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# Proficiency Testing in Forensic Toxicology: A Feasibility Study

**REFERENCE:** Peat, M. A., Finnigan, J. S., and Finkle, B. S., **"Proficiency Testing in Forensic Toxicology: A Feasibility Study,"** *Journal of Forensic Sciences*, JFSCA, Vol. 28, No. 1, Jan. 1983, pp. 139-158.

**ABSTRACT:** This study has shown that a national proficiency testing program in forensic toxicology is feasible. Samples that resemble typical case specimens were prepared and shipped to approximately 100 laboratories. Participation varied between 61 and 73%. Tissue samples obtained from laboratory animals can be used to simulate those encountered by forensic toxicologists. This has been demonstrated using liver homogenates from animals administered pentobarbital and methaqualone and propoxyphene and acetaminophen. There was a large coefficient of variation however, for the quantitation of acetaminophen in liver. The qualitative data obtained during the course of this study showed a very low incidence of false positives. However, there was a disappointingly low percentage of positive responses for (a) low concentrations of secobarbital and (b) the opiate narcotics (morphine and codeine) in blood, despite the fact that sensitive immunoassay procedures are available for detecting these particular compounds in blood samples. The quantitative determination of drugs and metabolites, other than ethanol, shows wide interlaboratory variation. This variation is presumably not a result of the use of different analytical techniques, since gas liquid chromatography was used by the majority of participants to quantitate drugs and metabolites. Forensic toxicologists are willing to participate in a voluntary proficiency testing program conducted by an independent agency. The performance data developed in this study can serve as a baseline for current forensic toxicology laboratory functional capability in the assessment of future changes and improvements in analytical forensic toxicology.

**KEYWORDS:** toxicology, drug identification, proficiency testing

It was the general purpose of the research described in this paper to undertake a nationwide assessment of the current ability of forensic analytical toxicologists to detect, identify, and quantitate drugs, their metabolites, and other chemical agents in biological specimens for medicolegal purposes. Drugs are by far the most commonly encountered poisons in forensic toxicology cases and toxicologists therefore have a key role to play in any investigation that purports to record or interpret drug involvement. These investigations require modern analytical procedures to detect and assay the drugs and metabolites in biological

Received for publication 5 June 1982; revised manuscript received 17 July 1982; accepted for publication 20 July 1982.

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fluids. Many forensic toxicologists subscribe to proficiency testing programs, such as those that support clinical toxicology or drug abuse testing.

Dinovo and Gottschalk [*1*] performed a limited proficiency testing survey of nine forensic toxicology laboratories. Their study showed that there was a wide interlaboratory variation, although it has been criticized on a statistical basis by Kelly and Sunshine [*2*], who demonstrated statistically that the test and reference laboratory results together represented a different population of values than the weighed-in concentrations. A more general critique of the study was published by McCloskey and Finkle [*3*], who concluded that the authors ignored essential details of variance, accuracy, and precision in their interpretation of the data. Perhaps the major practical criticisms to be made of that study are that "atypical samples" (lyophilized urine and serum albumin) were forwarded to the laboratories; these samples contained an unrealistic number of drugs and no metabolites were included in any of the samples.

The program described in this paper, however, was designed to simulate case samples seen in typical forensic toxicology laboratories and included hemolyzed blood, urine, gastric contents, and tissue homogenate samples. A primary aim of the research project was to evaluate the feasibility and effectiveness of having forensic toxicologists subject themselves to external proficiency testing. An Advisory Board (Table 1), consisting of respected forensic toxicologists, was appointed to assist in the selection of drugs and metabolites to be included in the study and to offer guidance as the study progressed.

## Study Design

The selection of test specimens for this one-year research program was conditioned by several important considerations. Firstly, it was intended not to provide specimens containing unrealistic combinations of drugs or unusual compounds. Selection of analytes was made after reviewing several annual reports by forensic toxicologists and after consultations with the Advisory Board, leading to the inclusion of drugs that were commonly encountered by toxicologists. A number of these agents were known to provide some difficulty for the analyst. As indicated previously, the test samples were prepared to simulate typical case samples. Participants were given between two and four working weeks to report results. To encourage participation, an interim report was sent to each participant after the results of each batch of samples had been received and processed at the Center for Human Toxicology

TABLE 1—*Advisory Board members who assisted both in the organization of the project and in reviewing the paper before it was submitted for publication.*

| | |
|---|---|
| Randall C. Baselt, Ph.D. | University of California, Davis |
| Leonard R. Bednarczyk, Ph.D. | Medical Examiners Department, Miami |
| Kurt M. Dubowski, Ph.D. | University of Oklahoma, Oklahoma City |
| Patricia H. Field, Ph.D. | State Laboratory of Hygiene, University of Wisconsin, Madison |
| Bryan S. Finkle, Ph.D. | Center for Human Toxicology, University of Utah, Salt Lake City |
| James C. Garriott, Ph.D. | Southwestern Institute of Forensic Science, Dallas; presently, Office of Medical Examiner, Bexar County, San Antonio |
| Arthur J. McBay, Ph.D. | Office of Chief Medical Examiner, Chapel Hill |

(CHT). This report included a statistical analysis of the data obtained by all methods used and by designated analytical procedures, such as gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC). In addition, a brief review of the methods used by the participants and those published in archival journals was presented. The major purpose of the interim report was to provide prompt feedback to each laboratory.

Potential participants were selected from the membership rosters of the American Academy of Forensic Sciences Toxicology Section, National Association of Medical Examiners, the Society of Forensic Toxicologists, The Southwestern Association of Forensic Toxicologists, the California Association of Toxicologists, and the Northwestern Association of Forensic Scientists. A letter sent to each potential participant outlined the scope of the proposed study, benefits of participation, and requested their cooperation in the project. Positive responses were received from 105 laboratories and every state, except Hawaii, was represented in the project.

To maintain confidentiality, the participants returned their results in a "double envelope" (that is, in a plain white envelope inside a previously addressed envelope) to a disinterested party, who then forwarded only the inner envelopes containing the results to CHT. Over a period of approximately nine months, four batches of five samples were shipped to each participant.

Table 2 shows the matrix, drug content, and concentration (weighed-in value) of each sample shipped. Quantitation of drugs and metabolites detected was requested on Samples 1, 2, 4, 7, 8, 9, 11, 12, 13, 14, 16, 17, 19, and 20; the remainder were to be qualitatively analyzed only. The time allowed for analysis of each batch varied, depending upon the analytical difficulty of the samples.

### Sample Preparation and Analysis

Appropriate amounts of drugs were dissolved in water and added to drug-free bovine blood or human urine. Both blood and urine were extensively screened by sensitive analytical procedures before the addition of drug or metabolite to assure absence of detectable drugs. Sample 16 (gastric contents) was prepared at CHT by adding a calculated amount of the pharmaceutical preparation to a simulated gastric content. The liver samples (9 and 18) were prepared by treating a population of rats with methaqualone and pentobarbital (Sample 9) and propoxyphene and acetaminophen (Sample 18) over a 30-day period. The animals were then killed and their livers removed, combined, and homogenized with water. An aliquot of this homogenate was then shipped to each participant. Samples were shipped in glass containers to the participants so that they reached the laboratories between 24 and 36 h after shipment.

The samples were analyzed at CHT throughout the course of the project to determine the stability of drugs and metabolites. After preparation, portions of the samples were stored at $-15°C$ and aliquots were taken and analyzed at regular intervals. Table 2 shows the results of these analyses.

For all analyses performed at CHT, the within-run coefficients of variation of the analytical methods used were less than 10%. It is apparent from Table 2 that when these analytes were repetitively assayed, the between-run coefficients of variation increased significantly with time. Volatiles were only determined at the time of shipment and during the period of analysis by the participant. Those samples that were to be screened were tested only qualitatively and found to be positive throughout the study. The results of quantitative analysis over an extended period of time indicated that, for some of the drugs and metabolites, it is unreasonable to prepare a sample pool on the first day of a proficiency testing program and expect the concentration to be within 10% of the weighed-in value several months or years later.

TABLE 2—*Stability studies in samples for quantitation.*[a]

| Sample | Drug | Weighed-in Value | Analytical Method[b] | Period of Analysis, Months[c] | Analytical Results | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | No. of Analyses | Mean | Standard Deviation | CV, % | Range |
| 1-Blood | diazepam | 1.0 mg/L | GC/MS and GC/ECD | 8 | 13 | 0.98 | 0.16 | 16.8 | 0.65–1.2 |
| | nordiazepam | 1.5 mg/L | GC/MS and GC/ECD | 8 | 13 | 1.49 | 0.13 | 8.6 | 1.37–1.71 |
| | ethanol | 50 mg/L | GC/FID | ... | 2 | 46 | ... | ... | ... |
| 2-Blood | carboxyhemoglobin | 60% | UV | 8 | 6 | 60 | 4.10 | 6.9 | 55–66 |
| | amitriptyline | 0.50 mg/L | GC/MS and GC/NPD | 8 | 5 | 0.46 | 0.04 | 8.6 | 0.41–0.5 |
| | nortriptyline | 0.75 mg/L | GC/MS and GC/NPD | 8 | 5 | 0.66 | 0.12 | 17.7 | 0.55–0.8 |
| | ethanol | 300 mg/dL | GC/FID | ... | 2 | 230 | ... | ... | ... |
| 4-Blood | ethanol | 100 mg/dL | GC/FID | ... | 2 | 87 | ... | ... | ... |
| | methanol | 50 mg/dL | GC/FID | ... | 2 | 60 | ... | ... | ... |
| | secobarbital | 2.5 mg/dL | GC/MS and HPLC | 8 | 10 | 2.24 | 0.30 | 13.6 | 1.9–2.7 |
| 7-Blood | ethanol | 100 mg/dL | GC/FID | 5 | 2 | 83 | ... | ... | ... |
| | flurazepam | 0.80 mg/L | GC/MS and GC/ECD | 5 | 12 | 0.91 | 0.14 | 15.9 | 0.7–1.1 |
| | desalkyl-flurazepam | 0.50 mg/L | GC/MS and GC/ECD | 5 | 12 | 0.58 | 0.08 | 12.9 | 0.5–0.7 |
| 8-Blood | methaqualone | 15 mg/L | HPLC | 5 | 9 | 11.90 | 1.10 | 9.30 | 10.6–13.5 |
| | metabolite I | 7.0 mg/L | HPLC | 5 | 6 | 4.70 | 0.60 | 12.30 | 4.1–5.6 |
| | pentobarbital | 10 mg/L | HPLC | 5 | 7 | 7.0 | 0.80 | 10.90 | 6.1–7.8 |
| 9-Liver | methaqualone | ... | HPLC | 5 | 8 | 8.10 | 1.30 | 15.70 | 6.2–10.2 |
| | metabolite I | ... | HPLC | 5 | 6 | 4.40 | 1.40 | 32.30 | 3.1–5.9 |
| | pentobarbital | ... | HPLC | 5 | 6 | 39.20 | 29.10 | 29.10 | 29–57 |
| 11-Blood | salicylate | 300 mg/L | colorimetric | 2.5 | 5 | 203 | 18.20 | 6.0 | 279–328 |
| 12-Blood | propoxyphene | 5.0 mg/L | GC/NPD and GC/MS | 2.5 | 9 | 5.20 | 0.80 | 15.20 | 4.3–6.9 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | norpropoxyphene | 4.0 mg/L | GC/NPD and GC/MS | 2.5 | 7 | 4.30 | 0.40 | 8.60 | 3.9–5.0 |
| | doxepin | 0.40 mg/L | GC/MS | 2.5 | 8 | 0.55 | 0.12 | 22.50 | 0.36–0.66 |
| | nordoxepin | 0.60 mg/L | GC/MS | 2.5 | 8 | 0.93 | 0.36 | 38.30 | 0.55–1.5 |
| 13-Blood | diazepam | 1.0 mg/L | GC/ECD | 2.5 | 6 | 0.94 | 0.05 | 4.8 | 0.87–1.0 |
| | nordiazepam | 1.5 mg/L | GC/ECD | 2.5 | 6 | 1.43 | 0.08 | 5.7 | 1.3–1.5 |
| | morphine | 0.05 mg/L | GC/MS | 2.5 | 4 | 0.058 | 0.005 | 8.7 | 0.05–0.06 |
| | codeine | 0.15 mg/L | GC/MS | 2.5 | 4 | 0.197 | 0.003 | 1.5 | 0.193–0.200 |
| 14-Blood | phenobarbital | 20 mg/L | HPLC | 2.5 | 8 | 18.40 | 2.10 | 11.8 | 15.8–21.0 |
| | carboxyhemoglobin | 30% | UV | 2.5 | 6 | 28.60 | 2.90 | 10.10 | 25–33 |
| 16-Gastric | propoxyphene | 325 mg | GC/NPD | 1 | 3 | 378 | ... | ... | ... |
| contents | acetaminophen | 3250 mg | HPLC | 1 | 8 | 2795 | 131 | 11.7 | 2462–3402 |
| | ethanol | 150 mg/dL | GC/FID | ... | 2 | 160 | ... | ... | ... |
| 17-Blood | propoxyphene | 5.0 mg/L | GC/NPD | 1 | 3 | 8.20 | ... | ... | ... |
| | norpropoxyphene | 4.0 mg/L | GC/NPD | 1 | 3 | 3.0 | ... | 15.0 | ... |
| | acetaminophen | 200 mg/L | HPLC | 1 | 9 | 139 | 46 | 32.9 | 77.6–206.2 |
| | ethanol | 80 mg/dL | GC/FID | ... | 2 | 77 | ... | ... | ... |
| 18-Liver | propoxyphene | ... | GC/NPD | 1 | 3 | 86 | ... | ... | ... |
| | norpropoxyphene | ... | GC/NPD | 1 | 3 | 12 | ... | 15.0 | ... |
| | acetaminophen | ... | HPLC | 1 | 8 | 23.85 | 9.90 | 41.4 | 12.6–35.0 |
| | ethanol | ... | GC/FID | ... | 2 | 80 | ... | ... | ... |
| 19-Urine | propoxyphene | 10 mg/L | GC/NPD | 1 | 3 | 17.0 | ... | 8.0 | ... |
| | norpropoxyphene | 25 mg/L | GC/NPD | 1 | 3 | 23.0 | ... | 15.0 | ... |
| | acetaminophen | 500 mg/L | HPLC | 1 | 5 | 683 | 55.10 | 8.10 | 629–749 |
| | ethanol | 100 mg/L | GC/FID | ... | | 93 | ... | ... | ... |
| 20-Blood | secobarbital | 2.0 mg/L | HPLC | 1 | 6 | 1.8 | 0.10 | 5.60 | 1.7–1.9 |
| | morphine | 0.50 mg/L | GC/MS | 1 | 6 | 0.55 | 0.06 | 9.90 | 0.51–0.63 |
| | codeine | 0.20 mg/L | GC/MS | 1 | 6 | 0.26 | 0.01 | 5.60 | 0.24–0.28 |

[a] Standard deviation, coefficients of variation, and ranges are not reported for an $n$ of less than 4.

[b] GC/MS—gas chromatography/chemical ionization mass spectrometry.
GC/ECD—gas chromatography/electron capture detection.
GC/NPD—gas chromatography/nitrogen phosphorous detection.
GC/FID—gas chromatography/flame ionization detection.

[c] Period between first and last analysis.

**Participation**

The degree of participation (that is, the number of replies received as a percentage of the number of samples shipped) was between 62 and 73% for the four batches and was one of the most encouraging aspects of the study. Previously, this degree of participation had only been reached when considerably greater periods of time for analysis were allowed.

**Qualitative and Quantitative Data**

The detailed qualitative and quantitative results are shown in Tables 3 and 4, respectively. Some drugs were included in different samples at similar concentrations; for example, Samples 4 and 20 contained secobarbital at weighed-in values of 2.5 and 2.0 mg/L, respectively. In addition to quantitative replicates, a number of the samples for which screening was requested contained drugs with similar chemical characteristics, for example amitriptyline (Sample 3) and imipramine (Sample 15). The qualitative and quantitative results will be discussed separately.

*Qualitative Results*

By far the most common analytical techniques used to screen biological samples for the presence of drugs and metabolites are chromatographic procedures. Most analytical forensic toxicologists use a combination of these to identify the drug, before quantitating the agent in biological fluids. During the past 10 to 15 years, GLC with a variety of detectors, including flame ionization and nitrogen phosphorus detectors, has become the technique of choice for the preliminary separation and identification of drugs in autopsy specimens; such detectors satisfy the sensitivity requirements for the detection of drugs and metabolites. Thin-layer chromatography (TLC), however, with a combination of spray reagents is still widely used to screen urine and gastric content. Together with the development of chromatographic procedures, there has been a major advance in the use of immunoassays to screen biological samples for a number of drugs, particularly drugs of abuse. The enzyme multiplied immunoassay technique (EMIT®, Syva Co., Palo Alto, CA 94304) can be used to screen urine samples for morphine and other opiate narcotics, methadone, propoxyphene, cocaine, phencyclidine (PCP), and some other drugs of abuse. Radioimmunoassays (Abuscreen®, Roche Diagnostics, Nutley, NJ 07110) are also available for screening drugs of abuse in urine samples. Certain analysts use immunoassays for the preliminary identification of these drugs in blood as well.

The qualitative results obtained during this study were generally satisfactory, with two exceptions. A significant percentage of false positive results were reported for Sample 12 and there was a low percentage of positive responses for Samples 4, 6, 10, 12, 13, 15, and 20. These two categories will be considered separately.

*False Positive Results*—The rate of false positive results was particularly low with one notable exception. Blood Sample 12 contained propoxyphene, norpropoxyphene, doxepin, and nordoxepin; of the 61 laboratories that performed a qualitative identification on this sample, only 43% detected doxepin and 21% nordoxepin. Eight laboratories (13%) falsely reported nortriptyline and seven (11%) amitriptyline. Doxepin and its *N*-demethylated metabolite (nordoxepin), amitriptyline, and nortriptyline are all tricyclic antidepressants, a group that is frequently encountered in forensic toxicology cases. Although the case history indicated depression, less than half of the laboratories identified doxepin, and a significant percentage misidentified these drugs as other tricyclic antidepressants. In contrast, 82% of the respondents identified propoxyphene and 69% norpropoxyphene, consistent with a history of abdominal pain.

GLC was used by most of the participants to screen and quantitate these particular drugs and metabolites. This technique should, however, be used with caution when identification

TABLE 3—*Qualitative analyses.*

| Sample | Analytes Present | Weighed-In Value | % Positive Response |
|---|---|---|---|
| 1-Blood | Ethanol | 50.00 mg/dL | 95 (70/74) |
| | Diazepam | 1.00 mg/L | 84 (62/74) |
| | Nordiazepam | 1.50 mg/L | 68 (50/74) |
| 2-Blood | Ethanol | 300.00 mg/dL | 100 (74/74) |
| | Carboxyhemoglobin | 60% saturation | 97 (72/74) |
| | Amitriptyline | 0.50 mg/L | 76 (56/74) |
| | Nortriptyline | 0.75 mg/L | 66 (49/74) |
| 3-Urine | Amitriptyline | 2.00 mg/L | 80 (59/74) |
| | Nortriptyline | 3.00 mg/L | 80 (59/74) |
| 4-Blood | Ethanol | 100.00 mg/dL | 97 (71/73) |
| | Methanol | 50.00 mg/dL | 92 (67/73) |
| | Secobarbital | 2.50 mg/L | 33 (24/73) |
| 5-Urine | Morphine | 2.00 mg/L | 88 (65/74) |
| | Methadone | 5.00 mg/L | 96 (71/74) |
| | Methadone metabolite | 10.00 mg/L | 68 (50/74) |
| 6-Urine | Propoxyphene | 20.00 mg/L | 88 (65/74) |
| | Norpropoxyphene | 30.00 mg/L | 84 (62/74) |
| | Salicylate | 100.00 mg/L | 38 (28/74) |
| 7-Blood | Ethanol | 80.00 mg/dL | 95 (69/73) |
| | Flurazepam | 0.80 mg/L | 84 (61/73) |
| | Desalkylflurazepam | 0.50 mg/L | 45 (33/73) |
| 8-Blood | Methaqualone | 15.00 mg/L | 89 (62/70) |
| | Methaqualone metabolite | 7.00 mg/L | 41 (29/70) |
| | Pentobarbital | 10.00 mg L | 80 (56/70) |
| 9-Liver homogenate | Methaqualone | ... | 84 (57/68) |
| | Methaqualone metabolite | ... | 34 (23/68) |
| | Pentobarbital | ... | 76 (52/68) |
| 10-Urine | Cocaine | 20.00 mg/L | 92 (67/73) |
| | Benzoylecgonine | 50.00 mg/L | 66 (48/73) |
| | Dextromethorphan | 2.00 mg/L | 27 (20/73) |
| 11-Blood | Salicylic acid | 300.00 mg/L | 98 (60/62) |
| 12-Blood | Propoxyphene | 5.00 mg/L | 82 (60/62) |
| | Norpropoxyphene | 4.00 mg/L | 69 (42/61) |
| | Doxepin | 0.40 mg/L | 43 (26/61) |
| | Nordoxepin | 0.60 mg/L | 21 (13/61) |
| 13-Blood | Diazepam | 1.00 mg/L | 90 (54/60) |
| | Nordiazepam | 1.50 mg/L | 73 (44/60) |
| | Morphine | 0.50 mg/L | 25 (15/60) |
| | Codeine | 0.15 mg/L | 25 (15/60) |
| 14-Blood | Phenobarbital | 20.00 mg/L | 98 (62/63) |
| | Carboxyhemoglobin | 30% saturation | 91 (57/63) |
| 15-Urine | Meprobamate | 75.00 mg/L | 56 (34/61) |
| | Imipramine | 2.00 mg/L | 87 (53/61) |
| | Desipramine | 3.00 mg/L | 75 (46/61) |
| 16-Gastric contents | Propoxyphene | 325.00 mg total | 69 (45/65) |
| | Acetaminophen | 3250.00 mg total | 49 (32/65) |
| | Ethanol | 150.00 mg/dL | 26 (17/65) |
| 17-Blood | Propoxyphene | 5.00 mg/L | 92 (60/65) |
| | Norpropoxyphene | 4.00 mg/L | 77 (50/65) |
| | Acetaminophen | 200.00 mg/L | 75 (49/65) |
| | Ethanol | 80.00 mg/dL | 88 (57/65) |
| 18-Liver homogenate | Propoxyphene | ... | 77 (48/62) |
| | Norpropoxyphene | ... | 61 (38/62) |
| | Acetaminophen | ... | 48 (30/62) |
| | Ethanol | 150.00 mg/dL | 24 (15/62) |
| 19-Urine | Propoxyphene | 10.00 mg/L | 54 (35/65) |
| | Norpropoxyphene | 25.00 mg/L | 48 (31/65) |
| | Acetaminophen | 500.00 mg/L | 43 (28/65) |
| | Ethanol | 100.00 mg/dL | 48 (31/65) |
| 20-Blood | Secobarbital | 2.00 mg/L | 44 (24/54) |
| | Morphine | 0.50 mg/L | 57 (31/54) |
| | Codeine | 0.20 mg/L | 31 (17/54) |

TABLE 4—*Quantitative analyses (statistical data included only for n ≥ 10).*

| Analyte Method | No. of Labs | Mean | Standard Deviation | CV, % | Range |
|---|---|---|---|---|---|
| | | SAMPLE 1-BLOOD | | | |
| Ethanol, mg/dL | | | | | |
| All methods | 77 | 53 | 11 | 21 | 20-90 |
| Gas chromatography | 70 | 54 | 10 | 19 | 20-90 |
| Gas chromatography internal standard | 46 | 55 | 8 | 15 | 30-71 |
| Enzymatic | 3 | ... | ... | ... | 31-46 |
| Diazepam, mg/L | | | | | |
| All methods | 55 | 1.2 | 0.57 | 48 | 0.3-3.3 |
| Gas chromatography | 46 | 1.1 | 0.61 | 55 | 0.3-3.3 |
| Gas chromatography internal standard | 30 | 1.1 | 0.56 | 51 | 0.45-3.1 |
| High pressure liquid chromatography | 5 | ... | ... | ... | 0.9-1.3 |
| Nordiazepam, mg/L | | | | | |
| All methods | 35 | 1.5 | 0.53 | 35 | 0.68-3.3 |
| Gas chromatography | 32 | 1.4 | 0.52 | 37 | 0.68-3.3 |
| Gas chromatography internal standard | 26 | 1.5 | 0.36 | 24 | 0.92-2.51 |
| High pressure liquid chromatography | 3 | ... | ... | ... | 1.71-2.2 |
| | | SAMPLE 2-BLOOD | | | |
| Ethanol, mg/dL | | | | | |
| All methods | 74 | 281 | 30 | 11 | 170-360 |
| Gas chromatography | 70 | 281 | 30 | 11 | 170-360 |
| Gas chromatography internal standard | 46 | 283 | 29 | 10 | 170-360 |
| Enzymatic | 4 | ... | ... | ... | 250-295 |
| Carboxyhemoglobin, % saturation | | | | | |
| All methods | 71 | 60 | 12 | 20 | 20-85 |
| Co-Oximeter | 17 | 63 | 7 | 11 | 50.3-81.8 |
| Spectrophotometry | 26 | 61 | 11 | 18 | 35-85 |
| Diffusion-palladium chloride | 15 | 56 | 17 | 30 | 20-75 |
| Gas chromatography | 6 | ... | ... | ... | 34.5-72 |
| Amitriptyline, mg/L | | | | | |
| All methods | 49 | 0.51 | 0.25 | 49 | 0.07-1.4 |
| Gas chromatography | 38 | 0.51 | 0.27 | 53 | 0.07-1.4 |
| Gas chromatography internal standard | 21 | 0.49 | 0.25 | 51 | 0.1-1.4 |
| High Pressure liquid chromatography | 8 | ... | ... | ... | 0.2-0.67 |
| Nortriptyline, mg/L | | | | | |
| All methods | 39 | 1.0 | 0.69 | 69 | 0.1-3.44 |
| Gas chromatography | 29 | 0.95 | 0.65 | 68 | 0.1-3.44 |
| Gas chromatography internal standard | 19 | 1.1 | 0.92 | 84 | 0.2-3.44 |
| High Pressure liquid chromatography | 7 | ... | ... | ... | 0.36-1.07 |
| | | SAMPLE 4-BLOOD | | | |
| Ethanol, mg/dL | | | | | |
| All methods | 71 | 102 | 22 | 21 | 40-170 |
| Gas chromatography | 67 | 103 | 22 | 21 | 40-170 |
| Gas chromatography internal standard | 42 | 103 | 23 | 22 | 44.4-170 |
| Enzymatic | 4 | ... | ... | ... | 65-104 |
| Methanol, mg/L | | | | | |

TABLE 4—*Continued.*

| Analyte/Method | No. of Labs | Mean | Standard Deviation | CV, % | Range |
|---|---|---|---|---|---|
| All methods | 63 | 59 | 13 | 22 | 30-87 |
| Gas chromatography | 62 | 59 | 13 | 22 | 30-87 |
| Gas chromatography internal standard | 36 | 59 | 13 | 22 | 30-87 |
| Secobarbital, mg/L | | | | | |
| All methods | 23 | 2.1 | 1.0 | 48 | 0.15-5.0 |
| Gas chromatography internal standard | 15 | 2.1 | 0.9 | 43 | 1.2-5.0 |
| | | SAMPLE 7-BLOOD | | | |
| Ethanol, mg/L | | | | | |
| All methods | 69 | 82 | 8.5 | 10 | 60-104 |
| Gas chromatography | 64 | 82 | 8.5 | 10 | 60-104 |
| Gas chromatography internal standard | 54 | 82 | 8.7 | 11 | 60-104 |
| Enzymatic | 2 | ... | ... | ... | 72-74 |
| Flurazepam, mg/L | | | | | |
| All methods | 54 | 0.97 | 0.56 | 58 | 0.1-3.3 |
| Gas chromatography | 46 | 0.91 | 0.54 | 59 | 0.1-3.3 |
| Gas chromatography internal standard | 40 | 0.93 | 0.56 | 60 | 0.1-3.3 |
| High performance liquid chromatography | 5 | ... | ... | ... | 0.65-2.2 |
| Desalkylflurazepam, mg/L | | | | | |
| All methods | 26 | 0.61 | 0.27 | 44 | 0.18-1.4 |
| Gas chromatography | 21 | 0.59 | 0.28 | 47 | 0.18-1.4 |
| Gas chromatography internal standard | 19 | 0.60 | 0.29 | 48 | 0.18-1.4 |
| High performance liquid chromatography | 4 | ... | ... | ... | 0.41-0.75 |
| | | SAMPLE 8-BLOOD | | | |
| Methaqualone, mg/L | | | | | |
| All methods | 56 | 13 | 4.4 | 34 | 2.7-21.1 |
| Gas chromatography | 48 | 13 | 4.2 | 32 | 2.7-21.1 |
| Gas chromatography internal standard | 37 | 13 | 4.0 | 31 | 2.7-20.0 |
| High performance liquid chromatography | 3 | ... | ... | ... | 12.5-16 |
| Methaqualone metabolite, mg/L | | | | | |
| All methods | 10 | 7.5 | 4.0 | 53 | 1.87-14.1 |
| Gas chromatography | 9 | ... | ... | ... | 1.87-14.1 |
| Pentobarbital, mg/L | | | | | |
| All methods | 53 | 7.6 | 2.3 | 30 | 1.3-13.8 |
| Gas chromatography | 44 | 7.7 | 2.4 | 31 | 1.3-13.8 |
| Gas chromatography internal standard | 35 | 7.7 | 2.4 | 31 | 1.3-12.3 |
| Ultraviolet spectrophotometry | 3 | ... | ... | ... | 6.0-9.0 |
| | | SAMPLE 9-LIVER | | | |
| Methaqualone, mg/L | | | | | |
| All methods | 45 | 8.3 | 3.7 | 45 | 1.5-20 |
| Gas chromatography | 39 | 8.2 | 3.7 | 45 | 1.5-20 |
| Gas chromatography internal standard | 32 | 7.9 | 3.3 | 42 | 1.5-14.5 |
| High performance liquid chromatography | 4 | ... | ... | ... | 8.6-11.3 |
| Methaqualone metabolite, mg/L | | | | | |
| All methods | 7 | ... | ... | ... | 2.7-12.03 |

TABLE 4—*Continued.*

| Analyte/Method | No. of Labs | Mean | Standard Deviation | CV, % | Range |
|---|---|---|---|---|---|
| Pentobarbital, mg/L | | | | | |
| All methods | 41 | 41.5 | 15 | 36 | 12-84.3 |
| Gas chromatography | 32 | 43 | 16 | 37 | 12-84.3 |
| Gas chromatography internal standard | 25 | 42 | 14.5 | 35 | 12-74 |
| | | SAMPLE 11-BLOOD | | | |
| Salicylic acid, mg/L | | | | | |
| All methods | 52 | 295 | 121 | 41 | 100-730 |
| Colorimetric | 22 | 270 | 93 | 34 | 100-400 |
| Ultraviolet | 19 | 296 | 86 | 29 | 190-430 |
| | | SAMPLE 12-BLOOD | | | |
| Propoxyphene, mg/L | | | | | |
| All methods | 42 | 4.63 | 2.0 | 43 | 0.8-10.0 |
| Gas chromatography | 41 | 4.64 | 2.0 | 44 | 0.8-10.0 |
| Gas chromatography internal standard | 35 | 3.83 | 1.9 | 39 | 1.0-10.0 |
| Norpropoxyphene, mg/L | | | | | |
| All methods | 36 | 4.29 | 2.7 | 63 | 0.2-11.0 |
| Gas chromatography | 35 | 4.29 | 2.7 | 63 | 0.2-11.0 |
| Gas chromatography internal standard | 30 | 4.04 | 2.5 | 62 | 0.5-11.0 |
| Doxepin, mg/L | | | | | |
| All methods | 24 | 0.43 | 0.23 | 54 | 0.14-1.0 |
| Gas chromatography | 21 | 0.46 | 0.24 | 52 | 0.14-1.0 |
| Gas chromatography internal standard | 16 | 0.46 | 0.24 | 52 | 0.14-1.0 |
| Nordoxepin, mg/L | | | | | |
| All methods | 11 | 0.70 | 0.38 | 55 | 0.2-1.48 |
| | | SAMPLE 13-BLOOD | | | |
| Diazepam, mg/L | | | | | |
| All methods | 50 | 1.04 | 0.50 | 48 | 0.2-2.6 |
| Gas chromatography | 40 | 1.00 | 0.50 | 50 | 0.2-2.6 |
| Gas chromatography internal standard | 29 | 0.91 | 0.42 | 46 | 0.2-2.4 |
| High pressure liquid chromatography | 6 | ... | ... | ... | 0.80-2.26 |
| Nordiazepam, mg/L | | | | | |
| All methods | 38 | 1.49 | 0.74 | 50 | 0.3-3.5 |
| Gas chromatography | 30 | 1.29 | 0.55 | 43 | 0.3-2.3 |
| Gas chromatography internal standard | 26 | 1.29 | 0.55 | 43 | 0.3-2.3 |
| High pressure liquid chromatography | 5 | ... | ... | ... | 1.32-3.4 |
| Morphine, mg/L | | | | | |
| All methods | 8 | 0.081 | 0.018 | 22 | 0.06-0.09 |
| Codeine, mg/L | | | | | |
| All methods | 14 | 0.28 | 0.13 | 46 | 0.10-0.60 |
| | | SAMPLE 14-BLOOD | | | |
| Phenobarbital, mg/L | | | | | |
| All methods | 60 | 17.3 | 5.6 | 32 | 7.41-36 |
| Gas chromatography | 34 | 15.6 | 6.0 | 38 | 7.41-33 |
| Gas chromatography internal standard | 32 | 16.7 | 5.0 | 30 | 8.07-33 |
| High pressure liquid chromatography | 8 | ... | ... | ... | 9.7-20.6 |
| Ultraviolet spectrophotometry | 7 | ... | ... | ... | 11.36-36 |
| Carboxyhemoglobin, % | | | | | |
| All methods | 51 | 29 | 11 | 38 | 13-50 |

TABLE 4—*Continued.*

| Analyte/Method | No. of Labs | Mean | Standard Deviation | CV, % | Range |
|---|---|---|---|---|---|
| Co-Oximeter | 12 | 34 | 13 | 38 | 16.2-48.4 |
| Spectrophotometry | 18 | 29 | 9 | 31 | 15-47.4 |
| Palladium chloride | 11 | 27 | 12 | 44 | 13-42 |
| Gas liquid chromatography | 6 | | | | 23-50 |
| | SAMPLE 16-GASTRIC CONTENTS[a] | | | | |
| Propoxyphene, mg | 45 | 290.4 | 198.2 | 68 | 35-900 |
| Acetaminophen, mg | 32 | 3228.0 | 1373.0 | 43 | 1400-7530 |
| Ethanol, mg/dL | 17 | 1303.0 | 187.0 | 14 | 1026-1800 |
| | SAMPLE 17-BLOOD[a] | | | | |
| Propoxyphene, mg/L | 60 | 4.7 | 2.2 | 46 | 0.4-10.2 |
| Norpropoxyphene, mg/L | 50 | 4.9 | 3.5 | 71 | 0.2-13.8 |
| Acetaminophen, mg/L | 49 | 179.3 | 57.9 | 32 | 76-332 |
| Ethanol, mg/dL | 57 | 78.0 | 8.2 | 10 | 60-105 |
| | SAMPLE 18-LIVER HOMOGENATE[a] | | | | |
| Propoxyphene, mg/L | 60 | 58.2 | 30.0 | 51.1 | 12.3-130.0 |
| Norpropoxyphene, mg/L | 38 | 16.7 | 10.8 | 64.7 | 1.4-48.0 |
| Acetaminophen, mg/L | 30 | 146.0 | 194.5 | 133.0 | 13.0-780 |
| Ethanol, mg/dL | 15 | 150 | 15.1 | 14 | 76-134 |
| | SAMPLE 19-URINE[a] | | | | |
| Propoxyphene, mg/L | 35 | 11.2 | 4.0 | 35 | 3.0-20.8 |
| Norpropoxyphene, mg/L | 31 | 28.9 | 52 | 52 | 10.6-76.0 |
| Acetaminophen, mg/L | 28 | 639.0 | 256.0 | 40 | 286-1327 |
| Ethanol, mg/dL | 31 | 97.0 | 11.6 | 12 | 70-110 |
| | SAMPLE 20-BLOOD[a] | | | | |
| Secobarbital, mg/L | 24 | 2.4 | 1.0 | 43 | 1.0-4.4 |
| Morphine, mg/L | 31 | 0.59 | 0.23 | 39 | 0.1-1.1 |
| Codeine, mg/L | 17 | 0.25 | 0.05 | 22 | 0.1-0.3 |

[a]The data for Samples 16 through 20 is for all methods. Some obvious outliers were omitted from certain of these data.

is made using a two-column system; Pierce et al [*4*] have reported the retention times relative to prazepam for these compounds on the commonly used OV-17 and OV-1 systems in Table 5.

While other techniques could have been used by the participants to obtain a positive identification, the most definitive procedure is gas chromatography/mass spectrometry (GC/MS), either in the electron impact (EI) or chemical ionization (CI) mode. Doxepin and amitriptyline are both tertiary amines and have base peaks at an $m/z$ value of 58, but their complete fragmentation pattern in the EI mode is characteristic and results in a positive identification. Use of GC/CIMS, with either methane or methane-ammonia as reagent gas, results in the formation of a protonated molecular ion. A number of forensic toxicology laboratories in the United States presently have GC/MS capabilities. Other laboratories might consider it beneficial to examine the use of HPLC for positive identification of the tricyclic antidepressants, although this technique is not altogether free of the problems associated with GLC when these drugs are considered.

*Low Percentage of Positive Responses*—A low percentage of positive responses (when less than 75% of the participants identified the parent drug) was obtained on Samples 4, 6, 10, 12, 13, 15, and 20. These will be considered in numerical order:

SAMPLE 4: Blood Sample 4 was sent to the participants with the following history:

> A 33-year-old truck driver was found dead in the cab of his truck. A bottle of what was suspected to be "wood alcohol" was found beside him. The pathologist requested a blood drug screen and quantitation of any drug detected.

TABLE 5—*Retention time relative to prazepam for these compounds on the commonly used 3% OV-17 and OV-1 (Supelco) systems.*

| Drug Name | 3% OV-17 | 3% OV-1 |
|---|---|---|
| Propoxyphene | 0.65 | 0.69 |
| Norpropoxyphene | 0.83 (0.85) | 0.83 (0.85) |
| Norpropoxyphene amide | 0.94 | 0.94 |
| Doxepin | 0.71 | 0.72 |
| Amitriptyline | 0.67 | 0.70 |
| Nortriptyline | 0.70 | 0.72 |

Ethanol (weighed-in value 100 mg/dL), methanol (weighed-in value 50 mg/dL), and secobarbital (weighed-in value 2.5 mg/L) were included in this sample. Of the laboratories responding 97% identified ethanol, 92% methanol, and only 33% secobarbital. Of the 33% that identified secobarbital, 65% used GLC to quantitate the drug. The other techniques used included ultraviolet spectrometry, HPLC, and immunoassay. Although the blood concentration of 2.5 mg/L is lower than that encountered in fatal cases, it is greater than that resulting from a single dose of the drug. This blood concentration should be detectable by GLC with a flame ionization detection [5], immunoassay procedures [6], and HPLC [7].

SAMPLE 6: Urine Sample 6 was shipped with the following history:

> A 50-year-old male with a history of lower back pain and epileptic seizures was found dead at the base of a set of stairs. An autopsy was performed and the medical examiner requested that a urine sample be screened to establish medication history. Do not quantitate any drugs and/or metabolites detected.

It contained propoxyphene (weighed-in value 20 mg/L), norpropoxyphene (weighed-in value 20 mg/L), and salicylate (weighed-in value 100 mg/L). Of the laboratories responding, 96% positively identified propoxyphene and 84% norpropoxyphene. The procedures used to identify these particular drugs included TLC, GLC, and EMIT. Only 38% positively identified salicylate in this sample; however, the concentration chosen for inclusion in this sample is close to the sensitivity limit of the commonly used color test.

SAMPLE 10: Urine Sample 10 had the following history:

> A 25-year-old male, on probation for drug abuse, was killed while riding his motorcycle. Cause of death was due to multiple injuries. A urine sample was taken, and a drug screen was requested to establish drug use.

It contained cocaine (weighed-in value 20 mg/L), benzoylecgonine (weighed-in value 50 mg/L), and dextromethorphan (weighed-in value 2 mg/L). Of the laboratories responding, 92% positively identified the cocaine and 66% its metabolite; however, only 27% reported the presence of dextromethorphan. The laboratories that identified dextromethorphan used a combination of TLC and GLC. Although the concentration of this drug is lower than that expected from an overdose, it is consistent with therapeutic ingestion for cough suppression, and it should have been detected by those participants using chromatographic techniques.

SAMPLE 12: Blood Sample 12 had the following history:

> A 46-year-old male, with a history of abdominal pain and depression, was found dead in bed by his daughter. A suicide note and several empty prescription bottles were found. Please screen the blood sample to determine the concentration of any drugs and/or metabolites detected. Cause of death: pending toxicology.

This sample was discussed earlier because a significant number of false positive results were reported by the respondents.

SAMPLE 13: Blood Sample 13 was shipped with the following history:

> A 19-year-old female died following a party. One hour before she had been given an injection by her boyfriend who was a known drug abuser. The deceased was known to take minor tranquilizers for anxiety. Please screen the blood sample and determine the concentration of any drugs and/or metabolites detected. Cause of death: pending toxicology.

This sample contained diazepam (weighed-in value 1.0 mg/L), nordiazepam (weighed-in value 1.5 mg/L), morphine (weighed-in value 0.05 mg/L), and codeine (weighed-in value 0.15 mg/L). Of the 60 laboratories responding, 90% positively identified diazepam, 72% nordiazepam, and only 25% morphine and codeine. Baselt [*8*] has reported that blood morphine concentrations range from 0.01 to 2.0 mg/L in heroin fatalities; the morphine concentration in this particular case is certainly at the low end of this scale. The most suitable screening technique for such low concentrations of narcotics in blood samples is radioimmunoassay. The commercially available I-125 kit (Abuscreen, Roche Diagnostics) is designed to react to morphine, but cross-reacts to codeine on approximately a one-to-one basis. Using this particular screening procedure, the participants should have been able to presumptively identify an opiate narcotic in the blood; in fact, one laboratory reported an opiate positive by radioimmunoassay.

SAMPLE 15: Urine Sample 15 was shipped with the following history:

> A 56-year-old female with a history of mental illness was killed in an automobile accident. An autopsy was performed and the medical examiner requested that the urine sample be screened to establish drug use. Do not quantitate any drugs and/or metabolites detected. Do not screen for volatiles.

This sample contained meprobamate (weighed-in value 75 mg/L), imipramine (weighed-in value 2 mg/L), and desipramine (weighed-in value 3 mg/L). Of the laboratories responding, 87 and 75%, respectively, identified imipramine and desipramine. However, only 56% correctly identified the sedative-hypnotic drug meprobamate. Although this drug may not be as widely used as it was several years ago, it is an agent with which forensic toxicologists have had considerable experience. The drug is susceptible to thermal decomposition in the injection port of a gas chromatograph; for this reason it is more reliable to use TLC as a screening technique. Furfural-hydrochloric acid can be used as a selective spray reagent for the detection of carbamates.

SAMPLE 20: This was a blood sample accompanied by the following history:

> A young man was brought comatose to a hospital emergency room by friends but died very quickly afterwards. He had a long history of multiple drug abuse, including opiate narcotics, and there were recent "track marks" noted at autopsy. Please screen the blood sample for drugs and quantitate any drugs and/or metabolites detected.

This sample contained secobarbital (weighed-in value 2.0 mg/L), morphine (weighed-in value 0.5 mg/L), and codeine (weighed-in value 0.2 mg/L). Of the 54 laboratories responding, 45% positively identified secobarbital, 57% morphine, and 21% codeine. Although this history may be considered typical of cases seen from continued drug abuse, and the drugs included in the sample are representative of those encountered on the street, less than half of the laboratories identified secobarbital and codeine, and only 57% positively identified morphine. There was, however, a significant increase in the number of laboratories that positively identified secobarbital when compared to Sample 4; for this sample only 33% posi-

tively identified this barbiturate. Morphine was included at a concentration approximately tenfold greater than that added to Sample 13 and this resulted in an increase in the number of positive responses (57 compared to 25% for Sample 13).

*Metabolite Analysis*—A number of samples contained metabolites of parent drugs; most of these were *N*-dealkylated products and are considered to be pharmacologically active. Several, such as nordiazepam and nortritpyline, are also available as therapeutic agents. Table 6 shows the results of qualitative metabolite analysis; the data have been tabulated as a ratio of the percent positive responses of the parent to the percent positive responses of the metabolite. Only in one case (Sample 3) was this ratio unity; in some instances the ratio was greater than two. The presence of metabolites may aid in the qualitative identification of a particular therapeutic agent and because they are, generally, pharmacologically active it is also desirable to determine their concentrations.

*Conclusion*—Inspection of these qualitative data reveal two major areas of concern: the identification of opiate narcotics in blood samples and the identification of low concentrations of barbiturates. Both these problems may be related to the sensitivity of the analytical procedures used. It is interesting to note that blood Sample 8 containing pentobarbital (weighed-in value 10 mg/L) caused little problem to the participants, with 80% of 70 laboratories identifying the barbiturate. This blood concentration of barbiturate is, of course, more typical of those encountered in fatal cases; however, with the introduction of

TABLE 6—*Metabolite analyses (qualitative).*

| Sample | Analytes Present | Positive Response for Parent: %Positive Response for Metabolite |
|---|---|---|
| 1-Blood | Diazepam | 1.23 |
| | Nordiazepam | ... |
| 2-Blood | Amitriptyline | 1.15 |
| | Nortriptyline | ... |
| 3-Urine | Amitriptyline | 1.00 |
| | Nortriptyline | ... |
| 5-Urine | Methadone | 1.40 |
| | Methadone metabolite | ... |
| 6-Urine | Propoxyphene | 1.04 |
| | Norpropoxyphene | ... |
| 7-Blood | Flurazepam | 1.86 |
| | Desalkylflurazepam | ... |
| 8-Blood | Methaqualone | 2.17 |
| | Methaqualone metabolite | ... |
| 9-Liver homogenate | Methaqualone | 2.47 |
| | Methaqualone metabolite | ... |
| 10-Urine | Cocaine | 1.39 |
| | Benzoylecgonine | ... |
| 12-Blood | Propoxyphene | 1.18 |
| | Norpropoxyphene | ... |
| | Doxepin | 2.00 |
| | Nordoxepin | ... |
| 13-Blood | Diazepam | 1.23 |
| | Nordiazepam | ... |
| 15-Urine | Imipramine | 1.08 |
| | Desipramine | ... |
| 17-Blood | Propoxyphene | 1.20 |
| | Norpropoxyphene | ... |
| 18-Liver homogenate | Propoxyphene | 1.26 |
| | Norpropoxyphene | ... |
| 19-Urine | Propoxyphene | 1.12 |
| | Norpropoxyphene | ... |

more potent therapeutic agents, the detection of drugs and metabolites at milligram per litre concentrations will become increasingly important.

*Quantitative Data*

The most common analytical techniques used for quantitation of drugs and metabolites were chromatographic ones. During the project an attempt was made to evaluate whether there was a statistical difference between those results obtained using internal standards and those obtained by other procedures, such as external standards. In the laboratories of the Advisory Board members, an internal standard is one that is added before the initial step in any extraction and separation procedure. Of the laboratories that indicated they quantitated drugs or metabolites or both by chromatographic techniques, the majority stated that they performed such analyses using internal standards. For example, of the 48 laboratories that quantitated methaqualone by GLC in Sample 8, 38 used an internal standard and, of the 41 laboratories that quantitated propoxyphene in Sample 12, 35 used an internal standard. It was felt, therefore, that an insufficient number of laboratories used alternative procedures to allow statistical evaluation of the advantages (or disadvantages) of using the internal standard procedure for quantitation.

Upon consideration of the quantitative data (Table 4), there was no statistical difference in the standard deviation and mean when individual procedures such as GLC and HPLC were considered; therefore these data are not subdivided into distinct analytical procedures. A number of points arise from a close study of these analytical data:

1. The quantitation of blood ethanol (histograms are shown in Fig. 1) was performed satisfactorily in all cases, as is seen in Table 7.
2. The quantitation of drugs and metabolites other than ethanol was not as consistent. In

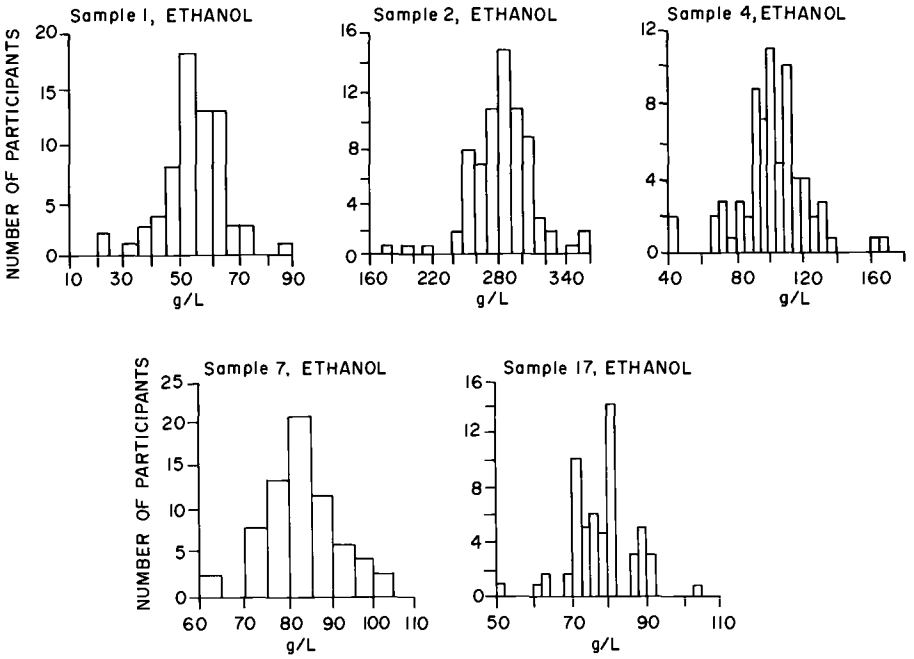


FIG. 1—*Histograms of ethanol data.*

TABLE 7—*Quantitation of blood ethanol.*

| Sample | Weighed-In Value, mg/dL | No. of Labs | Mean, mg/dL | CV, % |
|---|---|---|---|---|
| 1 | 50 | 70 (95%) | 53 | 21 |
| 2 | 300 | 74 (100%) | 281 | 11 |
| 4 | 100 | 71 (97%) | 102 | 21 |
| 7 | 80 | 69 (95%) | 82 | 10 |
| 17 | 80 | 57 (88%) | 78 | 10 |

general, the coefficients of variation were large and no improvement was seen throughout the course of the study. Three particular examples will demonstrate this:

a. The quantitation of diazepam and nordiazepam in Samples 1 and 13 (histograms are shown in Fig. 2). The data obtained by the participants are in Table 8. The coefficient of variation for diazepam in Sample 13 is the same as that for Sample 1 although the mean was closer to the weighed-in value. The coefficient of variation for the quantitation of nordiazepam in Sample 13 was greater than that in Sample 1.

b. Propoxyphene and norpropoxyphene in Samples 12 and 17 (histograms are shown in Fig. 3). The data are in Table 9. These results are similar to those obtained for diazepam and nordiazepam, the coefficient of variation for propoxyphene being approximately the same for Samples 12 and 17, whereas that for the normetabolite increased slightly from Sample 12 to 17. It is interesting to note that there was a greater percent positive response for Sample 17 for both parent and metabolite; the history for this sample indicated that the

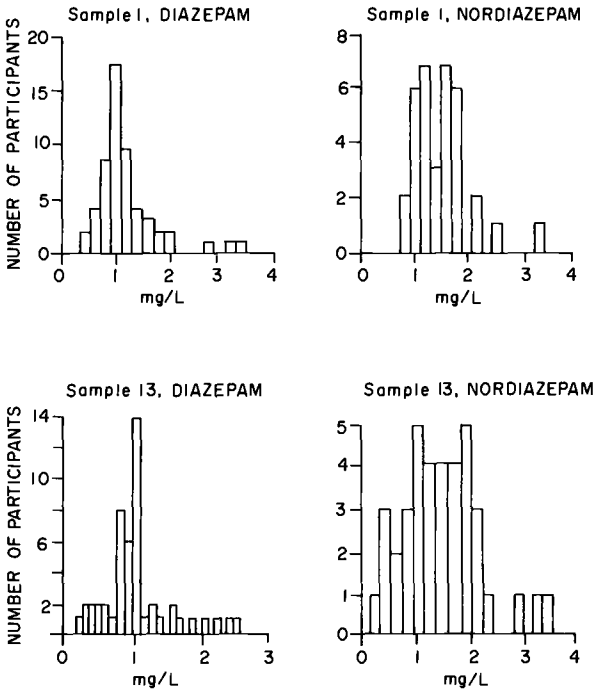


FIG. 2—*Histograms of diazepam and nordiazepam data.*

TABLE 8—*Quantitation of diazepam and nordiazepam in Samples 1 and 13.*

| Sample | Drug | Weighed-In Value, mg/L | No. of Labs | Mean, mg/L | CV, % | Range, mg/L |
|---|---|---|---|---|---|---|
| 1 | diazepam | 1.0 | 55 (74%) | 1.2 | 48 | 0.3-3.5 |
| 13 | diazepam | 1.0 | 50 (83%) | 1.0 | 48 | 0.2-2.6 |
| 1 | nordiazepam | 1.5 | 35 (47%) | 1.5 | 35 | 0.68-3.3 |
| 13 | nordiazepam | 1.5 | 38 (63%) | 1.5 | 59 | 0.3-3.5 |

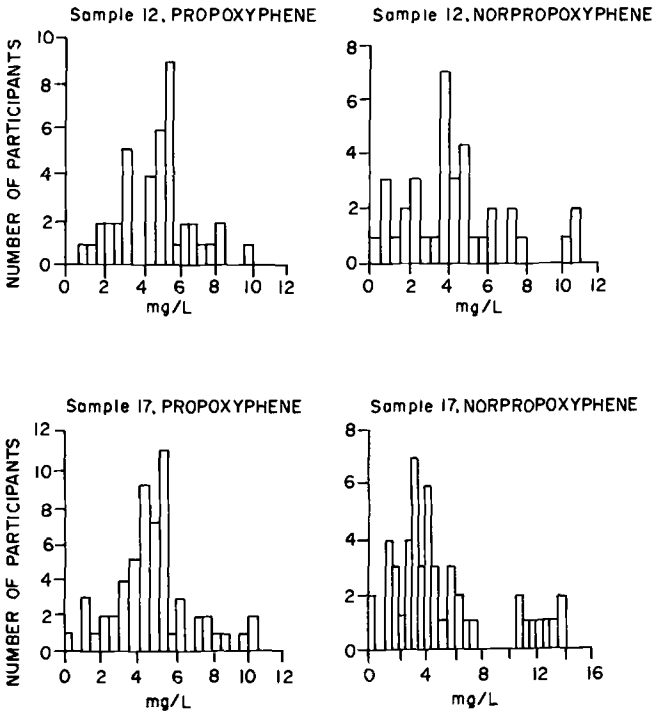


FIG. 3—*Histograms of propoxyphene and norpropxyphene data.*

TABLE 9—*Quantitation of propoxyphene and norpropoxyphene in Samples 12 and 17.*

| Sample | Drug | Weighed-In Value, mg/L | No. of Labs | Mean, mg/L | CV, % | Range, mg/L |
|---|---|---|---|---|---|---|
| 12 | propoxyphene | 5 | 42 (69%) | 4.6 | 43 | 0.8-10 |
| 17 | propoxyphene | 5 | 60 (92%) | 4.7 | 46 | 0.4-10.2 |
| 12 | norpropoxyphene | 4 | 36 (59%) | 4.3 | 63 | 0.2-11 |
| 17 | norpropoxyphene | 4 | 50 (76%) | 4.9 | 71 | 0.2-13.8 |

deceased had been prescribed Darvocet®. However, the coefficients of variations for quantitation were similar even though a greater number of laboratories responded.

c. Secobarbital in Samples 4 and 20 (histograms are shown in Fig. 4). The data are in Table 10. The coefficients of variation for Samples 4 and 20 were similar.

Two samples, 9 and 18, were aliquots of a liver homogenate prepared from rat liver. Sample 9 contained methaqualone, methaqualone metabolite I, and pentobarbital and Sample 18 contained propoxyphene, norpropoxyphene, acetaminophen, and ethanol. In general, the coefficients of variation for the quantitative determination of these drugs in liver homogenates were similar to those for the same analyses in blood. However, there was a noticeable increase in the coefficient of variation for the analysis of acetaminophen in liver homogenate when compared to that from the analysis of blood. For blood the coefficient of variation was 32%, whereas for liver it was 133%. The reason for this is unknown, and the phenomenon warrants further investigation.

In addition to ethanol and other drugs and their metabolites, two blood samples were also partially saturated with carbon monoxide. The percent of carboxyhemoglobin in Sample 2 was 60% and that in Sample 14 was 30%. The coefficient of variation for the sample with

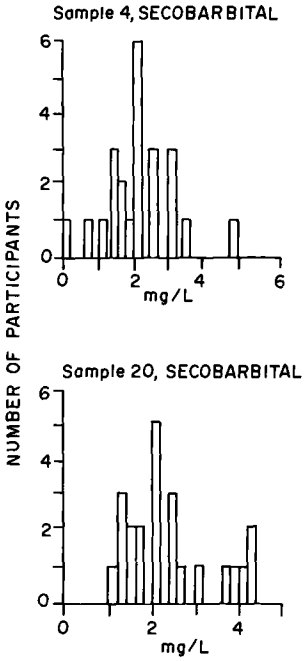


FIG. 4—*Histograms of secobarbital data.*

TABLE 10—*Quantitation of secobarbital in Samples 4 and 20.*

| Sample | Drug | Weighed-In Value, mg/L | No. of Labs | Mean, mg/L | CV, % | Range, mg/L |
|---|---|---|---|---|---|---|
| 4 | secobarbital | 2.5 | 23 (32%) | 2.1 | 48 | 0.15–5 |
| 20 | secobarbital | 2.5 | 24 (44%) | 2.4 | 43 | 1–4.4 |

the higher concentration was 20% and for the lower sample was 38%. It is difficult to explain this increase in the coefficient of variation when both samples contained significant amounts of carboxyhemoglobin. It is notable that the use of a CO-Oximeter® in Sample 14 resulted in a coefficient of variation of 38%, whereas the same technique had a coefficient of variation of 11% in Sample 2.

These particular examples demonstrate the considerable interlaboratory variation in quantitation. Comparison with results from other proficiency testing programs, particularly the College of American Pathologists Advanced Toxicology Survey Program, however, is illuminating. When chromatographic techniques are used by participants in these proficiency testing programs, coefficients of variation similar to those seen in this study are observed. For example, a serum sample containing 1 mg/L of propoxyphene and norpropoxyphene was analyzed in 1981. The coefficients of variation for quantitation by GLC were 49 and 64%, respectively. It is true, however, that much lower coefficients of variation are obtained in these programs when techniques such as EMIT are used for quantitating drugs in plasma samples. It must be remembered, however, that such quantitative immunoassay techniques are presently designed for the analysis of plasma or serum samples and not for the direct analysis of hemolyzed blood samples.

Examination of the coefficients of variation obtained during this study for the quantitation of chemical agents in biological fluids is interesting in view of a recent article by Horwitz [9]. Using a summary of interlaboratory data obtained from over 150 independent Association of Official Analytical Chemists collaborative studies, he reported that precision could be represented by the following equation, which is independent of analytical technique used and such external influences as sampling and contamination:

$$CV(\%) = 2^{(1 - 0.5 \log C)}$$

where $C$ is the concentration expressed as an exponent (for example, 1 ppm = $10^{-6}$). The coefficient of variation $CV$ doubles for each decrease of concentration of two orders of magnitude. The between-laboratory $CV$ at 1 ppm is 16% ($2^4$). Although, this represents the ideal, none of the quantitative determinations performed during the course of the study in the mg/L ($10^{-6}$) range has a $CV$ within this range. In addition, determination of ethanol in the majority of samples is outside the $CV$ expected for a concentration of 100 mg/dL.

Although the precision studies demonstrate a wide interlaboratory variation in the quantitation of drugs and metabolites from biological media, the mean concentrations obtained are similar to the weighed-in values. In general, therefore, the method used by the participants satisfies requirements of accuracy.

*Acknowledgments*

This study would not have been possible without the cooperation of forensic toxicologists throughout the United States. The advice and guidance of the Advisory Board (Drs. Randall C. Baselt, Leonard R. Bednarczyk, Kurt M. Dubowski, Patricia H. Field, Bryan S. Finkle, James C. Garriott, and Arthur J. McBay) are also gratefully acknowledged. The authors thank Ms. Cindy Daybell for her secretarial assistance. This study was supported by the National Institute of Justice Grant 80-IJ-CX-0072.

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