This document sets forth background materials on the scientific research supporting examinations as conducted by the forensic laboratories at the Department of Justice. It also includes a discussion of significant policy matters. This document is provided to assist a public review and comment process of the related Proposed Uniform Language for Testimony and Reports (posted separately). It is not intended to, does not, and may not be relied upon to create any rights, substantive or procedural, enforceable by law by any party in any matter, civil or criminal, nor does it place any limitation on otherwise lawful investigative and litigative prerogatives of the Department.

SUPPORTING DOCUMENTATION FOR DEPARTMENT OF JUSTICE PROPOSED UNIFORM LANGUAGE FOR TESTIMONY AND REPORTS FOR THE FORENSIC TOXICOLOGY DISCIPLINE

Background

Toxicology is the study of how drugs and poisons affect a living system. Forensic toxicology is defined as the application of toxicology for the purposes of the law. It is considered to be a hybrid of analytical chemistry and basic toxicology. The field consists of subfields, including postmortem forensic toxicology (i.e., cause of death investigations), human performance toxicology (e.g., driving under the influence investigations, drug-facilitated crimes), workplace drug testing, court-ordered toxicological testing (i.e., probation and parole, child services), and general toxicological testing (e.g. poisoning investigations where the victim survives, testing food samples suspected of containing a poison).[1]

Although the first systematic studies of toxicology began in France in the 1800's, the study and knowledge of poisons has existed for thousands of years. References exist in both ancient Egyptian and Greek literature which detail poisonings due to plants and food, for example, the state sponsored execution of Socrates via the deadly hemlock plant. During the Middle Ages, poisonings by opium, arsenic, and cyanide were often reported. The French doctor M.J.B. Orfila began the modern era of toxicology in 1814, publishing a treatise *Traité des Poisons ou Toxicologie Generale*, which established six classes of poisons based upon their effects.

In the United States, forensic toxicology's roots date back to the beginning of the 20th century. Under Charles Morris' guidance, New York's newly established medical examiner system included a dedicated toxicology laboratory. Alexander Gettler directed this laboratory and trained the nation's first generation of forensic toxicologists. Some of the earliest work from this era generated the principles by which blood alcohol analyses are still performed today.

[1] The Department conducts all testing listed except workplace drug testing and court-ordered toxicological testing. In the Department, testing of food samples may fall to either the Toxicology Discipline or to the General Chemistry Discipline.

Advancements in forensic toxicology have kept pace with advancements in analytical chemistry, pharmacology, physiology, and basic toxicology. The modern forensic toxicologist has access to advanced instrumental techniques, libraries of published laboratory and epidemiological studies, and well-established peer and accreditation organizations. All of these combine to allow today's forensic toxicologist to arrive at well-founded conclusions that are based upon firmly established analytical principles.

Theory of Forensic Toxicology Examinations

When an individual is exposed to a drug or poison, trained medical personnel may be able to evaluate what that individual was exposed to by studying the individual's physical signs and symptoms. However, a more definitive determination about what someone has been exposed to can be reached if biological specimens from that person are collected and tested for drugs and/or poisons. In cases of suspected driving under the influence of alcohol, breath may be collected and analyzed for the presence of ethanol. Blood and urine are commonly collected from living subjects to determine if exposure to drugs has occurred. Hair may also be collected to document long term or historical exposure of a person to a drug or poison. Additional specimens such as vitreous humor, liver, brain and stomach contents may be collected from deceased individuals to aid in cause of death determinations. In addition to testing specimens to determine *what* is present, quantitative analyses may be performed to determine *how much* is present. Quantitative results of drugs, metabolites, and poisons in a specimen may be compared to listed concentrations of the same in the scientific literature to assist in determining if a measured concentration corresponds to therapeutic treatment or an overdose.

A. Screening Techniques

Toxicological examinations typically begin with a screening technique. The purpose is to rule out the presence of analytes that are detectable by these techniques, or to indicate when further testing may be warranted. Screening techniques have minimum detection limits for analytes of interest that will include therapeutic concentrations for drugs and lethal concentrations for chemicals. Examples of typical toxicology screening techniques include:

- Gas Chromatography (GC) - A chemical separation technique that isolates chemicals from a complex mixture based on their interaction with a stationary phase and a gaseous mobile phase.
- Liquid Chromatography (LC) - A chemical separation technique that isolates chemicals from a complex mixture based on their interaction with a stationary phase and a liquid mobile phase.
- Color Tests - Simple chemical tests that produce a distinctive color when a reagent is mixed with a biological sample containing a drug of interest.
- Immunoassay - A chemical test that produces a color reaction based on the binding between a drug (antigen) and an antibody that interacts with that drug and/or drug class.

B. Confirmation Techniques

As a general matter of scientific and forensic toxicology principles, the identification of drugs and other toxic substances is confirmed (whenever possible) by a second technique based upon a different chemical principle. Generally, the confirmatory test for the target analyte is more specific than the first assay. The confirmatory test includes analysis of positive and negative controls for the analyte of interest. When a screening technique indicates the possible presence of a drug or chemical in one biological specimen (i.e. urine), confirmation of the identity of the analyte in a second specimen from the same individual (i.e. blood) is acceptable, as is confirmation of a second portion of the same specimen. Whenever possible and practical, the use of mass spectrometry is recommended for confirmation. Confirmatory techniques include:

- Gas-chromatography / mass spectrometry (GC-MS) – Gas chromatography is used to introduce an unknown sample to a mass spectrometer, which fragments the compounds into identifiable fragments that can be compared to the fragments of known standards.
- Liquid-chromatography / mass spectrometry (LC-MS) – Liquid chromatography is used to introduce an unknown sample to a mass spectrometer, which fragments the compounds into identifiable fragments that can be compared to the fragments of known standards.
- Inductively-coupled-plasma mass spectrometry (ICP-MS) – Samples are ionized in a high temperature plasma in order to introduce the elements in an unknown sample to a mass spectrometer where they can be detected and quantified. This technique is used for the analysis of heavy metals such as arsenic.

C. Quantitative Techniques

Quantitation is the determination of the amount of drug or poison present in a given sample. Quantitative analyses are performed based on case history, specimen volume, and the derived interpretive value in assessing the toxicological significance. A typical example would be the measurement of the amount of ethanol in a driver's blood. Many of the same chemical analysis techniques that are employed in screening and confirmation can be used to quantify the amount of analyte present in the tested sample. Additionally, the following are utilized in quantitative assays:

- Internal Standardization
- Multi-point Calibration
- Control Limits and Historical Performance Tracking
- Estimation of Measurement Uncertainty
- Confidence Intervals

Toxicology Process

There are different methodologies and processes for conducting a toxicology examination. The Department shares information regarding some appropriate processes below. The Department does not suggest that the processes outlined here are the only valid or appropriate processes.

A. General Approach

Forensic toxicological examinations are conducted on a variety of specimens for a wide range of drugs and other substances. These examinations begin with a thorough review of each case's history, as signs and symptoms and other details surrounding a case are needed to determine what examinations are appropriate. Training, case specific details, and professional judgment are used to determine the sequence of assays that will be performed for a given case. Some common scenarios include:

- Cases of suspected drug-related homicide typically include a blood ethanol analysis and a standard drugs-of-abuse screen for commonly abused drugs with confirmation of any relevant findings.
- Fatalities involving motor vehicle drivers often involve a blood ethanol determination, a drugs-of-abuse screen, and a more comprehensive screen for recreational and prescription drugs. Relevant positive findings are usually quantitated in blood.
- Suspected drug-facilitated sexual assault (DFSA) analysis routinely incorporates screening urine for drugs-of-abuse, alcohol, and other central nervous system depressants (i.e. benzodiazepines and barbiturates), and may include targeted screens for other DFSA drugs (i.e. GHB, flunitrazepam, chloral hydrate).
- Suspected poisoning cases without an alleged poison may call for a review of medical records to guide the analytical scheme. These cases may require a wide ranging analytical approach that may include screening for volatiles chemicals, cyanide, pesticides, etc.

B. Forensic Toxicology Standards

Quality assurance and quality control standards are vital parts of forensic toxicology analyses within the Department. The Departments's standards are based upon standards developed within the field of forensic toxicology over several decades. All methods are thoroughly validated before implementation in casework. Additionally, controls are incorporated each time a procedure is conducted to verify that the method is working properly on the day that case samples are analyzed.

In 1991, members of the Society of Forensic Toxicologists, Inc. (SOFT) and the American Academy of Forensic Sciences (AAFS) Toxicology Section, drafted and approved the *SOFT/AAFS Forensic Toxicology Guidelines*. [2] This document applied the

[2] SOFT/AAFS Forensic Toxicology Laboratory Guidelines. 2006 Version. (http://www.soft-tox.org/files/Guidelines_2006_Final.pdf)

requirements of the recently published *Federal Workplace Drug Testing Program*[3] (applicable to urine drug testing for employment purposes) to postmortem and human performance toxicology. The *SOFT/AAFS Forensic Toxicology Guidelines* addressed areas such as quality control, safety, and requirements of laboratory staff. This document was subsequently updated four times over the next 15 years.

The American Society of Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) offers accreditation to laboratories practicing forensic science. The American Board of Forensic Toxicology (ABFT) offers a separate accreditation program specific to laboratories practicing forensic toxicology. Both ASCLD/LAB and ABFT hold laboratories in their programs to written standards.[4] Laboratories seeking accreditation and reaccreditation undergo onsite inspections at set intervals, during which practicing scientists from other laboratories perform audits of data, instrumentation, and laboratory documentation to determine if the laboratory is compliant with the accreditation standards. Currently, Laboratories within the Department are accredited by both ASCLD/LAB and ABFT where appropriate.

The Scientific Working Group for Forensic Toxicology (SWGTOX) was established in 2009 by the Forensic Toxicology Council. Among SWGTOX's objectives are to establish minimum standards of practice for forensic toxicology in the areas of quality assurance, quality control, education and training, accreditation, and certification.

The Department uses validated methods to perform testing on case work specimens. Validation is the process of performing a set of experiments that reliably estimates the efficacy and reliability of an analytical method or modification to a previously validated method. The goal of validation is to establish objective evidence that demonstrates a method is capable of successfully performing at the level of its intended use and to identify the method's limitations under normal operating conditions. Until recent years, while there were few documents published on method validation specific to forensic toxicology, ample published guidance in the fields of analytical chemistry and clinical toxicology existed.[5] In May of 2013, SWGTOX published a document titled *Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method*

[3] Current version of the Mandatory Guidelines for Federal Workplace Drug Testing Program can be found at: *Federal Register*, 75:83, Friday, April 30, 2010 Notices, 22809-22810 (http://www.gpo.gov/fdsys/pkg/FR-2010-04-30/pdf/2010-10118.pdf)

[4] See *ABFT Forensic Toxicology Laboratory Accreditation Manual*, v. May 31, 2013 (available at www.ABFT.org), *ASCLD/LAB-International - Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories* (AL-PD-3040; 11/22/2011) and *International Standard ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories* (2005-05-15).

[5] Eurachem Guide, The Fitness for Purpose of Analytical Methods, (1998); Thompson M, et al. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, IUPAC Technical Report, *Pure Appl Chem*, 74:5, (2002), 835-855; U.S. Department of Health and Human Services, Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation, (2001); Drummer O, Requirements for Bioanalytical Procedures in Postmortem Toxicology, *Anal Bioanal Chem*, 388 (2007) 1495-1503; Peters F, et al. Validation of New Methods, *Forensic Sci Int*, 165 (2007) 216-224.

Validation in Forensic Toxicology[6] that details the minimum requirements to thoroughly validate forensic toxicology methods.

Current validation requirements are based on whether or not a procedure is for screening, confirmation, or quantitation. For screening and confirmation procedures, the method's limit of detection, processed sample stability, and selectivity are routinely evaluated. For quantitative procedures, the following are routinely evaluated (if applicable): accuracy, calibration model, carryover, ionization suppression/enhancement, limit of detection, limit of quantitation, precision, processed sample stability, recovery and selectivity. All validation plans and validation results are peer reviewed.

One of the challenges of the field of forensic toxicology lies in the number of potential drugs and poisons that can be detected. Each year, dozens of new drugs receive FDA approval for clinical use. Further, because "the dose makes the poison," practically every chemical substance known to man has to the potential of being used as a poison. Since there are over 50 million registered chemicals, each year the Toxicology Subunit is required to develop and validate new analytical procedures due to the suspicion and/or appearance of one of these chemicals in a given case. Such efforts are done following a well-defined, established, and scientifically accepted process.

In situations where a new drug or poison is identified, attempts are made to purchase a certified reference standard of the analyte of interest from a reliable supplier that can provide information about its traceability. Such reference standards are furnished with a *Certificate of Analysis* that specifies, through independent analysis, the identity and purity of the substance. If a certified reference standard is unavailable, the Laboratory will either contact a pharmaceutical company or the Drug Enforcement Administration (DEA) to attempt to obtain a reference standard. When the reference standard of a new analyte is not "traceable", internal characterization of the identity and purity is performed before using the reference standard for method development and validation purposes.

C. Conclusions within the Forensic Toxicology Discipline

Depending upon the case history, the specimens available, and the assays performed, the examiner may arrive at different fact conclusions upon the completion of testing procedures. Such conclusions may include:

Identification

- Positive results have been obtained for an analyte in two separate samplings of a biological specimen, or in two specimens from the same person, and;
- The predefined decision criteria were met for the procedure(s) that gave positive results, and;
- Mass spectrometry has been used as a part of the testing procedure

[6] Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. SWGTOX Doc 003, Revision 1, Published May 20, 2013. (http://www.swgtox.org/documents/Validation3.pdf)

Detection

- Positive results are obtained for a mass spectrometric method for an analyte in one sampling of a biological specimen but there is not enough remaining sample volume to perform a second confirmatory analysis, or;
- No certified reference material is available for mass spectral comparison but the mass spectral results compare favorably to a library entry

Inconclusive

- An immunoassay screening result is positive but there is insufficient sample remaining to perform a second confirmatory analysis.

Not Detected

- The results of a screening and/or confirmatory procedure are negative or below the method's detection limit.

In addition to reporting findings of toxicology examinations, toxicology examiners are often asked to interpret those findings. Such interpretations generally fall into one of the following categories:

- Pharmacokinetic and/or pharmacodynamic principles
    - Questions posed in an individual case may include whether or not a specific dose of a drug would be detected in a toxicology specimen within a particular time period after exposure or how high a blood alcohol concentration might be after a set number of drinks.[7]

- Effects of drugs on the average person
    - Effects of a drug on performance are often helpful to a jury when deciding if the behavior of a suspect or a victim may have been caused by a drug. An examiner may be asked if certain symptoms are consistent with those caused by a particular drug.[8]

- The significance of a blood concentration of a drug
    - Reported blood concentrations may be correlated to those in the published literature to provide interpretation about whether or not the concentration is consistent with reported therapeutic, toxic or fatal levels. If a limitation of this interpretation includes a comparison of a postmortem blood

[7] References used to answer these questions vary widely. Medicolegal Aspects of Alcohol, ed. J.C. Garriott is a common first source for information related to the forensic toxicology of ethanol. Baselt's Disposition of Toxic Drugs and Chemicals in Man is a good starting point for information on other drugs and poisons.

[8] Data on effects of drugs and poisons on the average person are available in pharmacological studies, case reports, and in many other sources.

concentration to published antemortem data, this limitation will be included in the report.[9]

- Back extrapolation of blood alcohol concentrations
    - Examiners may be asked what the blood alcohol concentration may have been at the time of an automobile accident, or other event, if the blood is not collected for a few hours.[10] When the results of such calculations are provided in a laboratory report, the examiner also includes a listing of factors and assumptions used in these calculations.

- Interpretations for the segmental analysis of hair specimens
    - The portion(s) along the length of a hair specimen where a drug or poison is identified may be used to estimate the time(s) of drug exposure.[11]

Policy Considerations

In 2006, Congress authorized the National Academy of Sciences (NAS) to conduct a study on forensic science which culminated in a 2009 report.[12] While the NAS report did not provide specific criticism or guidance regarding toxicology, it did refer to it as a laboratory based discipline.[13] An overall criticism of forensic science by the NAS was the need for research and empirical data to support conclusions drawn by examiners during forensic analysis.

Position statements published in the field of forensic toxicology

- Consensus opinion summarizing the current applicability of hair analysis to testing for drugs of abuse[14]
- Proposed SOFT position statement on the misuse of volume of distribution calculations for drugs in postmortem cases[15]

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[9] The following references may be used to guide in these interpretations: Musshoff, F., et al. Fatal blood and tissue concentrations of more than 200 drugs. *Forensic Sci. Int.*, 142 (2004) 161-210. Schulz, M., et al. Therapeutic and toxic blood concentrations of nearly 1000 drugs and other xenobiotics. *Critical Care*, 16:R136 (2012). Winek, C.L., et al. Drug and chemical blood-level data 2001. *Forensic Sci. Int.*, 122 (2001) 107-123.

[10] The basis for these calculations is that ethanol is excreted at a constant concentration per unit time. This has been documented in the scientific literature since the 1970s.

[11] The basis for these calculations is that head hair grows an average of 1 cm per month, as described in LeBeau M.A, et al. The Role of Variations in Growth Rate and Sample Collection on Interpreting Results of Segmental Analysis of Hair; *Forensic Sci In*t 210 (1-30) (2011).

[12] National Research Council. (2009) Strengthening Forensic Science in the United States: A Path Forward, National Academy Press, Washington, D.C. (http://nap.edu/catalog/12589.html)

[13] *Id.* at 7.

[14] ToxTalk, vol. 14, no. 3, 1990

[15] ToxTalk, vol. 29, no. 2, 2005

These two statements cover two areas of interpretation in forensic toxicology casework, specifically hair testing and calculations performed to estimate the dose of a drug based on a single blood drug concentration.