UNITED STATES OF AMERICA
U.S. DEPARTMENT OF HOMELAND SECURITY
UNITED STATES COAST GUARD

UNITED STATES COAST GUARD
Appellant

vs.
SIMONE JOYCE SOLOMON
Appellee

Docket Number 2012-0351
Enforcement Activity No. 4405978

AMICUS CURIAE BRIEF IN SUPPORT OF PROPOSITION THAT URINARY CREATININE CAN DEGRADE IN TEMPERATURES LOWER THAN 300 DEGREES CELSIUS

1. INTEREST OF AMICUS CURIAE

Amicus Curiae is a scientist and Professor of Pathology and the Director of Clinical Chemistry, Toxicology, and Point of Care Testing for the University of Florida Health Science Center, Jacksonville, FL with a B.S. in Chemistry from James Madison University, an M.S. in Chemistry from the University of Virginia, and a Ph.D. in Biochemistry from the University of Virginia. He is certified in clinical chemistry and toxicological chemistry by the American Board of Clinical Chemistry, and is a qualified and experienced forensic toxicology laboratory director.

This case may have an important impact and set a scientifically incorrect precedent for anyone conducting an inquiry on the stabilility of urinary creatinine in a drug testing case.

2. SUMMARY OF ARGUMENT

The previous decision rendered in this matter seemed largely based upon a statement of a physician that creatinine is "heat stable" and that "sitting in the desert [during shipment] is not going to cause the urine to degrade and cause . . . the creatinine to disappear, or to break down."

The physician also stated that creatinine has a melting point of 300 degrees Celsius. This statement is not true. Creatinine decomposes at 300 degrees Celsius; decomposition temperature and melting point are not equivalent. Melting point refers to the temperature at which a chemical compound transitions between solid (crystalline) and liquid states. Also, while the decomposition temperature of pure creatinine is 300 degrees Celsius, this statement is irrelevant in relation to the stability of urinary creatinine. Urinary creatinine can be degraded by various chemical reactions occurring in urine, at temperatures well below 300 degrees Celsius.

3. ARGUMENT

A. 300 degree Celsius Melting Point or Degradation Point of Pure Crystalline Creatinine is not Directly Related to the Stability of Urinary Creatinine

The Merck Index is an authoritative reference for chemical data on organic and inorganic compounds. It was first published in 1889. It is currently in its 15th edition. In the 11^{th} Edition (1989) of the Merck Index, the entry for creatinine lists a decomposition temperature of 300 degrees Celsius for this compound. What the decomposition temperature means is that creatinine will begin to chemically decompose at temperatures equal to or exceeding 300 degrees Celsius (572 degrees Fahrenheit).

The decomposition temperature for compounds such as creatinine has no relationship to their stability in solution. Stability in solution depends on the reactivity of the compound with the solvent (e.g., water) and any other constituents present (e.g., enzymes) that may react with the compound. In the case of creatinine, it can react with both water (urine is >99% water) and enzymes present in urine due to bacterial contamination (e.g., creatininase enzyme from Pseudomonas, or creatinine deaminase enzyme from Corynebacterium—both bacteria are widely distributed in nature). All of these chemical processes have the capability of breaking down creatinine at temperatures far below 300 degrees Celsius, and all of the reactions would be accelerated as temperature increases. Generally, these reaction are halted in refrigerated (5 degrees Celsius) or frozen specimens (-20 degrees Celsius).

B. Degradation of Urinary Creatinine is Accelerated When Exposed to Heat over Time

Based on chemical and thermodynamic principles, it is reasonable to expect that the rate of chemical or metabolic decomposition of creatinine would increase with temperature. Nearly all chemical reactions occur more rapidly at higher temperatures.

There is experimental evidence that the creatinine concentration can decrease over time in urine specimens, and the rate of decrease in creatinine concentration correlates with temperature. *See* e.g., URINE PH: THE EFFECTS OF TIME AND TEMPERATURE AFTER COLLECTION (Cook, J: Journal of Analytical Toxicology, Vol. 31, October 2007). The study assessed the stability of creatinine after storage at various temperatures using a pooled urine specimen from three healthy donors that had a creatinine concentration over 30 mg/dL. The creatinine concentration decreased after storage for 8 days at 37 degrees Celsius or higher; the maximum decrease in creatinine was observed in the aliquot stored at 93 degrees Celsius. Temperatures between 37 and 93 degrees Celsius were not tested. The creatinine concentration in the specimen stored at 37 degrees Celsius decreased from over 30 mg/dL to about 27 mg/dL, while in the specimen stored at 93 degrees Celsius, the creatinine concentration decreased to about 17 mg/dL. The former represents approximately a 10% decrease, whereas the latter represents a 43% decrease. Based on these published data, it is possible for a urine specimen with a creatinine concentration over 2 mg/dL at the time of collection, after extended storage at temperatures at or above 37 degrees Celsius (about 100 degrees Fahrenheit) to have a creatinine concentration below 2 mg/dL when tested.

A proportional decrease in creatinine at elevated temperatures would be expected if creatinine degradation is strictly due to a chemical reaction with components in urine, such as water. However, if creatinine degradation is due to consumption by enzymes in microbial contaminants, it may not be proportional, but instead absolute based on the degree of bacterial contamination. Therefore, urine specimens stored at 37 degrees Celsius may experience a loss of creatinine corresponding to 4 mg/dL, or greater at higher temperatures, based on the published study. For example, a urine specimen may have a creatinine concentration of 5 mg/dL when collected, but less than 2 mg/dL after storage for several days at 37 degrees Celsius or higher.

C. Application of Science to Facts of Instant Case

It is possible for a urine specimen with a creatinine concentration over 2 mg/dL at the time of collection, after extended storage at temperatures at or above 37 degrees Celsius (about 100 degrees Fahrenheit) to have a creatinine concentration below 2 mg/dL when tested.

Chemically tampering with a urine specimen to artificially produce a specimen with a specific gravity within normal limits, while diluting the creatinine to a sub-physiological but measureable concentration, would require significant knowledge of chemistry and precise measuring devices for both weight and volume.

It is possible for a creatinine measurement of 1.3 mg/dL and specific gravity of 1.0223 to be consistent with human urine which was left in temperatures of 37 degrees Celsius or above for an extended time before testing. Temperatures of 300 degrees Celsius or higher are not required for urinary creatinine degradation. The true rate of degradation of creatinine in a specific urine sample cannot be accurately predicted due to variables of bacterial contamination and enzymatic reactions.

4. CONCLUSION

The opinion rendered in this case on May 15th, 2013, contains scientific inaccuracies regarding the degradation of urinary creatinine by finding that creatinine is "heat stable" in urine. The opinon adopts misleading and incorrect findings regarding the stability of creatinine in urine exposed to heat over time. The opinion on creatinine degrading at temperatures of 300 degrees Celsius seems to come from the melting point or degradation temperature for pure crystalline creatinine, and not creatinine in a urine matrix. The heat stability of pure creatinine is not indicative of the heat stability of uninary creatinine.

It is possible for a urine specimen with a creatinine concentration over 2 mg/dL at the time of collection, after extended storage at temperatures at or above 37 degrees Celsius (about 100 degrees Fahrenheit) to have a creatinine concentration below 2 mg/dL when tested.

The opinion rendered in this case contains inaccuracies regarding urinary creatinine that should not be established as precedent in any future cases where a urine sample was exposed to high heat and shows low creatinine.

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Date: Thursday, June 27, 2013

BY SIGNING, I CERTIFY THAT A TRUE AND CORRECT COPY OF THE FOREGOING HAS BEEN SERVED UPON Mr. Eric Bauer, Eric.A.Bauer@uscg.mil, as dated above.