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MEMORANDUM
To: Walt Vogl Ph.D., Drug Testing Section Division of Workplace Programs
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Subj: Comments for DHHS proposed guidelines FR Doc. 04-7984 Filed 4-6-04 12:39pm

Encl: Publication proof of **Mechanisms of Drug Deposition in Hair and Issues for Hair Testing** Review article by J.A. Ruth and P.R. Stout accepted for publication in Forensic Science Reviews.

My comments relate specifically to the proposed inclusion of hair as a matrix for testing regulated testing. While hair testing has some limited applicability in situations where there are comparable data from traditional matrices, its utility for large scale drug testing is questionable. Hair ultimately may be of utility when the understanding of the chemistry of drug deposition in hair is appropriately understood, the current understanding is inadequate for the level of scrutiny to which testing under the federal program will be subjected. The confounders to hair testing limit its ability to be utilized for wide spread testing to accomplish the goal of identifying individuals in security or safety sensitive positions who have used drugs.

Numerous publications have now indicated in both animal *in vivo* and *in vitro* models and also in human models that hair color is not only important in drug deposition, but that eumelanin may be the primary site of drug binding in hair. (see attached review). This binding may go so far as covalent adduction of some dugs to eumelanin during melanogenesis. Papers indicating a lack of color effect have often 1) relied upon qualitative and subjective determination of hair color rather than quantitative determination of eumelanin and pheaomelanin content; 2) relied upon self reported dosages or limited controlled dosages. Additionally it is likely that recoveries from hair even from stringent digestion methods (some of which not all analytes will tolerate) are low do to the strong binding and physical occlusion in melanosomes. This also may have impacted studies indicating minimal or no hair color effect as their variable or poor recovery may have masked an effect which was present. While it is indeterminate if an "ethnic bias" exists in hair testing, is likely within medical certainty that hair testing is more sensitive in individual with eumelanotic pigmented hair.

The background information of the guidelines state that ". . .[issues] of particular concern are environmental contamination and the role of hair color. Concern has been raised about environmental contamination where a person may claim, for example, that the drug is present because the individual was in a room where others were using marijuana or cocaine. While washing the hair sample may remove some of the contamination,

ultimately we can differentiate environmental contamination from actual use because of the presence of the metabolite, which is not present when environmental contamination is the source of the drug." This is inadequate to rely on the presence of metabolites to preclude the presence of environmental exposure for several reasons. Numerous publications (see attached review) have indicated that the predominant species for most drugs is the parent compound. Thus, the sensitivity of the test is compromised by the requirement for the presence of a metabolite. The most crucial instance of this is THC-COOH which has been demonstrated to not bind well to hair at all (see attached review). Thus, this reduces the utility of hair testing for its intended purpose to identify individuals in security or safety sensitive positions who have used drugs.

Additionally, and more importantly this criteria is not sufficient to rule out environmental contamination. As demonstrated by Nakahara and Kikura (Hair analysis for drugs of abuse VII. The incorporation rates of cocaine, benzoylecgonine and ecgonine methyl ester into rat hair and hydrolysis of cocaine in rat hair, *Arch Toxicol* 68:54-59, 1994), benzoylecgonine (BE) was present as a hydrolytic product derived from cocaine *after* deposition of cocaine. Thus it would follow that detected metabolites, required under the proposed regulations for the determination of a positive sample, could well be hydrolytic products derived from exogenously deposited cocaine and thus an inappropriate result. The chemistry of compounds once in hair is poorly understood and, as this article indicates, this could be a substantial confounder to the attempt to control for external contamination by the detection of metabolites.

While the proposed regulations include requirements for the type of instrumentation and controls needed, there are no requirements for the type of sample preparation or methods of control for external contamination. The attached review article illustrates that there is vast variety in the preparative methods for hair. While the precise methodology should not be stipulated in the regulation, due to the unique nature of hair and the potential for external contamination that is not adequately accounted in the analysis of metabolites, for appropriate results to be assured across the program more specifics are necessary for this matrix. Specifically, that a decontamination strategy must be employed and controlled to positively demonstrate that external contamination is effectively removed, also that an adequate sample preparation strategy is employed (digestion/extraction) of the hair which is also controlled to ensure that adequate recovery is achieved for adequate sensitivity. What constitutes an adequate control of these processes will be controversial. It is unclear what an appropriate control for recovery is. Externally applied drug to drug free hair would not be an adequate recovery control as the incorporation is not analogous to *in vivo* conditions. Thus a *in vivo* produced control would be necessary.

The manufacture of control materials for proficiency testing of laboratories, blind proficiency testing and internal QC and calibration is a substantial problem for hair testing. A matrix matched control can be challenging in other aspects of toxicology (e.g. decomposition fluids or tissue samples) and have substantial issues for the interpretation of these results. Hair is a unique matrix in that its physical structure and chemistry are drastically different during its formation than from what is harvested for sampling. Thus it is neither reasonable nor appropriate to assume that applying drug to drug free mature

hair is an analog to drug incorporation into hair (see attached review). Moreover, if a laboratory uses drug free hair with externally applied drug (as is a common practice) as a control, subjects this control to the laboratory's decontamination procedure as a sample would be treated and is still able to detect the drug in the control, this has positively demonstrated that the decontamination procedure failed. Conversely if the decontamination procedure is sufficient, the control would be rendered useless. An appropriate control that parallels *in vivo* deposition of drug currently is impractical if not impossible to produce. An additional constraint on the production of an *in vivo* control other than ethical and practical considerations, is that little or no evidence is published of a dose response relation of the concentrations of most drugs in hair with dosage administered (contrary to the comment in the background section suggesting that the drug deposited in hair is proportional to the concentration in the blood.

Unless regulation can be written to account for confounding factors due to melanin binding, appropriate controls and exogenous drug contamination control, at this point the understanding of drug deposition in hair is not sufficient to support testing which is likely to be aggressively challenged in the court system.

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