CLINICAL & FORENSIC

# Toxicology News

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## Abused Inhalants Testing
### *Diverse Substances and Complex Procedures Challenge Laboratories*

*By Michael Wagner, PhD, Kim Manlove, and Thomas Andrew, MD*

Inhalants continue to present significant health risks—but in a new twist, more adults are engaging in this behavior once thought to be predominantly adolescent in nature.

More than 1 million Americans 18 and older acknowledge using inhalants. In fact, this age group accounted for more than 54% of inhalant-related admissions for treatment in 2008. Among these adult admissions, the largest proportion was 18- to 29-year-olds at 52%, followed 30- to 44-year-olds at 32%, and those 45 and older at 16%. The data in Table 1 demonstrates that inhalants have surpassed other commonly abused drugs in the number of adult users (1,2).

In terms of demographics, adults admitted for treatment were primarily non-Hispanic whites (72%) and males (72%). Some 38% lacked a high school education. Other ethnic groups included Hispanic, 11%; American Indian, 9%; and non-Hispanic black, 6%.

One million children age 12 to 17 also abuse inhalants each year (3). According to some estimates, one in four American students has used a household product to get high prior to reaching the eighth grade (4). Inhalants are the gateway drug for these children, with some beginning before ten years of age. By age 12, 59% of children know of someone who has abused inhalants. Inhalants are the fourth most commonly abused substances, behind alcohol, tobacco, and marijuana.

Rates of use among adolescents vary little by age, ranging from 4.7% for 14- and 15-year-olds to 3.5% among 16- and 17-year-olds. Table 2 shows that use is fairly evenly distributed among ethnic groups, with American Indians/Alaska Natives highest at 5.5% and African-Americans lowest at 2.5% (3). These rates differ from the adult demographic distribution inferred from treatment admissions referenced above.

A few of the street terms for inhalants and their abuse include huffing, air blast, bagging, bang, highball, poor man's pot, black hole, poppers, and glading. The terms reflect the diverse substances used. Some 1000 common household products are available for huffing abuse. Table 3 lists a few of the more commonly abused inhalants and their constituents (5).

The huffer may use medical grade gases (typically associated with workplace abuse by a medical professional), various propellant products sold over the counter, or rags soaked in the inhalant or in a mixture with a solvent such as gasoline.

#### A Plethora of Effects

The effects of inhalants vary dramatically. Symptoms of intoxication range from mild euphoria similar to low-dose alcohol impairment to intense euphoric presentations involving vivid hallucinations. Dose and chemical properties play roles in the various types of responses that subjects experience. Many factors contribute to the diversity of effects, including the large variety of compounds inhaled and the subjects' own co-morbidities. Even so, there are general pharmacological observations associated with inhalant abuse.

Inhalants generally act as central nervous system depressants, and like alcohol, act on the GABAnergic (gamma-aminobutyric acid) system, although the exact modes of action are unknown. Several abused solvents appear to target N-methyl-

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## Inside...

# Inhalants Testing

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D-aspartate, glycine, nicotine, and 5-$HT_3$ receptors, with evidence from animals suggesting the involvement of other receptor subtypes and nerve membrane ion channels. Finally, some inhalants interact at the cellular level, solvating the hydrophobic membrane and disrupting membrane potentials in a way that changes the membrane's permeability and increases the cell's susceptibility to oxidative stress (6).

Symptoms occur both centrally and systemically. They include horizontal gaze nystagmus, vertical nystagmus (at high doses), lack of convergence, slow pupil reaction, blurred vision, altered blood pressure (up or down), elevated pulse rate, psychomotor reduction, lack of coordination, dizziness, unsteady gait, depressed reflexes, general muscle weakness and lethargy, confusion, slurred speech, wheezing, mucous membrane irritation (sneezing, coughing, and excessive salivation), conjunctival injection, nose bleeds, and rhinorrhea (7,8,9).

The behavior of users can be as diverse as the various compounds abused. Users may get high alone or with others (10). Criteria for identifying dependence include the presence of three or more of these symptoms: tolerance, withdrawal, increase in dosage, repeated attempts to quit, significant time devoted to obtaining the drug or recovering from its use, and persistent use despite physical or psychological problems. The withdrawal symptoms from inhalant abuse are somewhat similar to those from cocaine, including hypersomnia, feeling weak and tired, depressed mood, elevated heart rate, and nausea (11).

### Dangerous Complications

Recent evidence indicates that inhalants cause changes in brain tissue. Brain imaging techniques show that they can cause thinning of the corpus callosum (a band of nerve fibers joining the cerebral hemispheres) and lesions in the white matter involved in brain cell communication. Other potential problems include leukoencephalopathy with multifocal myelin loss and birefringent inclusions (8,12).

These neuropathic changes are not exclusive to inhalant abuse; ethanol use can lead to similar problems. The demyelination that occurs seems to differ from that caused by other demyelinating diseases, such as multiple sclerosis and Guillain-Barre syndrome.

Other organs that can be damaged, depending on the parent inhalant and its metabolites, include the lungs, heart, liver, and kidney. Bone mineral loss, bone marrow suppression, and compromised immunity are all linked to inhalant abuse (13,14).

**Table 1. Adult Drug Use Estimates**

| | |
|---|---|
| Inhalants | 1,100,000 |
| Crack cocaine | 988,000 |
| LSD | 637,000 |
| Heroin | 571,000 |
| PCP | 75,000 |

Source: Reference 1

**Table 2. Adolescent Inhalant Use**

| | |
|---|---|
| American Indian or Alaska Native | 5.5% |
| Hispanic or Latino | 4.6% |
| White | 4.4% |
| Asian | 2.7% |
| Black or African American | 2.5% |

Source: Reference 3

Pulmonary dysfunctions linked to inhalant use include asthma, bronchitis, pneumonia, and sinusitis. Adolescents who abuse inhalants face an increased risk of developing a respiratory illness (4). An interesting aspect of this effect is that, although African Americans abuse inhalants at the lowest rates, they face the greatest risk for developing respiratory illnesses. The reason for this is not known, but environmental, genetic, and immunological factors could play a role.

Fatal cardiac arrest is common enough to be referred to as "sudden sniffing death syndrome." Vagal inhibition can lead to arrhythmias and also cause sudden death. Some inhalants can sensitize the myocardium to catecholamines, leading to hypoxia and additional risks for other types of arrhythmias and vascular tissue damage.

### Metabolic Markers

Although inhalants bypass first metabolism because their initial contact is with the cardiopulmonary system, the liver converts them to their primary metabolites via phase I and II reactions. Many metabolites contribute to additional tissue toxicity and long-term damage. This review will focus on the general features of a few of the many chemical compounds involved.

Inhalants fall into several general categories, including aliphatic hydrocarbons, aromatic hydrocarbons, halogenated hydrocarbons, and nitrites. Aliphatic hydrocarbons, such as hexane, produce three metabolites (2-hexanol, 2-hexanone, and 2,5-hexanedione) that involve liver enzymes like CYP P450 3A4 and 2E1. Hexan-2-ol-glucuronide can be detected in urine as a metabolic marker of hexane exposure. The metabolite 2,5-hexanedione is associated with toxic effects (15).

Aromatic hydrocarbons are common chemical agents found in inhalants. Xylene, toluene, and benzene are just a few examples with known toxic effects. These compounds are metabolized via complex routes, and their toxic metabolites offer opportunities for detection. For example, benzene metabolism is thought to be associated predominantly with CYP P450 2E1, and its phase I and phase II metabolites are numerous (16).

The benzene oxepin and oxide intermediates are in equilibrium with each other and undergo a nonenzymatic rearrangement to phenol. Other phase I metabolites, such as o/p-benzoquinone and 1,2,4-trihydroxybenzene, are precursors for phase II metabolism. These metabolites are mostly excreted as urine conjugates of glucuronide and sulphate, including phenylmercapturic acid and trans-trans muconic acid (1,3 butadiene-1,4 dicarboxylic acid). These two urine metabolites are the main markers of benzene exposure (17).

Xylene and toluene are metabolized by CYP P450 2E1; however, toluene metabolism involves additional liver enzymes (CYP P450 1A2 and 2B6). The primary metabolite of xylene used as a biomarker is the phase II-generated methylbenzoic acid glycine adduct, methyl hippuric acid. Toluene produces a number of minor glutathionated adducts, but the major product is hippuric acid (17).

Halogenated hydrocarbons can contain a number of different halothanes, ranging from trifluoroethanes and difluoroethanes to brominated compounds. Liver metabolism studies seem to indicate that CYP 2E1 and CYP 2A6 are involved in the oxidation of these compounds (phase I) to alcohol and acetic acid products. For example, trichloroethylene and 1,1,1-trichloroethane convert to 2,2,2-trichloroethanol and trichloroacetic acid and can be detected in blood and urine. The half-lives of these compounds are 12 hours and 100 hours, respectively (18).

Alkyl nitrites, which are generally referred to as poppers, are inhaled rapidly, with the effects felt within seconds. Metabolism is rapid, most likely through hydrolyzing enzymes in the liver, kidney, lungs, and vascular tissue. Hepatic glutathione-organic nitrate reductase appears to be the major route. The metabolites formed are the corresponding alcohols—for example, butyl nitrite converts to butyl alcohol and pentyl nitrite converts to pentyl alcohol. Typically, the nitrites and corresponding alcohols can be detected in urine. In addition, methemoglobin can be used to monitor exposure.

## Comprehensive Testing Required

Testing patients suspected of inhalant exposure requires a comprehensive approach because the sub-

**Table 3. Agents in Commonly Abused Inhalants**

| Category | Agents |
|---|---|
| **Glues and Adhesives** | |
| Airplane glue | Toluene, ethyl acetate |
| Rubber cements | Hexane, toluene, methyl chloride, acetone, methyl-ethyl-ketone, methyl butyl ketone, benzene, xylene, trichloroethylene, tetrachloroethylene, chloroform |
| **Aerosols** | |
| Spray paint | Butane, propane, fluorocarbons, toluene, hydrocarbons, xylene |
| Hair spray and deodorants | Butane, propane, chlorofluorocarbons |
| Analgesic spray | Chlorofluorocarbons |
| Asthma spray | Chlorofluorocarbons |
| Fabric spray | Butane, trichloroethane |
| Computer cleaner | Dimethyl ether, hydrofluorocarbons |
| Video head cleaner | Ethyl chloride |
| **Anesthetics** | |
| Gaseous | Nitrous oxide |
| Liquid | Haloethane, enflurane, desflurane, isoflurane |
| Local | Ethyl chloride |
| **Food** | |
| Whipped cream | Nitrous oxide |
| **Cleaning Products** | |
| Dry cleaner and degreaser | Tetrachloroethylene, trichloroethane, trichloroethylene |
| Spot remover | Xylene, petroleum distillates, chlorohydrocarbons |
| Lacquer, thinner | Acetone, methanol, ethyl acetate, methyl chloride, toluene |
| Paint remover | Toluene, methylene chloride, methanol, acetone, ethyl acetate |
| Paint thinner | Petroleum distillates, esters, acetone |
| **Solvents and Gases** | |
| Nail polish remover | Acetone, ethyl acetate, methyl methacrylate, toluene (rarely) |
| Correction fluid | Trichloroethylene, trichloroethane, isoparaffins |
| Fuel gas | Butane, isopropane |
| Lighter fluid | Butane, isopropane |
| Fire extinguisher | Bromochlorodifluoromethane |
| Gasoline | Benzene, n-hexane, toluene, xylene, petroleum distillates |
| Poppers | Amyl nitrite, butyl nitrite, cyclohexyl nitrite, isobutyl nitrite, isopropyl nitrite |

Source: Reference 5

stances can have wide-ranging effects. To support a detailed medical history and clinical evaluation, blood tests should include electrolyte (sodium and potassium) levels, liver function tests, BUN levels, creatinine concentrations, cholesterol levels, and anion gap. Comprehensive blood testing, incorporating red blood cell counts with differential and platelet counts, complements these tests. Urine tests should include hippuric acid, drug screening, rapid plasma regain testing, thyroid hormones, creatine kinase, and heavy metals.

Forensic analyses for inhalants require sophisticated testing capabilities. As Table 3 illustrates, inhalants include a variety of compounds that could challenge a laboratory's ability. Immunoassays cannot detect these compounds. Detection requires hybridized chromatographic techniques, such as gas chromatographs fitted with flame ionization detectors, electron capture detectors, ultraviolet detectors, or mass spectrometers. High performance liquid chromatography-ultraviolet/visible photo diode array or liquid chromatography-tandem mass spectrometry can provide additional help in detecting biomarkers (19,20).

Special sample preparation can improve the performance of these hybridized techniques. The analysis of nonpolar, low-molecular-weight, volatile compounds requires temperature control during sample collection and preparation. Extracting these compounds from biological matrices requires careful consideration, including the use of techniques without organic solvents because solvents can generate false-positive results. Gas chromatograph-heated head space and solid-phase microextraction are two techniques often used (21). To increase sample stability, collections should use gray-top tubes containing fluoride and oxylate. The tubes should be filled to minimize head space and transported with cold packs.

## Case Report: Cardiac Arrest

The following case report illustrates the complexity of these cases. A 16-year-old male had a three-year history of alcohol and cannabis abuse. His family environment consisted of supportive parents and a sibling. The subject indicated that his drug counseling was not effective. His treatment facility's standard compliance testing drug menu did not screen for inhalants, so he evidently knew he could use them without being detected.

On the day of his death, he purchased a computer duster containing 1,1-difluoroethane. Witnesses saw him inhaling the propellant then diving and submerging his body repeatedly in a friend's pool. He claimed that he discovered this technique on the Internet as a way to enhance the inhalant high. After submerging four times, he did not resurface.

Friends rescued him from the pool. Paramedics and emergency room physicians attempted advanced life-support measures without success. A postmortem evaluation revealed that he died of cardiac arrest associated with "sudden huffing syndrome." His parents indicated that they had recently voiced concerns to him about this type of abuse.

This case demonstrates how complicated drug addiction treatment can be and to what extent patients may go to hide their addiction. Comprehensive drug screening and confirmation may provide treatment centers with more information about a patient's status of true abstinence. Clinical and forensic testing should be considered as an adjunct to other treatment options. Analytical costs can be prohibitive if the abused agent is unknown, thus it is imperative that a thorough investigation narrow down the scope of the testing menu. This case is unusual because witnesses could give detailed accounts of the events. Otherwise, the death may have been mistaken for a drowning.

## Clues at the Scene

Because the pathology results may be unremarkable, death-scene investigations involving inhalant abuse often require the investigator to draw clues from the surroundings as well as interview relatives and friends. These clues include chemical odors on the breath or clothing; paint or other stains on the face, hands, or clothes; empty spray paint or solvent containers and chemical-soaked rags and clothing; frostbite around the mouth, lips, or cheeks; and perioral or perinasal dermatitis. If the scene reveals no such hints, the true cause of death may not be discovered (8).

The tremendous number of compounds that can be inhaled and the complex instruments and procedures needed to identify them make comprehensive inhalant analyses a daunting challenge. In addition, we lack knowledge of the pharmacodynamics, pharmacokinetics, toxicokinetics, and general toxicology of many of them. Despite these difficulties, advances in laboratory technology and science offer hope for future improvements.

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# Alcohol Abuse

## *How Useful Are Fingernails for Monitoring Long-term Exposure?*

*By Joseph T. Jones, MS, NRCC-TC*

Alcohol abuse remains a significant public health issue worldwide, with rates of alcohol use disorder, risky drinking, maternal drinking, and underage drinking contining to rise (1). Alcohol abuse contributes to a number of public health issues, such as traffic accidents, liver disease, heart disease, cerebrovascular disease, and cancer (2). Drinking during pregnancy can lead to fetal alcohol spectrum of disorders, which is the leading cause of mental retardation in the U.S. and the only cause that is completely preventable (3). Alcohol use disorder affects an estimated 18 million Americans, compared with 4.2 million people for other drug use disorders (4). Because of this impact, healthcare professionals need objective tools to identify and monitor patients who have issues with excessive alcohol consumption.

### Historical Measures

Historically, healthcare professionals have monitored patients for excessive ethanol consumption using various self-reporting questionnaires, the direct measurement of ethanol, and indirect alcohol biomarkers. Self-reporting tools, such as the Alcohol Use Disorder Identification Test, CAGE questions, and Michigan Alcohol Screening Test, however, have limited utility in the clinical setting because of patient deception and the stigma attached to alcohol abuse.

The usefulness of direct measurement of ethanol in breath, blood, urine, and oral fluid also is limited by its short window of detection: about one hour per drink. On the other hand, indirect alcohol biomarkers, such as carbohydrate deficient transferrin, gamma-glutamyl transferase, and mean corpuscular volume, have limited sensitivity and specificity, especially in the presence of conditions such as pregnancy, cancer, or liver disease.

Recently, ethyl glucuronide (EtG), ethyl sulfate (EtS), and phosphatidylethanol (PEth) have come into use as long-term direct alcohol biomarkers. Substance abuse treatment programs, including drug courts, professional health programs, and residential treatment settings, routinely perform urine EtG and EtS to monitor compliance. The markers offer a window of detection in urine of two to five days following alcohol consumption. The tests are sensitive enough to pick up a single drink a day later, but incidental exposure to ethanol in food, medicine, and personal hygiene products can trigger a positive result.

Another method relies on measuring phosphatidylethanol, an abnormal phospholipid that forms during periods of elevated blood ethanol concentrations. It remains in the phospholipid membranes of the blood cells, decomposing with a half-life of 4.5 days. It can be detected up to three weeks after chronic heavy drinking. There is no evidence that it is detectable as a result of social drinking.

### New Specimen Type: Fingernail

Although hair testing for EtG has gained popularity in family court and addiction treatment settings because it has a three-month detection window, growing evidence indicates that the newest long-term alcohol biomarker assay to become commercially available—for EtG in fingernail—could be better.

Fingernail has been considered an alternative or backup specimen for individuals lacking sufficient hair to test. Made of keratinized protein very similar to hair, nail is porous and entraps compounds within its structure.

Figure 1 shows the four anatomical features relevant to this discussion: the germinal matrix, the nail plate, the nail bed, and the free edge. The nail grows outward toward the fingertip from its origin at the germinal matrix, where compounds may be incorporated. The hardened material forming the nail plate grows across the nail bed, which is rich in capillary blood flow and gives fingernail its pinkish

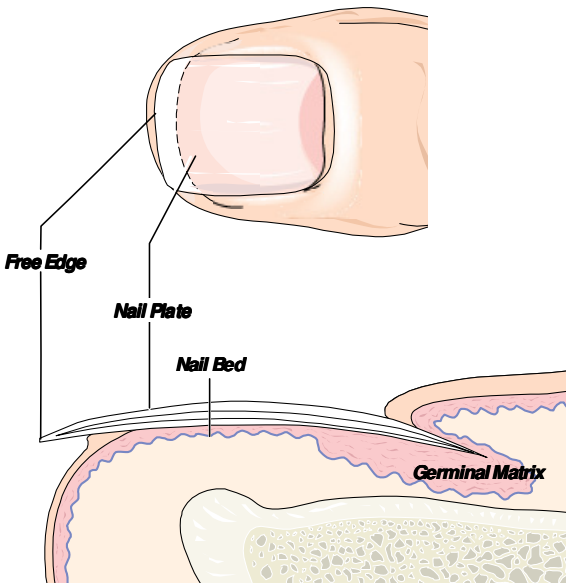


**Figure 1. Fingernail Anatomy**
Source: Blamb/Shutterstock.com; used with permission.

color. Material joins from underneath to thicken the nail as it grows toward the fingertip (5).

The nail plate continues to grow past the nail bed toward the fingertip, forming the part that can be clipped, called the free edge. The process of growing from germinal matrix to free edge takes up to six months, depending on a person's health.

## Fingernail Validation

Morini et al. published the first fully validated liquid chromatography-tandem mass spectrometry method for the detection of EtG in fingernail clippings in 2012 (6). They analyzed the nails of 15 subjects who had provided their drinking history using a test with a limit of quantitation of 10 pg/mg.

The researchers found no EtG in the nails of 10 subjects who reported consuming less one alcoholic drink per day. The nails of two subjects who consumed an average of one to two drinks per day contained 12.3 pg/mg and 18.7 pg/mg of EtG. Two subjects who drank two to four drinks per day had 44.1 pg/mg and 84.3 pg/mg. The single self-reported heavy drinker (more than four drinks per day) had 92.6 pg/mg of EtG in his fingernail clippings.

A more recent, larger study suggests that nail might be preferred as a specimen over hair because of gender differences in EtG hair test results (7). Jones et al. obtained extensive self-reported, 90-day histories of alcohol consumption from 606 college students and analyzed matched pairs of hair and fingernail specimens for the presence of EtG. The concentrations of EtG in the nails of both genders and the EtG concentration in the male hair were strongly associated with their drinking histories (Table 1). The concentrations of EtG in female hair were only weakly associated with their drinking histories. Morini et al. previously reported that various cosmetic hair treatments interfered with the detection of EtG, which could explain this difference between genders (6).

**Table 1. EtG Concentration Correlations by Gender**

| Specimen type | Gender | Pearson Correlation Coefficient |
|---|---|---|
| Nail | Male | 0.6402 |
| Nail | Female | 0.5152 |
| Hair | Male | 0.5562 |
| Hair | Female | 0.2777 |

Specimens from male and female fingernails and male hair correlated with the subjects' 90-day alcohol consumption histories. Female hair specimens correlated weakly with their histories.

Source: Reference 7

## Promise for the Future

The EtG fingernail assay offers a promising new way to identify and monitor those engaged in risky alcohol drinking. EtG has not been reported in the fingernails of teetotalers, and self-reported social drinkers have levels less than current standard cutoffs (U.S. = 20 pg/mg; Europe = 30 pg/mg). Only individuals who report consuming more than two standard drinks per day have been identified as positive. As with all long-term ethanol biomarkers, a single positive result does not justify taking action. The tests must be used in conjunction with clinical observation.

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# Hip Replacement Metal Toxicity Risks

## *Patients with Metal Implants May Need to Be Monitored*

*By Michael A. Wagner, PhD*

With the aging population's increase in degenerative joint disease, the demand for surgical implants of prosthetic devices continues to grow. Injured veterans returning from deployments in the Middle East constitute another important patient population requiring prosthetic joint replacement.

Currently, metal alloys provide some of the best options for prosthetic design. Metal alloys offer biocompatibility with host material and many functional properties. They feature a low corrosion profile, but are not corrosion-free. The bio-alloy corrosion process leads to metal ions being released into tissue centrally, as well as systemically. Therefore it is important that alloys release only low levels of metal ions with non-toxic properties. Manufacturers have gone through several generations of metal prosthetics, with most containing cobalt-chrome alloys or titanium alloys (1).

### Hip Types

The metal-on-metal total hip replacement system is one of the most common types, with some 40,000 implanted in the U.S. from 2005 to 2010 and 31,000 implanted in England and Wales from 2003 to 2011 (2). In this system, a metal ball with a stem in the thighbone replaces the femoral head. The ball fits into a metal cup socket that replaces the acetabular component (hip bone).

Another metal-on-metal approach is called a resurfacing hip system or ASR (articular surface replacement). This approach features the bone's femoral head being capped by a metal cover that is set into a metal acetabular hip component. Surgeons have implanted about 100,000 of these systems internationally since 2004.

Providers of these systems include DePuy of Johnson & Johnson, Reuters, Smith & Nephew, Stryker Corporation, Zimmer Holdings, and Wright Medical Group.

### Metal Recall

In 2010, the U.S. Food and Drug Administration issued a recall of all metal-on-metal systems. Over time, the metal surfaces create debris and shed metal particles into the local tissue and systemic circulation. The metallic wear leads to corrosion through several steps: an oxidative reaction, the flow of electrons across the surface of the metal, and a reduction reaction.

The reactions can lead to different products being formed depending on the environment. In an aqueous environment, soluble metallic ions form that can react with tissue locally and systemically.

Local tissue damage leads to muscle and nerve pain, loosening of the implant, bone damage, and performance failure. Symptoms of systemic reactions can include skin rash, cardiomyopathy, auditory and visual neurological damage, and impaired renal performance. Metal infection can also impair thyroid function and alter psychological status (3).

As previously mentioned, the prosthetic alloys commonly contain cobalt and chromium. Cobalt has 28 isotopes, with cobalt-59 the stable isotope used in implant alloys. It has two common oxidation states (+2 and +3) and can form multiple biogenic complexes with ligands, such as histidine, lysine, glycine, EDTA, and more (4).

Chromium has multiple isotopes, with Cr-52, Cr-53, and Cr-54 being stable. Cr-52 is the most stable and abundant, approximately 84%. Among its oxidation states, Cr +6 is genotoxic and causes hepatic and renal failure. Gastric juices, ascorbic acid, and glutathione reduce Cr +6 to Cr +3, which is an essential nutrient. Like cobalt, chromium undergoes oxidation/reduction reactions and becomes biologically soluble.

### Testing for Exposure

Urine is the preferred matrix for monitoring exposure to these metals, although feces can be used for some forms of cobalt. Other matrices researchers have explored include whole blood, bone, bile, and hair.

Laboratories use a variety of analytical methods such as the single-element techniques, differential pulse anodic stripping voltammetry and graphite furnace-atomic absorption spectrometry. Multi-element techniques are used more frequently, led by inductively coupled plasma-atomic emission spectrometry and inductively coupled plasma-mass spectrometry (ICP-MS). The ICP-MS technique can be coupled with high performance liquid chromatography (HPLC) to separate and identify metals with multiple oxidation states, organometallic species, and metalloid species.

ICP-MS and ICP-HPLC-MS offer the best sensitivity and specificity for monitoring cobalt and chromium exposure. A patient exposed to a failed prosthetic often has metal concentrations ranging from the sub parts per billion range to the parts per million range, so a method's ability to distinguish between endogenous concentrations and background contamination is critical. Background contamination

for cobalt can come from syringes, low-grade anticoagulants, and storage containers.

Sample background interference appears to be matrix-driven. Urine samples can typically be diluted to reduce interference; however, blood samples require an acid digestion followed by either dilution and centrifugation or cleanup on column material, such as a chelating or ion-exchange matrix. Because both metals can form oxides, polyatomic interferences occur under ICP-MS analytical conditions. Specifically, the plasmagen gas, argon, can react with carbon-12 to form an isotope of atomic weight 52, which can interfere with chromium-52 detection. Analysts can eliminate this interference by operating the ICP-MS in chemical collision technology mode and adding a mixture of hydrogen and helium to the argon plasmagen. The hydrogen and helium collide with and break up the polyatomic isotopes to lessen their interference.

Standard operating procedures include using matrix-matched blanks, as well as external standard calibration with internal standards.

## Tests Before Implantation

Prior to device implantation, patients should have their endogenous cobalt and chromium concentrations measured to establish a fingerprint. Because the metal debris takes time to accumulate in circulation, a reliable biological history requires results from multiple pre-implant samples. Most patients suffering from implant failures were probably unaware of the need for this precaution, but perhaps analysts can use hair samples to establish a longer history of metal exposure to compare with current urine samples.

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