

On the other hand, molybdenum, at high levels, can compete with sulfate in its activation step to PAPS as well as for carrier mediated renal tubular reabsorption, thus lowering sulfate levels and impairing sulfation capability. Molybdenum status is therefore important in the assessment of poor sulfation capability.²⁶

It is worth noting that standard treatments for acetaminophen overdose are cysteine or the glutathione precursor, N-acetylcysteine (NAC).²⁷ These compounds, therefore, are likely candidates for nutritional supplementation.²⁸

Figure 2

Glucuronidation Because of the ready availability of UDP-glucuronate in vivo, glucuronidation is considered an important detoxification mechanism when sulfation or glycation is diminished or saturated.²⁹ For most individuals, glucuronidation is a supplemental pathway. The kinetics of glucuronidation cause it to be a secondary, slower process than sulfation or glycation. Glucuronidation appears to be enhanced in obese patients, with the capacity for glucuronidation related almost linearly to total body weight.³⁰ Thus, obese people have enhanced capacity for detoxification of molecules that utilize this pathway.

As free radical stress can lead to damage of the mitochondrial oxidative phosphorylation mechanism, it is reasonable to hypothesize that people suffering from oxidative stress may have diminished capacity for Phase II glucuronide conjugation. An interesting example of decreased glucuronidation occurs in subjects with Gilbert's syndrome. It is caused by diminished bilirubin glucuronosyl transferase activity, leading to accumulation of bilirubin in vivo.²⁹

Detoxification-intestinal permeability relationship

The intestinal mucosa is the primary barrier to permeation of toxic compounds and macromolecules. Abnormalities of the intestinal barrier system as detected by intestinal permeability assessment may lead to enhanced uptake of inflammatory luminal macromolecules, endotoxins and xenobiotics. Impairment of intestinal integrity dramatically increases mucosal absorption of substances that are normally excluded. These foreign chemicals are presented to the liver's detoxifying system for processing and elimination. They can stress the detoxification capability of the liver or be partially processed and accumulate in the liver and adipose tissue. It has been speculated that the combination of leaky gut and dysfunctional liver detoxification can lead to increased tissue stores of toxic compounds and depressed immune status.

Using the Detoxification Profile

In the Detoxification Profile, one caffeine caplet (200 mg) is taken in the morning and its clearance is assessed from two salivary specimens collected two and eight hours after ingestion.

Aspirin and acetaminophen are ingested in the evening and the products of detoxifying reactions are assessed in a 10-hour overnight urine specimen. The challenge dose consists of two capsules of aspirin (650 mg total) and two capsules of acetaminophen (650 mg total). The only side effect is potential drowsiness.

In the Comprehensive version of this profile, the urine specimen is analyzed for levels of lipid peroxides. In addition, glutathione, glutathione peroxidase, superoxide dismutase, plasma cysteine, and plasma sulfate are assessed from fasting blood specimens.

Interpreting the Detoxification Profile

For more detailed information, refer to our Detoxification Profile Interpretive Guidelines.

Low caffeine clearance (Phase I): Indicates slow P-450 enzyme activity and metabolic detoxification difficulty; may also reflect use of medications such as amphetamines, cimetidine, and oral contraceptives.

High caffeine clearance (Phase I): Reflects excessive P-450 enzyme induction, possibly due to toxin exposure; also implies greater production of free radicals.

Low acetaminophen mercapturate, salicylic acid, acetaminophen sulfate or acetaminophen glucuronide (Phase II): Indicate inadequate Phase II conjugation reactions. Low levels may reflect depletion of the particular amino acids or nutrient cofactors used in the reactions, or diminished enzymatic capacity for conjugation.

Elevated Phase I/Phase II ratios: May reflect elevated (induced) Phase I processes or diminished Phase II conjugation reactions. The ratio of Phase I to Phase II detoxification processes is important in determining the toxicity of certain drugs, and these ratios may be significant indicators of the balance of biological processes.

Substances which may induce P-450 enzymes

- Acetate
- Alcohol
- Barbiturates
- Carbon Tetrachloride
- Charcoal-broiled meats
- Dioxin
- Exhaust fumes
- High protein diets
- Niacin
- Oranges
- Organophosphorus pesticides
- Paint fumes
- Riboflavin
- Sassafras
- Saturated fats
- Steroid hormones
- Sulfonamides
- Tangerines

The following markers are included in the comprehensive version of the Detoxification Profile. For more detailed information on the oxidative stress markers, refer to our Oxidative Stress Interpretive Guidelines.

Elevated plasma cysteine/sulfate ratio: Suggests possible impairment of the sulfoxidation reaction that converts cysteine to the inorganic sulfate required for sulfation. Elevated ratios have been noted in neurological disorders such as Parkinson's disease, Alzheimer's, and motor neuron disease.

Elevated plasma cysteine: Indicates sulfoxidation impairment, other blocks in cysteine metabolism, excess intake of cysteine and related molecules, or excessive catabolism. Checking the levels of plasma sulfate and glutathione provides further information.

Depressed plasma sulfate: Suggests sulfoxidation impairment, especially when plasma cysteine is elevated. Organic sulfate precursors such as L-cysteine, N-acetyl cysteine, or glutathione may be contraindicated in these cases.

Low glutathione: Suggests low amount of glutathione available for removal of toxic intermediates, generation of cysteine and sulfate reserves, and antioxidant activity.

Low glutathione peroxidase (GSH-Px): Implies inadequate defense against accumulation of oxidized lipids in cell membranes. Low levels are found in conditions such as Down's syndrome, Alzheimer's dementia, and beta-thalassemia minor and may indicate insufficient nutrient cofactors.

High/low superoxide dismutase (SOD): Decreased in conditions associated with inflammation, impaired glucose metabolism, and zinc deficiency. High levels occur in systemic sclerosis, myositis and malignant melanoma, and may also indicate exposure to agricultural pesticides.

Elevated Urine Lipid Peroxides: Suggest increased cellular lipid peroxidation and the need for antioxidant protection of body lipids.

High Hydroxyl Radicals: Indicate the potential for oxidative damage, such as that occurring in the pathogenesis of diabetes and other illnesses. May also reflect the presence of an inflammatory process.

How do I order this test?

For Detoxification Profile kits or information, please call a Client Services representative at 800-522-4762 or order online at www.GDX.net.

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Detoxification Profile Application Guide



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