Anderson Materials Evaluation, Inc.

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XPS, SEM/EDX, FTIR, Optical Microscopy, Thermal Analysis, Electrochemistry

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TO Homeowners, Redacted

Local Government Code Enforcement Officials, Redacted

FROM Charles R. Anderson, Ph.D.

SUBJ FTIR Analysis of a Dark Orange Liquid Sample

Summary

The liquid and the low-temperature dried residue of the unknown liquid sample were analyzed with FTIR infrared spectroscopy and the principal results are:

- The liquid is primarily water, but it contains other constituents, some of which appear to be of a denser nature and tend to clump together, making small lumps in the liquid. This is especially evident upon moving the liquid across the interior of the glass jar surface it was packaged in and examining the temporary layer of materials left on the surface over which the liquid has been transported.

- The liquid has many organic materials in it, including proteins, which is expected for urine samples. The dried residue is rich in amine bonds, carboxyl bonds, and amino acids, consistent with urine content.

- The liquid residue is a near match with that of a fresh human urine sample.

- Dog urine residue should be a near match with the residue of a human urine sample. It will not be quite the same and the urine samples of different dogs or the same dog over time will not be entirely the same either.

- This liquid was said to have traveled over flooring materials and surely did pick up some additional chemicals leached from those materials along the way. In addition, the time the liquid spent in the flooring is likely to have been extended,

which has aging consequences and may have brought it under the action of urine utilizing bacteria or fungi.

- The similarity of the liquid residue to that of a fresh human urine residue sample is thought close enough to justify the rational conclusion that this liquid is primarily due to urine.

Samples and Background

The homeowner shipped a dark orange liquid sample to Anderson Materials Evaluation, Inc. for our characterization and identification of the liquid. We were told the liquid had been collected from drippings through flooring and floor support materials of the homeowner's home into their basement. Their home is one half of a duplex or double house. The sample as we received it is shown below in Fig. 1.

On opening the jar, I attempted to smell the liquid. Before I could actually smell it, my nose suffered such a burning sensation that I was unable to smell it. However, while evaporating one drop of the sample on the diamond window of the ATR attachment of our FTIR instrument under low temperature heat from an inverted coffee cup warming device a couple of inches above it over about a 30 minute period, I smelled the sample at my desk in my office 55 feet away through four open doors to the laboratory room in which the FTIR spectrometer is housed. That smell was rather like an aged urine smell. The smell was disgusting enough that I opened the front door of our laboratory and used a large fan to blow the air of the entire 5,375 sq. ft. outside for a period of 3 hours. Expecting that this liquid might be repugnant, I had made the choice to perform this analysis over the 4th of July weekend so my colleagues at the lab would not be exposed to any awfulness. This is one of the most vile and potent liquids I have ever handled.

Fig. 1. The liquid for identification and analysis received from the homeowner is shown above as it was received. The jar was well-sealed. Note that the reflection of light from the right side of the liquid surface in the upper right image makes it clear the liquid is more dense than water and has constituents in it which are not uniformly dissolved. The lower image was made soon after returning the jar to its upright position and a thick and rather lumpy residue is seen on the glass surface which has not yet had time to descend into the lower pool of liquid.

The homeowner expressed the opinion that this liquid was the urine of four dogs kept in the other half of the duplex home. Urine is primarily water, but it is really a soup of many ingredients. Among these ingredients are:

Creatinine
Proteins (albumin and many more)
Glucose
Bilirubin
Ketones
Blood

Urobilinogen
Urea
Uric acid
Amylase
Citric acid
Cortisol
Homovanillic acid
Leucine aminopepidase
Porphyrins
Amino acids (many)

The precise mix of these ingredients varies between species such as humans, dogs, and cats. It also varies over time for a given animal and is dependent on the time of day, diet, age, and health. Some ingredients in urine will degrade with time. Consequently, a general spectrographic analysis of the residue of a urine sample filtered through and traversed over flooring and floor support materials will not provide an exact match to a given urine reference sample. We do not have a fresh, let alone an aged, canine urine sample reference. Certainly the residue of aged dog urine will not be an exact replica of fresh human urine either. Nonetheless, it should have many similarities.

FTIR Spectroscopy Analysis

All FTIR data were collected using a JASCO 6100 infra-red spectrometer equipped with a Golden Gate GS-10515 Attenuated Total Reflectance (ATR) Cell with KRS-5 lenses. The FTIR spectrometer has a ceramic mid-infrared source and a temperature controlled DLATGS detector. The Michelson interferometer has KRS-5 lenses and the detector has DTGS windows. Spectra were analyzed using BioRad's KnowItAll Informatics System, Jasco IR Edition. Spectra were matched using the libraries contained in Fiveash Data Management's ATR Polymers and Adhesives Database. Each sample was scanned 128 times to obtain a high-signal-to-noise ratio at a resolution of 4 cm^{-1}.

FTIR infra-red spectroscopy analysis was performed on the unknown liquid as-received from the homeowner, on the residue of the liquid after evaporating it slowly over a 30 minute period while in place on the diamond window of the ATR, on a fresh liquid human urine sample, and on the similarly dried residue of that fresh human sample of urine. It was clear that both the unknown liquid sample and the human urine sample are primarily composed of water, as shown in Figs. 2 and 3. This is expected to be the case for the known urine sample. It was also immediately clear that both residue samples were rich in organic materials. This was expected for the residue of the human urine sample.

Our database of about 30,000 FTIR spectra of various materials does not contain a reference spectrum for dog or human urine or for that of any other species of animal. However, it does have spectra of some organic materials of the kind we would expect to find in a urine sample residue. The best correlations of spectra in the database to the dried residue of the unknown liquid were L-alanyl-L-glutamic acid, protamine, and vitamin B-12. The first two are proteins and all of them have many NH and carboxyl (C=O) bonds in them. It is clear that these are characteristic chemical bonds in the unknown sample and that such bonds are expected to be prolific in urine. See Fig. 4 for the comparison of the dried residue of the unknown sample with the two proteins it shares similarity with from the database reference spectra.

In Fig. 5., the FTIR-ATR infra-red absorption spectra of the low-temperature dried unknown liquid sample residue is compared to that of the low-temperature dried fresh human urine residue sample. These spectra are similar, given the expected possible differences in urine samples for a given animal, let alone animals of a different species, and given that the unknown sample was aged, contaminated with possible, even likely building material leachates, and is likely the product of four different dogs contibuting variable amounts of urine in time. Urine is always a complex and variable chemical mix, but it is always rich in nitrogen-containing chemicals, including proteins.

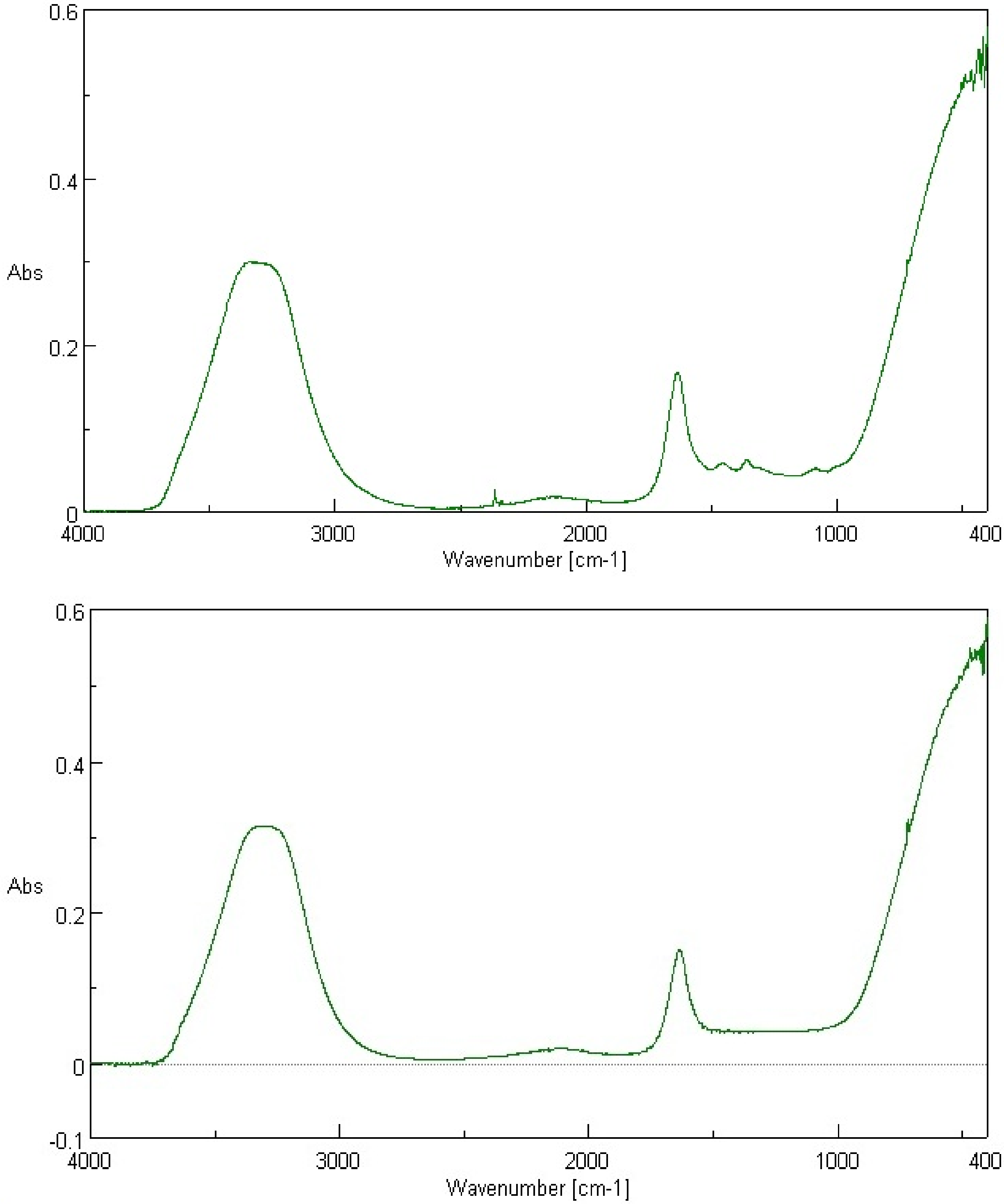

Fig. 2. The FTIR ATR spectrum of the liquid unknown sample is shown above, while that of water is shown below. Clearly, most of the unknown sample is water.

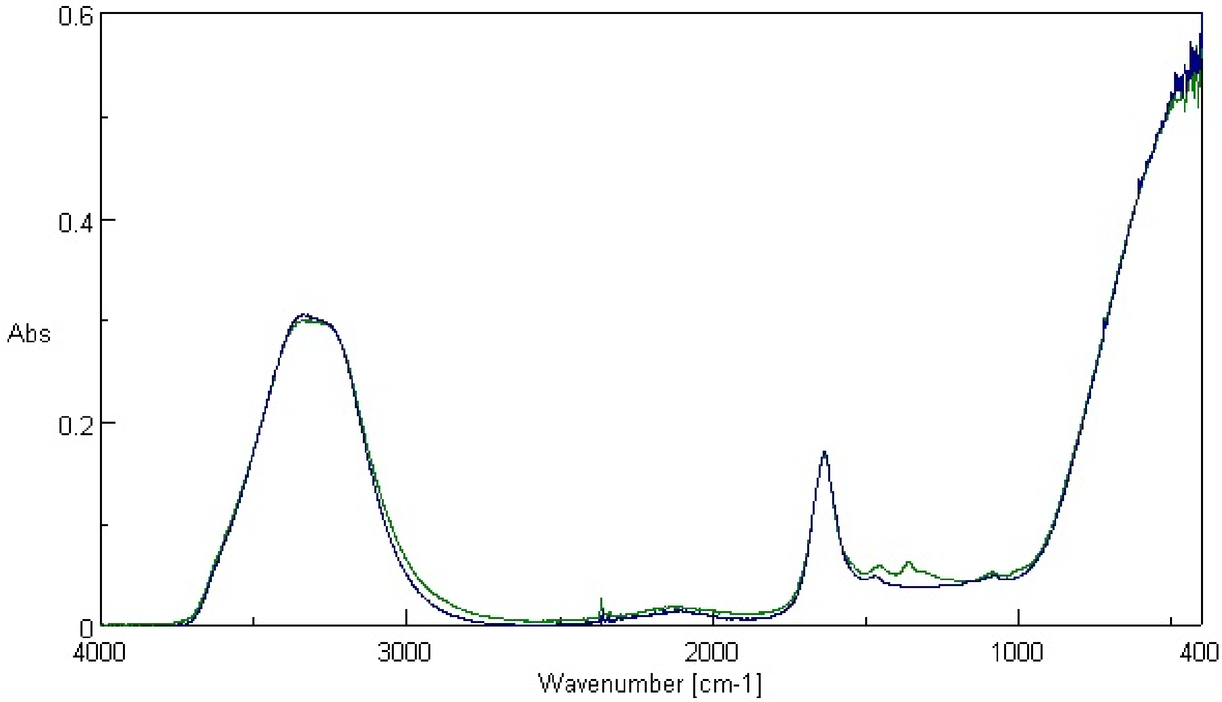

Fig. 3. The unknown liquid sample FTIR ATR infra-red absorption spectrum is in green and that of the fresh human urine sample is in blue. The minor absorptions at 1455, 1360, and 1082 cm^{-1}in the unknown liquid sample were the significant differences compared to pure water. Two of these are matched with minor absorptions in the fresh human urine sample.

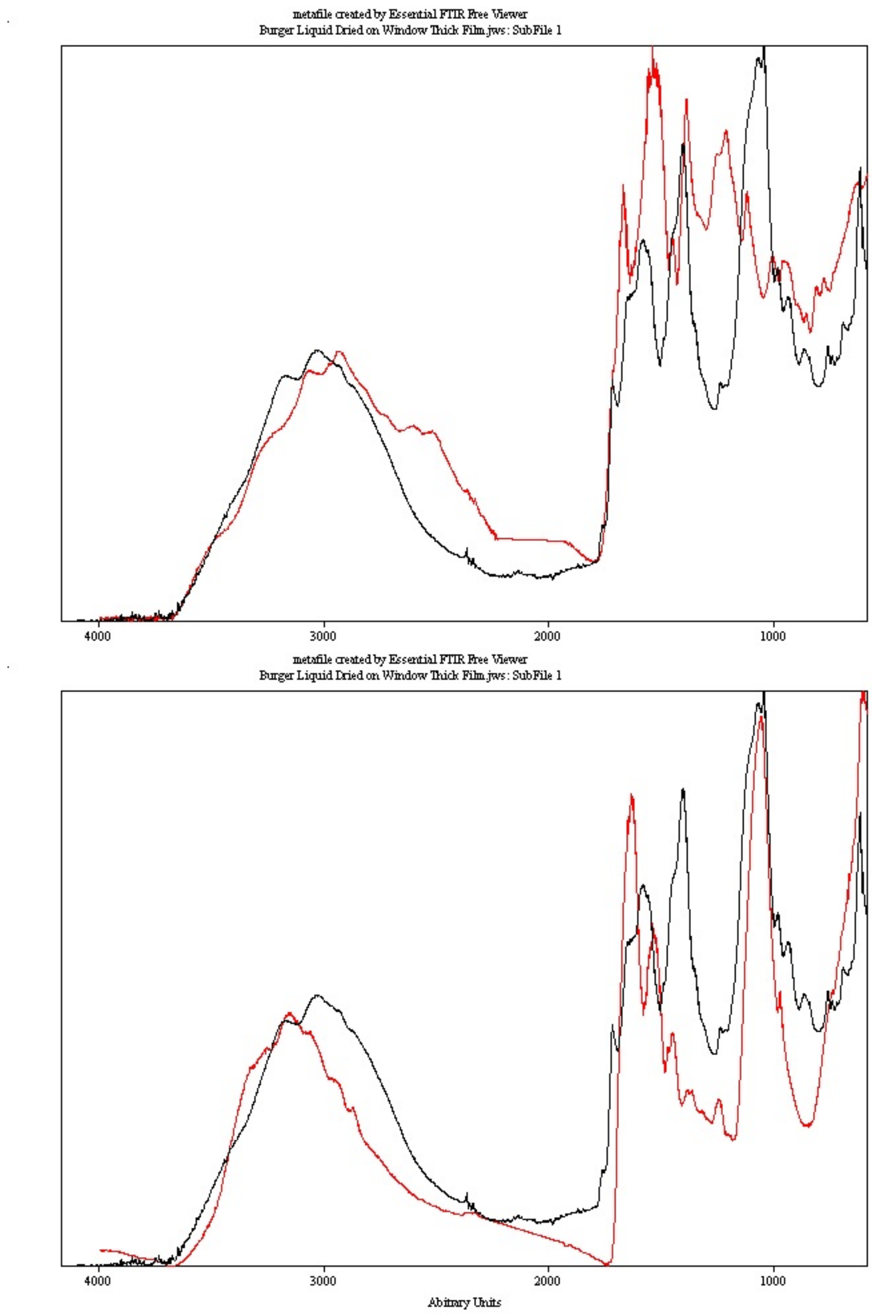

Fig. 4. In the two images above the low-temperature dried unknown sample FTIR absorption spectrum is shown in black. It is compared to one of the best matches from our database of spectra in each case, shown in red. In the upper spectrum, the similar spectrum of H-Ala-Glu-OH ($C_8H_{14}N_2O_5$, also called L-Alanyl-L-Glutamic acid) is compared to the dried sample. In the lower image the comparison to the spectrum of a protamine is given. A protamine is a small, arginine-rich nuclear protein. Both H-Ala-Glu-OH and protamines have amino acid groups in them, which are generally common in proteins. This is evidence that the unknown liquid content is largely protein in nature, aside from the water. Creatinine, another predominant ingredient in urine, also has amine and carboxyl (C=O) bonds in it and will share some similarities with the dried unknown sample spectrum.

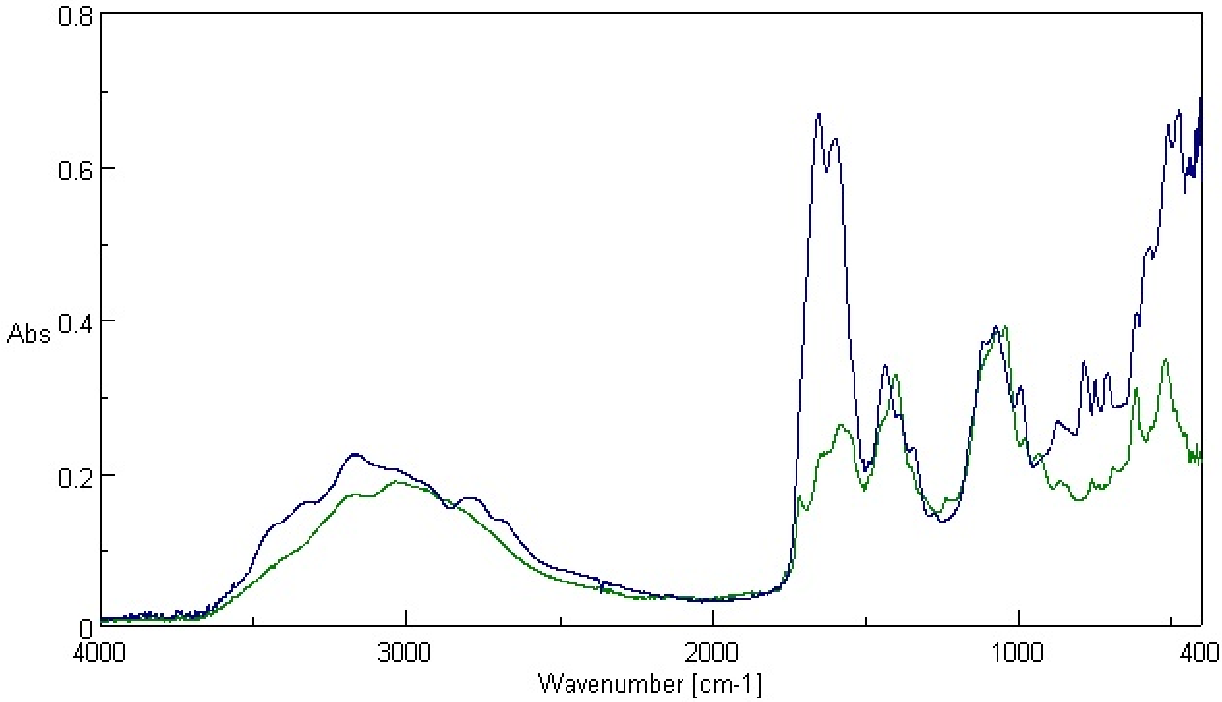

Fig. 5. The low-temperature dried residue of the unknown sample spectrum in green is compared to the similarly dried fresh human urine sample residue shown in blue. Though there are differences, the spectra are similar enough to make it likely that the unknown sample is also primarily urine. It may come from a different species of animal, it may show effects due to ageing, and it may have leached some chemicals out of the flooring materials it flowed through, but it appears most likely to be urine.