

# STRAIGHT TOX

## Ethanol, Ethyl Glucuronide, and Ethyl Sulfate in Urine

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Over the past couple of years I have found myself increasingly involved in interpreting the results of urine ethanol, ethyl glucuronide and ethyl sulfate tests for attorneys. One reason for this is because, through some twist of fate, I became the “go-to guy” for these things for our State Board of Nursing. The individuals being monitored by this and similar agencies are often, due to past alcohol abuse issues, required to be alcohol abstinent as a condition of their continued licensure. However, I suspect that the most likely reason is that in October 2006 the Wall Street Journal printed a story entitled “Federal Agency says Urine-Alcohol Test isn’t Totally Reliable”, following a Substance Abuse Treatment Advisory issued by SAMHSA regarding ethyl glucuronide testing. I will talk more about that later, but let’s first re-examine some of the issues of alcohol abstinence monitoring.

### Urine Ethanol:

It is estimated that less than 5% of an ethanol dose is excreted unchanged in man. However, due to ethanol’s relatively high concentration in the body after ingestion of pharmacologically significant quantities, it is readily detectable in the urine after use. As with most urinary drug concentrations, the prediction of impairment or a corresponding blood concentration from a urine concentration is a practice that should be shunned, or at the very least, approached with considerable caution.

Urine alcohol concentrations generally lag behind those of blood during the absorptive phase until around the time of peak BAC, when the urine concentration exceeds that of blood. Urine alcohol concentration continues to exceed that of blood throughout the subsequent decline in blood ethanol concentration. The ratio of urine to blood ethanol concentration in the post-absorptive phase can be quite variable, but in general it has a mean of about 1.3 to 1.4. Though not recommended as a routine practice, one can theoretically estimate an equivalent BAC in the post-absorptive phase by having an individual void his bladder and then subsequently collect a urine specimen some 20 to 30 minutes later. Dividing the determined urine ethanol concentration by 1.3 – 1.4 would theoretically represent an average BAC over the time period between the voids. Perhaps



a more practical and less contentious use of a urine alcohol concentration in alcohol abstinence environments would be to simply use a randomly collected urine specimen and divide the urine alcohol concentration by 1.3 – 1.4 as an estimate of “at least” how high an individual’s BAC was since his last void. In all cases, these estimates should be used with caution.

The detection window for ethanol in urine is rather short in comparison to many other drugs. In general, one would expect for an individual to have detectable ethanol in her urine beginning shortly after drinking and for as long as her blood alcohol was positive and continuing until her next void. Practically, the detection time would probably be no more than 2 – 3 hours after her blood alcohol became negative. In general, assuming a peak BAC of 0.10 g/dL, one would expect a person’s urine alcohol to be detectable for about 8 – 10 hours after the cessation of drinking; longer, of course, with a higher peak BAC. This leaves little opportunity for effective compliance monitoring, particularly in an individual who drinks in moderation and knows when she will be tested.

It is widely recognized that the presence of significant levels of urinary glucose, as may be found in an uncontrolled diabetic, along with various yeasts and/or bacteria may result in the in-vitro formation of ethanol in unrefrigerated and unpreserved urine specimens. It is this phenomenon, as well as the short detection window of parent ethanol, that has given rise to the pursuit of ethanol metabolites that would ideally only be produced in-vivo and that possess longer detection windows.

### **Ethyl glucuronide:**

Although sometimes referred to as a biomarker, ethyl glucuronide (ETG) is a minor metabolite of ethanol, accounting for approximately 0.5 – 1.5% of total ethanol elimination. ETG is formed when ethanol is conjugated with uridine diphosphate glucuronic acid. ETG is detectable in urine approximately one hour after ethanol intake and is detectable for 80 – 120 hours or more after urine ethanol is no longer detectable. ETG is reportedly stable in urine at room temperature for up to 4 days. However, it has been shown to be possible to hydrolyze ETG by the action of bacteria often present in urinary tract infections. Furthermore, it has also been shown to be possible to form ETG, in-vitro, in the presence E. coli and ethanol. Presumably this ethanol could arise from in-vitro fermentation of glucose in an uncontrolled diabetic, as described above.

### **Ethyl Sulfate:**

Ethyl Sulfate (ETS) is formed by sulphotransferases and is a minor metabolite of ethanol. ETS is typically found in lower urinary concentration than is ETG and has a detection time of approximately 80 hours after ethanol ingestion.

ETS has not been shown to be formed in-vitro nor has it been shown to be degraded by bacterial action.

## U.S Department of Health and Human Services Advisory:

In September 2006, the U.S. Department of Health and Human Services issued a Substance Abuse Treatment Advisory with the warning:

*Currently, the use of an EtG test in determining abstinence lacks sufficient proven specificity for use as primary or sole evidence that an individual prohibited from drinking, in a criminal justice or regulatory compliance context, has truly been drinking. Legal or disciplinary action based solely on a positive EtG, or other test discussed in this Advisory, is inappropriate and scientifically unsupportable at this time. These tests should currently be considered as potential valuable clinical tools, but their use in forensic settings is premature.*

This statement was quickly seized-on by attorneys as evidence that ETG testing cannot be used for the purpose of monitoring and enforcing abstinence in affected individuals. Ironically, if one reads the fine print on the last page of the Advisory, it states, “The content of this publication does not necessarily reflect the views or policies of SAMHSA or HHS.” This begs the question, “Then whose view does it reflect?” That notwithstanding, it is unfortunate that this Advisory was handed down in such a fashion. Such statements from government entities tend to circumvent the scientific peer-review process, and instead conjure up the specter of Big Brother.

The issues raised by the Advisory mostly pertain to the possibility of passive exposure and positive predictive value. These issues, of course, are related to the selection of a suitable threshold; one having the right balance of sensitivity and specificity. These are all valid issues. However, the discussion in the Advisory of positive predictive value (PPV) is somewhat troubling. While the Advisory itself makes no attribution, the Wall Street Journal attributes the authorship to Dr. Kenneth Hoffman, the agency physician. In the Advisory, Dr. Hoffman seeks to point out “the critical role played by prevalence in determining positive predictive value”, with the following quote: “Although the base rate of drinking among healthcare professionals required to refrain from drinking to maintain their license to practice is unknown, it is likely quite low.” Ironically, earlier in the Advisory the author makes the statement, “Relapse is unfortunately rather common in alcohol treatment, especially in the early stages of recovery.” Be that as it may, in support of the former statement the author references an article by Domino, et al. JAMA, 293(12), 1453-1460. In this study, Domino, et al. found the cumulative relapse rate at 5 years for alcohol in their cohort of health care professionals to have a mean of 24%. However, the author in making his point regarding PPV states, “in keeping with the ‘quite low’ assumption, if the prevalence of drinking is in fact **10 percent**...” (*emphasis added*). This assumption, likely chosen for the sake of demonstration, seems a bit disingenuous compared to Domino et al.’s value.

Furthermore, the critical reader must consider how Domino, et al. arrived at their relapse rate. The authors determined relapse by, “self-report, behavioral monitoring, chemical monitoring, workplace monitoring, regulatory board reports, or other”, with 31% of the detections of relapse being detected by chemical monitoring. While the nature of the chemical monitoring for ethanol is not provided, due to the fact that the cohort in the

study entered the monitoring program between January 1, 1991 and December 1, 2001, it is likely that a large percentage, if not all, of the chemical monitoring for alcohol abuse was by the detection of ethanol in urine; the same inadequate monitoring process that ETG/ETS testing seeks to correct. If this is true, the actual relapse rate may in fact be far greater than 24%. Additionally, in this same discussion, the author, in a continuing attempt to make his point about PPV chooses by way of example a test with a sensitivity of 100% and a specificity of 90%. This is a somewhat specious selection of values. Even though the author refers to this as “excellent specificity”, I know of no forensic toxicologist who would seek to obtain a 100% sensitivity at the cost of a 90% specificity? Ninety percent specificity may be “excellent” for a medical-diagnostic test, but in forensic urine drug testing, where rights and liberties are at risk, specificity is almost universally preferred over sensitivity. Using these assumptions, Dr. Hoffman derives a PPV of only 53%. However, if one more realistically chooses and applies the numbers, a 24% relapse rate, and let’s say an 80% sensitivity, and a 99% specificity, the calculated PPV becomes 96%. If the relapse rate is actually 45%, the PPV jumps to 99%. This is the very reason forensic toxicologists choose higher thresholds over lower ones. I believe the author sought only to dramatize a point in his selection of values. However, I fear that in doing so, these values will be taken by attorneys to be representative of the actual PPV of ETG testing as applied in alcohol abstinence monitoring.

It is not my purpose to be overly critical of the Advisory, nor Dr. Hoffman. It is likely that some laboratories were overly enthusiastic in a rush to market with ETG testing. However, the issues addressed in the Advisory are not new territory in the realm of urine drug testing. There is very little difference between ETG/ETS testing in regard to passive exposure, sensitivity, specificity, and PPV, than other drug testing, such as second-hand marijuana smoke and poppy seed ingestion. One unique feature of ETG/ETS testing, however, is that it is somewhat disproportionately applied to health care professionals. While caution is always advised, this author sees the Advisory as too strongly-worded, and the subsequent discussion tends to provide attorneys with unrealistic and misunderstood statistics that will likely be used in attempt to discredit ETG and ETS testing entirely.

### **Passive exposure:**

So what of passive exposure to ethanol? There is no doubt that beside alcoholic beverages there are plenty of opportunities to knowingly or unknowingly ingest or be passively exposed to ethanol. Mouthwash,



medicines, perfumes and colognes, foods, and skin sanitizers are but a few of the products that a consumer may encounter that contain ethanol.

An important aspect of any alcohol abstinence monitoring program should be patient education regarding products containing ethanol along with a signed agreement as to the understanding of this issue and the patient’s intended abstinence from the same. Beyond that, several studies have been performed on common products to assess their potential for producing ETG and ETS



positive results.

The following is intended to be an overview of some of the available studies and informal experiments on passive exposure to ethanol and is not intended to be exhaustive:

- Rohrig, et al. Letter to the Editor, Journal of Analytical Toxicology. Vol. 30, 2006, 703-704

Summary:

Four individuals applied Germ-X (62% ethanol) hand sanitizer to their hands in increments of 15, 30, and 60 minutes throughout the workday. The 60 and 30 minute interval participants did not demonstrate ETG at an LOQ of 50 ng/mL. The 15 min interval participants did not demonstrate ETG by midday, but one subject tested positive for ETG at the end of the day with a concentration of 62 ng/mL.

- Constantino, et al. Journal of Analytical Toxicology, Vol. 30, 2006, 659-662

Abstract:

Two studies were performed to evaluate the effect of alcohol containing mouthwash on the appearance of ethyl glucuronide (EtG) in urine. In the first study, 9 volunteers were given a 4-oz bottle of mouthwash, which contained 12% ethanol. They gargled with all 4 oz. of the mouthwash at intervals over a 15-min period. All urine samples were collected over the next 24 hours. Of 39 provided urine samples, there were 20 > 50 ng/mL, 12 > 100 ng/mL, 5 > 200 ng/mL, 3 > 250 ng/mL, and 1 > 300 ng/mL. The peak concentrations were all within 12 hours after the exposure. In the second study, 11 participants gargled 3 times daily for 5 days. The first morning void was collected. Sixteen of the 55 submitted samples contained EtG concentrations of greater than 50 ng/mL. All of them were less than 120 ng/mL. These studies show that incidental exposure to mouthwash containing 12% ethanol, when gargling according to the manufacturer's instructions, can result in urinary EtG values greater than 50 ng/mL. All specimens were negative for ethanol. The limits of detection and quantitation for the EtG testing were 50 ng/mL.

- Skipper, et al. Journal of Addiction Medicine. Vol. 3, No. 2, 2009, 1-5

Abstract:

**Context:** Ethylglucuronide (EtG), a minor metabolite of alcohol, is an important new marker that can detect alcohol use for several days or more after alcohol itself leaves the body. The test has rapidly gained widespread use where alcohol abstinence is desirable (e.g. in health professional monitoring programs, alcohol

treatment programs, high schools, criminal justice settings, liver transplant clinics etc). As with any new test, it is important to understand its limitations, especially, it turns out, regarding non-beverage sources of alcohol that can affect EtG levels. We describe a case and follow-up studies in which ethanol-based hand sanitizing gel (EthGel) caused elevated EtG levels for a pharmacist who disputed disciplinary actions by her licensing board.

**Objective:** To document that EthGel causes elevated EtG levels and to identify the route of absorption.

**Design, Setting, Participants:** Following discovery of the index case in 2004, twenty-four subjects were tested for EtG before and 30 min and 6 hours after exposure to EthGel in four groups: controls, skin exposure only, vapor exposure only, and both skin and vapor exposure. Breathalyzer was used to measure breath alcohol levels.

**Results:** EthGel caused elevated EtG and breathalyzer primarily from alcohol vapor. For “Skin Only”, “Vapor Only”, and “Both” Groups the mean EtG levels at 30 min were 42ng/ml (range 0-102ng/ml), 106ng/ml (18-328ng/ml), and 176ng/ml (0-348ng/ml) respectively. Breathalyzer levels of .01-.02gm% persisted for up to 40-60min in subjects with who had high EtG levels.

**Conclusion:** EthGel exposure, particularly inhalation of fumes, caused positive EtG levels. Subjects being monitored with EtG testing should be warned to avoid products containing alcohol, including fumes from EthGel and similar compounds. Further studies should be conducted to better quantitate the amount of ethanol absorbed from EthGel to determine if frequent use, particularly in poorly ventilated areas, might cause toxicity, especially for fetuses, where zero tolerance to alcohol is desirable.

- Jones, et al. United State Drug Testing Laboratory Research Monograph 2006.02

Summary:

Study participants applied 0.5 g of Purell (62% ethanol) gel to the hands once an hour for eight hours. One participant achieved a peak urinary concentration of 103 ng/mL of ETG at 8 hours. The same participant achieved a peak urinary concentration of 51 ng/mL of ETS at 4 hours. In a separate study a single participant applied 2 g of Purell to the hands and lower arms up to her elbows once an hour for eight hours. The authors note that this amount “was considered to be excessive.” A peak urinary concentration of 713 ng/mL of ETG was achieved at 9 hours and a peak urinary concentration of 14 ng/mL of ETS was achieved at 12 hours.

- Jones, et al. United State Drug Testing Laboratory Research Monograph 2006.01

Summary:

Two participants used a 20 mL dose of Target Brand Antiseptic Mouthrinse (ethanol 21.6%) as described by package directions, swishing between the teeth for 30 seconds, once an hour for eight hours. One participant achieved a peak urinary concentration of 366 ng/mL of ETG at 6 hours. The same participant achieved a peak urinary concentration of 73 ng/mL of ETS at 8 hours.

- Rosano and Lin, Journal of Analytical Toxicology. Vol. 32, 2008, 594-600

Abstract:

Ethyl glucuronide (EtG) is a direct ethanol biomarker and U.S. Department of Health and Human Services has advised that specificity studies at low EtG levels are needed for distinction of ethanol consumption and incidental exposure. The authors report urinary EtG excretion with ethanol abstinence, dermal exposure and oral consumption. EtG concentration by sensitive liquid chromatography-tandem mass spectrometry measurement in 39 urine specimens from adult alcohol abstainers (< 10-62 µg/L) and in urine from 13 children (< 10-80 µg/L) indicates either unrecognized ethanol exposure or endogenous ethanol metabolism. With repetitive daily dermal exposure to hand sanitizer (60% ethanol) by 9 adults, EtG concentration ranged from < 10 to 114 µg/L in 88 first-morning void specimens. EtG excretion following a 24 g ethanol drink by 4 adults revealed maximum urine EtG concentration (12,200-83,200 µg/L) at 3 to 8 h postdose and an EtG detection window up to 25-39 hours, compared to an ethanol window of only 2 to 4 hours. Oral ethanol use also showed an increase in the percent (molar equivalent) ethanol excreted as EtG with increasing oral ethanol doses. Human excretion studies show, 1. EtG detectable at low concentration (< 100 µg L) when ethanol use or exposures is not evident, 2. EtG concentration less than 120 µg/L in first morning specimens from adults with repeated dermal exposure to ethanol, 3. EtG levels maximally elevated within 3-8 h and above baseline for up to 39 hours after a 24 g ethanol drink, and 4. a dose-dependent increase in the percentage of ethanol excreted as EtG with increasing oral ethanol use.

- Ethanol in Food products – Dwain Fuller (Unpublished results)

Summary:

The claim of ingestion of rum cake was tendered as a defense to a positive ETG/ETS case brought before the State Board of Nursing. In an effort to establish or dispute the veracity of that claim the author prepared a “Bacardi Rum Cake” based on a recipe available off the internet. The cake contained ½ cup of

Bacardi® Dark Rum (40% ethanol by volume) in the mix prior to baking at 325°F for one hour. A separate glaze was prepared containing another ½ cup of Bacardi® Dark Rum. The rum was added to the boiling glaze mixture after removing it from the heat. GC headspace analysis of the cake and glaze demonstrated that the cake contained 11 mg/g residual ethanol and the glaze contained 61 mg/g residual ethanol. The cake weighed 956 g total and the total glaze weighed 415 g. The residual ethanol in the cake/glaze as intended to be served was 35.8 g total. Although significant amounts of ethanol could be consumed in this fashion, the fact that it is called a “Rum” cake and the fact that the presence of ethanol was readily apparent by smell and taste, would tend to exclude this as a legitimate source of unknowing ethanol ingestion. See Augustin, et al. *Journal of the American Dietetic Association*. 92(4), 1992, 486-488 for further residual alcohol in food product information.



### **Threshold Selection:**

It is apparent that much of the validity and future acceptance of ETG and ETS testing lies in the proper selection of threshold values. The chosen thresholds must rule out all but the most unlikely scenarios for passive exposure, while retaining the advantage of a longer window of detection over urine ethanol. Several thresholds have been proposed. I will refrain from opining on this subject lest I be seen to be issuing my own advisory. I trust that we as toxicologists will work this out, as we have with other urine drugs of abuse.

### **Summary:**

Urine ethanol testing has been around for a very long time, but ETG and ETS tests are rapidly taking precedence, particularly in the monitoring of healthcare professionals. Will one of these analytes supplant the others? My suggestion would be to perform all three tests, as well as a urine glucose test. By doing so one is armed with as much information as possible. As my mentor always said, “Forensic toxicology is not practiced in a vacuum.” Look at the totality of the data and the circumstances, then form your opinion, not the other way around.

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