Letters to the Editor

Centralia Massacre

I realize history is not the main focus of your journal. Still, I must point out that in Jan Beck's biography of Luke May (Journal of Forensic Sciences, Vol. 37, No. 1, Jan. 1992, pp. 349–355), a fact has been turned on its head. The "Centralia Massacre" began with an attack by the American Legion and other vigilantes against the Industrial Workers of the World (I.W.W.), not the other way around. The I.W.W. did fight back and there were casualties on both sides, notably Wesley Everest, a veteran in uniform who was nevertheless lynched by the Legionnaires.

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Author's Response

Dear Sir:

Dr. Roth correctly points out that the Journal of Forensic Sciences is not a historical publication. Because the "Centralia Massacre" was only a minor point in an article about a pioneer in forensic science, my response will be commensurately short.

I am aware that arguments persist over who was to blame in certain labor conflicts of the period, but no matter what one's biases may be in favor of one side or the other, there is no dispute that I know of concerning the fact of who fired on whom that day in Centralia. Therefore, I stand by the correctness of my statement that the Wobblies "opened fire on unarmed veterans."

My source is *Wobbly War—The Centralia Story* by John McClellan, Jr. (Washington State Historical Society, Tacoma, WA, 1987; pp. 73–74), an account by no means unsympathetic to the IWW side of the story.

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Laboratory Guidelines in Analytical Toxicology: How To Approach Qualitative Analysis

Dear Sir:

In recent years there has been a tremendous increase in the demand for toxicological analysis. Apart from the needs in the more classical areas such as forensic and clinical toxicology, the analysis of urine samples is now being regarded as an indispensable tool to Stem the spread of drug abuse, improve the quality of work, provide safer conditions in various sectors of our society, and assure fair competitions in sports.

This has put analytical toxicology in the limelight. The legal imperatives induced by the outcomes of the analyses have broad social implications and large percentages of the total population will become involved as subject to undergo drug testing. Thus, the analytical toxicologists must make sure that their approaches and methodologies are legally defensible and that their results are scientifically correct and undisputable.

In this regard, scientists and institutions in the USA were the first to recognize the need for adequate procedures and quality assurance. It started with the drug testing

programs of the U.S. Armed Forces in the early seventies (Winter, P.E., et al. "Drug Excretion in the Urine of Military Separatees: A Pilot Study," Journal of Forensic Sciences, Vol. 19, No. 2, 1974, pp. 317–324.) later followed by the Mandatory Guidelines for Federal Workplace Drug Testing (the NIDA Guidelines), the Accreditation Program of the College of American Pathologists and the AAFS/SOFT Laboratory Guidelines. Also at the international level laboratory guidelines are now being considered a very important issue, for example within the European Communities (EC), the International Federation of Clinical Chemists (IFCC) and The International Association of Forensic Toxicologists (TIAFT).

However, when evaluating the initiatives taken up till now, it becomes apparent that the qualitative side of analytical toxicology, that is, the screening for, and the identification of substances of potential toxicological relevance, has received insufficient attention. This may well be due to the complexity and scope of this domain. On the one hand, how to deal with the large number of substances and the variety of substance classes; on the other hand, how to differentiate between the minimal structural differences within a given class, the vast number of homologs, or between enantiomers.

Limited Screening

Obviously, the broader the number and classes of substances one has to screen for, the more difficult proper qualitative analysis will be. Yet, even a limited scope already raises a number of issues. The NIDA Guidelines [*1*], for example, are limited to marijuana, cocaine, opiates (such as morphine and codeine), amphetamines (that is, amphetamine and methamphetamine) and phencyclidine. Qualitative analysis must be done by a two-tier approach, the *initial test* by immunoassays and the *confirmatory test* by gas chromatography/mass spectrometry (GC/MS).

Interpretation of the results is geared towards avoiding false positive results as can be deduced from the instruction:

> The laboratory shall report as negative all specimens which are negative on the initial test or negative on the confirmatory test. Only specimens confirmed positive shall be reported positive for a specific drug.

An array of immunoassays is available from various manufacturers to detect the named substances. Yet, according to a member of the NIDA Advisory Panel on these Guidelines, the FDA requirements that these tests should meet do not yet exist [*2*]. As for the confirmation, it is surprising that—in contrast to the initial test—no criteria are given on how the GC/MS is to be carried out and what should be considered a positive match. On the other hand, although it remains important to avoid false positives, as a matter of equal justice the question of how to avoid false negatives ought to be considered as well. Especially when the scope of the screening becomes broader, which appears to be the intention [*3*], it will become impossible to have immunoassays that detect all substances with abuse potential in a given class. Also, what can or should be done with a positive immunoassay, for example, on amphetamines, and the GC/MS test cannot be matched to amphetamine or methamphetamine (think about the potent methoxyamphetamines).

Broad Screening

The AAFS/SOFT Laboratory Guidelines [*4*] are intended for a much broader screening, not only for drug testing in urine, but also for postmortem and human performance toxicology. The requirements for qualitative analytical procedures have been formulated as follows:

—Whenever possible, two tests should be performed for each analyte detected.
—Second test based on a different physical or chemical principle from that of the first test.
—Second test to offer a higher degree of specificity for the analyte in question.
—Second test (confirmatory step) particularly important when the initial test is designed to determine presence or absence of an analyte class (e.g. immunoassays).
—Detection limit of second test equivalent or lower than that of the initial test.

Here too, the two-test approach is followed, but the broader scope is clearly reflected in that the first test is not restricted to immunoassays, nor is GC/MS mandatory for the confirmation. No further details are given for the initial test, but the Appendix to [*4*] is more explicit on the confirmatory test. The following requirements/recommendations are given:

1. Scientific and forensic principle requires confirmation whenever possible.
2. Confirmation based on different physical or chemical principle.
3. Confirmation more specific and sensitive than first test.
4. Whenever possible and practical, mass spectrometry is recommended.
5. Confirmation using the same GC system as the first might be acceptable if chemical derivatization is used to change the retention times.
6. Confirmation using a second GC system with a different column than in the first is not acceptable, since the retention indices of many analytes may not differ substantially from one column to the other.
7. The quantitation of an analyte may serve as acceptable confirmation of its identity if it was initially detected by a significantly different method (e.g. GLC or HPLC quantitation of a drug detected by immunoassay).
8. Confirmation in a different specimen from that used for the first test (for example, urine and blood) is acceptable.
9. Confirmation in a second aliquot of the same specimen is acceptable.
10. Confirmation in the same original extract is not normally regarded as acceptable, since it will not rule out the possibility that the vial or extraction tube used was contaminated.

The majority of these recommendations are self-evident or appear to be so, but a closer inspection raises various questions:

—Chemical derivatization will add an additional substituent to a compound which may result in a change in its GC retention. However, there is no guarantee that this will allow to differentiate the substance from a similar molecule (for example, a homologue). The derivatized homologue may show the same change in GC retention (rec. 5).
—There are various instances in which confirmation using a second GC system may be the only viable option, for example, a chiral second column to differentiate between enantiomers. Also, in the analysis of solvents and other volatiles, a second GC system can be very helpful to distinguish positional isomers (rec. 6).
—The quantitation of an analyte can never be accepted as confirmation of its identity. (This is not to say that quantitation can be omitted but its results are to be used for interpretation, not for confirmation.) If one thinks to have morphine on the basis of an immunoassay and "quantitation" seems possible by GLC, it only means that the GC peak one is seeing has a similar retention as morphine (rec. 7).
—Recommendation 8, that initial test and confirmation can be done in different biological fluids (for example, urine and blood) is very dangerous. One can think of many situations were only urine or only blood will be positive (concentrations below cut-off levels, parent vs. metabolite, different metabolic patterns, etc.). Moreover, what to think of urine vs. saliva, or blood vs. saliva? If a confirmatory test in these instances is found negative, one would have to rule the entire case negative.

—Finally, as with the NIDA Guidelines, no criteria are given as to what constitutes a positive confirmation and what should be considered a negative confirmation.

Drawbacks of the Two-Test Approach

The above two sets of Guidelines, as well as other initiatives in the area of screening, give the impression that the two-test approach be best suitable for qualitative analysis. For example, the European Community advocates the same principle for the analysis of residues in food [5]. However, if the ultimate aim of qualitative analysis is defined as *to exclude the presence of all (relevant) substances, except one*, a number of drawbacks of the two-test approach become apparent:

—It hinges to a very large extent on the initial test. If the latter is not chosen properly, a false negative will result. This does not only apply to immunoassays (for example, too low cross reactivities) but to chromatographic screening techniques as well (TLC: below detection limit; GC: substance not volatile or unstable; HPLC: substance with low UV-absorption).

—If a positive initial test is obtained, the Guidelines seem to imply that this be translated into a drug candidate that is presumed present. This presumption then needs to be confirmed in the second test. However, if the initial presumption was not correct, the "confirmation" will be negative.

—A "positive" confirmation implies in essence that the results of the second test are *not against* the presumption derived from the first test. Yet, it does not rule out other substances that give similar results, for example, homologs, isomers, enantiomers, or other resembling substances.

—What are the criteria for a yes/no decision in the confirmatory test. For example, if MS is used, one is faced with various options: ionization technique; full spectra vs. condensed spectra; commercial libraries vs. user-generated libraries; inlet via chromatography vs. direct inlet; matching algorithms vs. visual comparison; and what is a positive match.

As indicated before, these drawbacks become more severe when the spectrum of relevant substances increases.

How to Exclude the Presence of All Substances Except One

This can best be accomplished by a two-phase approach instead of a two-test approach:

1. Screening phase, consisting of a *series* of different screening tests, run in parallel, to detect analytically positive specimens.
2. Identification/exclusion phase, a sufficient number of additional tests to provide unequivocal identification of the substance(s) present, at the same time excluding all other relevant substances.

At present, the most rational screening techniques are immunoassays and chromatographic techniques combined with appropriate detection modes, such as TLC with color reactions on the plate; GC with FID, NPD, ECD; HPLC with diode array, fluorescence or electrochemical detection. The screening tests should be chosen and combined in such a way that as many as possible relevant drugs can be detected. Naturally this depends on the scope of the analysis. Specimens that test positive in one or more techniques should then be run in phase II for full identification/exclusion. These additional tests will preferably include mass spectrometry, but it is incorrect to believe that MS is an absolute necessity. A proper combination of screening and additional tests may be equally suitable, provided that the validity of the various tests is adequately documented. However, this

also would apply to MS and we have seen above that the latter leaves much to be desired at the present time. Thus, the combined results of all tests from phase I and phase II should yield unequivocal identification, at the same time excluding all other relevant substances. A rational approach towards this kind of systematic toxicological analysis has been described [*6*].

Ideally, to finish a case, a reference sample of the identified substance—if available—should be rerun in all the tests applied and the results must be in agreement with the initial ones.

What Needs to be Done

In order to be able to correctly apply to two-phase approach, a number of technical requirements need to be fulfilled:

—Analytical methodologies for the phases I and II must be standardized (IA, TLC, GC, HPLC, UV, MS, etc.) and validated towards their identification power, interlaboratory reproducibility, robustness, etc.

—After having standardized the best suitable methodologies for qualitative analysis, computerized data bases for each methodology must be developed. They should not only contain data on as many as possible hazardous parent substances, but also on metabolites, endogenous and exogenous interferences (matrix compounds, plasticizers, etc.), toxicologically non-relevant drugs (foodstuffs, OTC medicines), etc.

—Stations should be established where reference samples of relevant substances can be obtained rapidly by accredited laboratories without time-consuming administrative procedures. Such stations may also play a useful role as information centers for analytical data.

It will be clear that the above requirements cannot be fulfilled by individual laboratories. Standardization, validation and the set-up and maintenance of data bases are such extensive and laborious tasks that it must be undertaken by interlaboratory cooperation, preferably at the international level. The Committee for Systematic Toxicological Analysis of TIAFT has already started to standardize TLC and GC methodologies [*7,8*], but much more additional work remains to be done. It should also be stressed, that the reference stations and data bases be accessible on an international basis and that the latter can only be searched meaningfully if the interlaboratory reproducibilities of the standardized methodologies are known, as exemplified if references 7 and 8. Thus, we are facing a number of challenging tasks in qualitative analytical toxicology with complex issues and laborious implications. Yet, if we join forces and rapid and diligent action is undertaken, they can be dealt with in a scientifically sound and practically feasible way.

References

[*1*] Mandatory Guidelines for Federal Workplace Drug Testing Programs. US Department of Health and Human Services. Federal Register, Vol. 53, No. 69, 11 April 1988, Di-D12.

[*2*] McBay, A. J., Personal Communication, October 1990.

[*3*] Consensus Report on Technical, Scientific and Procedural Issues of Employee Drug Testing. National Institute on Drug Abuse, HHS Publication Number (ADM) 90-1684, US Government Printing Office, Washington, D.C., 1990.

[*4*] Report of the Laboratory Guidelines Committee, American Academy of Forensic Sciences/ Society of Forensic Toxicologists, 1990.

[*5*] European Community, Directive of the Commission of 14 November 1989 (89/610/EEC).

[*6*] Franke, J. P., de Zeeuw, R. A., and Schepers, P., Retrieval of analytical data and substance identification by the Mean List Length approach, *Journal of Forensic Sciences*, Vol. 30, 1985, pp. 1074–1081.

[*7*] TIAFT/DFG Gas chromatographic retention indices of toxicologically relevant substances on SE-30 or OV-1, 2nd Ed., R. E. Ardrey et al. (Eds.), VCH, Weinheim-Deerfield Beach, 1985.

[8] TIAFT/DFG Thin layer chromatographic Rf values of toxicologically relevant substances on standardized systems, A. C. Moffat et al. (Eds.), VCH, Weinheim-New York, 1987.

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Facial Reconstruction

Dear Sir:

In the January 1992 issue (Vol. 37, pp. 155–162) Dr. Ubelaker and Mr. O'Donnell presented an excellent review of the literature concerning facial reconstruction. They also presented their technique of computer assisted facial reconstruction. I commend their report of a method that decreases time in the studio and still presents an image that is realistic and corresponds to the bony structure of the skull.

Dr. Karen Burns and I first presented the technique of video image capture assisted reconstruction in 1990 at the annual meeting of the International Association for Identification. The method was mildly criticized by several experienced composite artists because the final images were *too* lifelike. They referred us to several documented studies regarding facial recognition that advised against using images that were photographic in nature. Reportedly there is a higher identification success rate with facial images that leave the viewer with a chance to use his or her imagination and recognition skills.

I would like to know if the authors considered this aspect while developing their technique of producing an image that is so strikingly lifelike. I personally think it is an excellent method, and I currently use it in cases where the actual skull cannot be used for 3-dimensional reconstruction. However, the number of positive results have not been overwhelming. It would be interesting to see the author's percentage of actual cases that have resulted in positive identifications.

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Author's Response

Dear Sir:

This brief communication raises several important issues relevant to our efforts to estimate facial features from underlying skeletal structure. One such issue is nomenclature. Like many others, Ms. Craig uses the term "facial reconstruction" to describe this forensic procedure. In our article, we followed the advice of Rhine [1] in using the term "facial reproduction." As noted by Rhine, reconstruction implies that one reassembles parts that were once intact. Such is not the case in our forensic work where the soft tissue is missing or modified and clay markers are substituted. We are trying to reproduce or estimate the appearance of the face, not reconstruct it.

An important point to remember in attempting a facial reproduction is that we can not expect to produce an exact likeness of the individual. The goal is to emphasize those

features that reasonably can be reproduced in the face and de-emphasize those that cannot. Hopefully, the likeness will be close enough that when presented to the public it will cause someone to come forward with information about a missing person. To some extent, the reproductions serve as visual reminders to the public that someone with these general characteristics is deceased and unidentified. Our philosophy is that those features that can be reasonably predicted from bone should be presented as accurately and as lifelike as possible because these are the features that likely will stimulate the identification. This is especially true of unique, unusual features such as dental morphology or facial trauma. Head hair, facial hair, form of the ears etc. should not be emphasized in the reproduction unless of course these elements survive and offer information.

The computer assisted technique that we presented in the January 1992 issue of this journal basically is a computerized version of the composite technique that I have used in collaboration with the FBI since 1977. The images are not developed from photographs but from artists' line drawings of facial components. The impression relayed in Ms. Craig's letter that the resulting images appear "photographic in nature" attests to the skills of FBI artists who collaborate in this work. It should be noted that the three-dimensional clay reproductions also use "real" wigs and glass eyes and most experienced sculptors attempt to make their work as accurate and lifelike as possible, including placing "real" clothes on them. I believe that all of these approaches have a common goal: to present to the public an image developed from the available evidence that will stimulate recollection of a missing person and lead to identification.

The issue of "positive results" is complex. With the FBI, we have used the composite technique on many cases for 15 years. Several of these (that we know of) have been subsequently identified and we have generally been pleased with the similarity between the reproduction and the photograph of the known individual. Rarely is it absolutely clear that our work "resulted in positive identification." I know only of three such cases. In each of these, the image was circulated in the media, individuals saw the image, came forward with information about the person they suspected it might represent and the individual was later positively identified by dental records or other means. One of these, a black man from Georgia is published in my book "Human Skeletal Remains." (Ubelaker, D.H., Human Skeletal Remains, Excavation, Analysis, Interpretation, Second Edition, Taraxacum, Washington, D.C. 1989.)

In other cases, the person for whom we made the reproduction may have been later identified but it is not clear the role our reproduction played in the identification. Frequently it is difficult to untangle the many factors involved in the investigation that culminates in identification. Did the informant come forward because the published image bore a remarkable resemblance to their acquaintance or simply because of reading about the details of the case. Conversely, a truly accurate reproduction may remain unidentified simply because the deceased was from a different area.

In publishing this new technique, we do not mean to imply that it is superior in all ways to clay three dimensional reproduction. In contrast, I believe that in the hands of a skilled and experienced sculptor, the three dimensional approach probably offers superior opportunity to reproduce the fine contours of the face. The advantage of the computer-assisted approach is that is more rapid, allows for easy manipulation and adjustment of the final image, and maximizes the opportunity for collaboration between the artist and physical anthropologist. I believe this final point is an important one because we are beginning to see products from physical anthropologists who believe they have the necessary artistic skills to attempt facial reproduction and from artists who assume they know enough skeletal anatomy to attempt the procedure by themselves. In my opinion, facial reproduction is a technique that calls for collaboration. Physical anthropologists have the knowledge of cranial development and variation that offers important perspective on any reproduction. Anthropologists generally lack the artistic skills and

awareness of artistic techniques that are instrumental to quality reproduction. We now use the computer-assisted approach because it facilitates such collaboration.

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Reference

[1] Rhine, J. S., "Coming to Terms with Facial Reproduction," *Journal of Forensic Sciences*, Vol. 35, No. 4, July 1990, pp. 960–963.