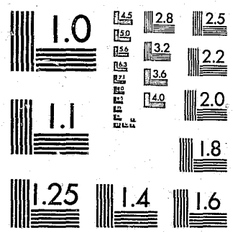

Report

University of Utah

FORENSIC TOXICOLOGY LABORATORY

PROFICIENCY TESTING RESEARCH PROGRAM

Grant No. 80-IJ-CX-0072

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Law Enforcement Assistance Administration
Office to Justice Assistance, Research,
and Statistics
Washington, D.C. 20531

FINAL REPORT

BY

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EXECUTIVE SUMMARY

This study has shown that:

1. A National Proficiency Testing Program in forensic toxicology is feasible. Samples that resemble typical case specimens were prepared and shipped to approximately 100 laboratories. The response rate varied between 62 and 73%.
2. Tissue samples prepared from laboratory animals can be used to simulate those encountered by forensic toxicologists. This has been demonstrated using homogenates containing pentobarbital and methaqualone, and propoxyphene and norpropoxyphene. There was a large coefficient of variation however, for the quantitation of acetaminophen in liver.
3. The qualitative data obtained during the course of this study showed a very low incidence of false positives. However there was a small percentage of positive responses for (a) low concentrations of secobarbital and (b) the opiate narcotics (morphine and codeine) in blood, despite the fact that immunoassay procedures are available for screening these particular compounds in blood samples.
4. The quantitative determination of drugs and metabolites, other than ethanol, shows wide interlaboratory variation. Although it could have been postulated that this was due to the use of different instrumental techniques, by far the most common technique used was gas liquid chromatography.

5. The Advisory Board feels that the results of this study were encouraging. In particular, this study has shown laboratories are willing to participate in such a proficiency testing program and that satisfactory analytical results were obtained.

INTRODUCTION

It was the general purpose of the research described in this report to make 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, obviously, toxicologists have a key role to play in any investigation which purports to record or interpret drug involvement. These investigations demand technical procedures which are at the forefront of modern analytical capability in order to detect and assay the drugs and metabolites in biological fluids. It was reasonable therefore, that an applied research project be undertaken to evaluate the proficiency of toxicologists to accurately determine these agents in biological fluids. Many forensic toxicologists currently subscribe to other proficiency testing programs, such as clinical toxicology or drug abuse testing. The program described in this report however, was designed to simulate case samples seen in typical forensic toxicology laboratories and included hemolyzed blood and tissue 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, leading ultimately to an improvement in the standards of laboratory practice.

In order to replicate typical cases seen in forensic toxicology laboratories the choice of drugs and metabolites chosen to serve as tests, in this one year research program, was conditioned by several important considerations. Firstly, it was not intended to provide test specimens containing unrealistic combinations of drugs or extremely unusual or bizarre compounds. Selection was made after reviewing several annual report prepared by toxicologists and after consultations with the Advisory Board members, leading to the inclusion of drugs that were most commonly encountered by toxicologists. A number of these agents were known to provide some difficulty for the analyst. Secondly, the tests samples should simulate typical case specimens and this was achieved by using whole blood, urine, gastric contents and homogenized tissues. In order to encourage participation an Interim Report was issued after the results of each batch of samples had been received and processed at the Center for Human Toxicology (CHT). This report included the analytical results of the batch of samples that had been previously sent out. Methodology used by the participants and a brief review of methods that have been published in the literature for the analysis of the included drugs and metabolites, together with a statistical analysis of the data, were also included.

This project could not have been completed without the advice and guidance of the Advisory Board. The purpose of this Committee was to provide recommendations for the preparation of samples and the selection of drugs and/or metabolites. The Board consisted of eight members and met three times during the course of the project. The first meeting was

held approximately six weeks after the project start date. At this time the detailed work plan was critiqued and decisions made on the samples to be included in the project. The second meeting was held after the receipt of the first set of results from the participating laboratories and was held solely to evaluate the initial data and to recommend any procedural changes that may have been required. The final meeting was held at the end of the project to review the final report and to approve the recommendations made therein. The Advisory Board consisted of:

Michael A. Peat (Chairman)	University of Utah, Salt Lake City
Members	
Dr. Randall Baselt	University of California, Davis
Dr. Leonard Bednarczyk	Medical Examiners Dept., Miami
Dr. Kurt Dubowski	University of Oklahoma, Oklahoma City
Dr. Patricia Field	State Laboratory of Hygiene University of Wisconsin, Madison
Dr. Bryan Finkle	University of Utah, Salt Lake City
Dr. James Garriott	Southwestern Institute of Forensic Science, Dallas
Dr. Arthur McBay	Office of Chief Medical Examiner, Chapel Hill

Potential partici ts in the project were contacted by letter. These people were selected from the membership lists 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.

The letter sent to each potential participant outlined the scope of the proposed study and benefits of participation; and requested their cooperation in the project. A copy of the letter is included in Appendix A. Positive responses were received from 105 laboratories; each State, except Hawaii, was represented in the project.

Two decisions resulted from the first Advisory Board meeting. Concern was expressed among the Advisory Board members regarding the confidentiality of the results, it was therefore unanimously agreed that the participants would be requested to return their results in a "double envelope" (i.e. in a plain white envelope inside a previously addressed envelope) to a disinterested party. The disinterested party would then forward the envelopes containing the results to the Center. This procedure was followed throughout the course of the project. The second decision concerned the number of batches of samples that should be shipped to the participants over a period of approximately nine months; after some discussion it was decided to send four (4) batches of five (5) samples to each participant. Table 1 represents the unaminous opinion of the Board members concerning (a) samples types, (b) drugs to be included and (c) suggested concentrations ranges. Quantitation was requested on samples 1, 2, 4, 7, 8, 9, 11, 12, 13, 14, 16, 17, 19 and 20; the remainder of the samples were to be screened only. This list was followed with one minor exception; because of problems encountered by the participants with sample 13 the content of sample 20 was changed to include morphine, codeine and secobarbital. The Advisory Board agreed that the turn around time of each batch be variable, depending upon the

difficulty of the test samples. The simpler analyses were to be completed in two weeks and the more difficult ones in three weeks. Together with each batch of samples a report form was issued to the participants. Copies of these report forms are included in Appendix B. These report forms were of the same format throughout the course of the study with one minor change being made after the first batch of samples. The change consisted of the addition of a column asking for information on the use of internal or external standards.

SAMPLE PREPARATION AND ANALYSIS

The blood and urine samples were prepared by dissolving appropriate amounts of the drugs or salts of the drug in water and then using these solutions to spike bovine blood or human urine. Both the blood and urine had been extensively screened by sensitive analytical procedures prior to the addition of drug or metabolite. Sample 16 (gastric contents) was prepared at CHT by adding an appropriate amount of the pharmaceutical preparation to a simulated gastric contents. 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 thirty day period. The animals were sacrificed, their livers removed, combined and homogenized with water. An aliquot of this homogenate was then shipped to each participant. Samples were shipped to the participants so that they reached the laboratories between twenty four and thirty six hours after shipment. All samples were shipped in glass containers at 4°C.

The samples were analyzed at the Center for Human Toxicology throughout the course of the project to determine the stability of drugs and/or metabolites. After preparation, the samples were stored at -15°C and at regular intervals aliquots were taken and analyzed. Table 2 shows the results of these analyses.

For all analyses performed at CHT, the within-run precision studies had coefficients of variation less than 10%. It is apparent from Table

2 that when these analytes were quantitated over a period of time, the coefficients of variation increased significantly for a number of them. Volatiles were only determined at the time of shipment and during the period of analysis. those samples that were to be screened only were tested qualitatively throughout the project and found to be positive.

If a longer proficiency testing program was to be established it would be inadvisable to prepare a sample batch at day 1, and expect the analyte concentrations to be within 10% of the weighed-in value two years later, for example, without question, further studies are needed to determine the optimum proceduring for stabilizing drug and/or metabolite concentrations in simulated forensic toxicology samples.

RESULTS

PARTICIPATION

The rate of participation for the four batches was one of the most encouraging aspects of this study. Previous attempts at proficiency testing in the forensic toxicology profession on a nationwide basis, by the American Academy of Forensic Sciences, resulted in a 66% response rate when considerably greater periods of time were given to reply. In this study, when the response time was limited to 3 weeks, a similar response rate was achieved on all batches of samples. A 73% response rate was achieved with Batches 1 and 2, a 62% response rate with Batch 3 and a 64% response rate with Batch 4.

QUALITATIVE AND QUANTITATIVE DATA

Although the detailed results, both quantitative and qualitative, are included in Appendix C, for the sake of clarity they have been re-tabulated in Tables 3 and 4. During the course of the project some drugs were included in different samples at similar concentrations; for example, samples 4 and 20 contained secobarbital, the weighed-in values were 2.5 mg/L and 2.0 mg/L respectively. Codeine and morphine were included in samples 13 and 20, diazepam and nordiazepam in samples 1 and 13, tricyclic antidepressants were included in samples 12, 16, 17, 18 and 19, and ethanol was included at various concentrations in a number of samples. In addition to these quantitative replicates, a number of the samples for which screening only was requested contained drugs with similar chemical characteristics. The qualitative and

quantitative results will be considered separately.

QUALITATIVE RESULTS

Introduction

By far the most common analytical techniques used to screen biological samples for the presence of drugs and metabolites are chromatographic procedures. Most practicing analytical forensic toxicologists use a combination of these procedures to identify the drug, before quantitating the agent in biological fluids. During the past 10 to 15 years gas liquid chromatography (GLC) with a number of detectors, including flame ionization and nitrogen phosphorous detectors, has become the technique of choice for the preliminary identification of drugs in autopsy specimens. These detectors satisfy the sensitivity requirements for the detection of drugs and metabolites in such samples. However, thin layer chromatography (TLC) with a combination of spray reagents is still widely used to screen urine and gastric contents. Together with the development of chromatographic procedures there has been a tremendous advance in the use of immunoassays to screen biological samples for a number of drugs, particularly the drugs of abuse. The enzyme multiplied immunoassays technique (EMIT ®, Syva) can be used to screen for morphine and other opiate narcotics, methadone, propoxyphene, cocaine, phencyclidine (PCP) and other drugs of abuse in urine samples. Radioimmunoassay techniques (Abuscreen ®, Roche Diagnostic) are available for screening the drugs of abuse in urine samples, and a number of groups have also used these techniques for

the preliminary identification of drugs in blood.

The qualitative results obtained during the course of this project were satisfactory, with some exceptions. These fall into two categories, those with a significant incidence of false positives reported and those samples in which there was a low rate of positive responses. These will be considered separately.

False Positives

The rate of false positives was particularly low throughout the course of this study with one noteable exception. Sample 12 was a blood sample which 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; of greater concern, however was the fact that eight laboratories reported nortriptyline and seven amitriptyline. Doxepin and its N-demethylated metabolite (nordoxepin), amitriptyline and nortriptyline are all members of the class of drugs known as the tricyclic antidepressants, a group that is becoming more frequently encountered in forensic toxicology cases. Although the history indicated depression less than half of the laboratories responding 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 the majority of the participants to screen and quantitate the particular drugs and met-

abolites. For these drugs, this technique should be used with caution when identification is made using a two column system; Pierce et al (1) have reported the following relative retention times (to prazepam) for these compounds on the commonly used OV-17 and OV-1 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

While other techniques could have been used by the participants to positively identify these particular drugs, the most definitive procedure is gas chromatography-mass spectrometry, either in the electron impact or chemical ionization mode. Although doxepin and amitriptyline are both tertiary amines and have base peaks at an m/z of 58, their complete fragmentation pattern in the electron impact mode, results in a positive identification. Use of chemical ionization mass spectrometry, with either methane or methane ammonia as reagent gas, results in the formation of a molecular ion at the corresponding molecular weights. Although a number of forensic toxicology laboratories in this country presently have GC-MS capabilities, these are still in the minority. Other laboratories might consider it beneficial to examine the use of high performance liquid chromatography (HPLC) for positive identification of the tricyclic anti-

depressants; although this technique itself has many problems when these drugs are considered.

Low Percentage of Positive Response

There were a number of samples in which there was a low percent of positive responses (when less than 75% of the participants identified the parent drug) these samples were 4, 6, 10, 12, 13, 15 and 20; they will be considered in numerical order:

Sample 4: Sample 4 was a blood sample which was sent to the participants in the first batch of samples, 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 drugs detected.

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. 97% of the laboratories responding identified ethanol, 92% methanol and only 33% secobarbital. Of the 33% that identified secobarbital 65% used GLC to quantitate the drug. Other techniques that were used to identify secobarbital included ultra violet spectrometry, HPLC and immunoassay techniques. Although the blood concentration of 2.5 mg/L is lower than that expected in toxic situations and therefore customarily encountered in fatal cases, it is higher than that resulting from a single dose of the drug. This concentration should be detectable by GLC with flame ionization detectors (2) immunoassay procedures (3) and HPLC (4).

Sample 6: This was a urine sample included in batch 2 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.

This sample 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 74 laboratories that responded, 96% positively identified propoxyphene and 84% norpropoxyphene. By far the commonest procedures used to identify these particular drugs were TLC, GLC and EMIT. Only 38% positively identified salicylate as being present in this sample; however, the concentration chosen for inclusion in this sample approaches the sensitivity limit of the commonly used color test.

Sample 10: This was a urine sample included in Batch 2 with 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.

This sample contained cocaine (weighed-in value 20 mg/L), benzoylecgonine (weighed-in value 50 mg/L) and dextromethorphan (weighed-in value 2 mg/L). 73 laboratories responded to this sample, of these 92% positively identified the cocaine and 66% its metabolite; however, only

27% reported the presence of dextromethorphan. The laboratories which positively identified dextromethorphan used a combination of thin layer and gas liquid chromatography. Although the concentration of this drug is lower than that expected from a overdose it is reasonable following therapeutic ingestion for cough suppression, it should be detected by those participants who used chromatographic techniques.

Sample 12: This was a blood sample included in Batch 3 with 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 was the sample discussed earlier (page 12) in which a significant number of false positives were reported by the respondents.

Sample 13: This was a blood sample included in Batch 3 together 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, 73% nordiazepam, and only 25% morphine and codeine. The case history for this sample represents the situation whereby a single dose of narcotic may have been given to the deceased. Baselt (5) has reported that blood morphine concentrations range from 0.01 to 3.0 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 in radioimmunoassay. The commercially available I-125 Kit (Abuscreen®, Roche Diagnostic) which is designed to react to morphine, cross-reacts to codeine on approximately a one-to-one basis. Using this particular technique for screening sample 13 the participants would have been able to presumptively identify an opiate narcotic in the blood; in fact one laboratory reported an opiate positive by RIA. It is strongly recommended that those laboratories, with access to a gamma counter, consider using RIA screening procedures for certain drugs in blood samples. This point is emphasized again when sample 20 is considered.

Sample 15: This was a urine sample included in Batch 3 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 61 laboratories who responded to this sample, 87% and 75% respectively identified imipramine and desipramine. However, only 56% identified the sedative hypnotic drug meprobamate. Although this drug may not be widely used in certain areas of this country, it is an agent with which the forensic toxicologist has had considerable experience. This drug itself is susceptible to thermal decomposition in the injection port of a gas chromatography, 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. It is noteworthy that the identification of imipramine and desipramine, two other examples of tricyclic antidepressants, did not cause any problem to the participants in this sample.

Sample 20: This was a blood sample that was included in Batch 4, the history was as follows:

> A young man was brought comatose to a hospital ER 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 44% positively identified secobarbital, 57% morphine and 31% codeine. Although this history may be considered typical of cases seen from continued drug abuse, and the drugs included in the sample representative of those

encountered on the street, less than half of the 54 laboratories replying identified secobarbital and codeine, and only 57% positively identified morphine. There was however, a significant increase in the number of laboratories who positively identified secobarbital when compared to sample 4; in that sample only 33% positively identified this barbiturate. Morphine was included at a concentration approximately ten fold greater than that added to sample 13, this resulted in an increase in the number of positive responses (57% compared to 25% for sample 13). The comments, however concerning the most suitable method for screening opiate narcotics in blood samples still apply.

Metabolite Analysis

A number of the samples included metabolites of parent drugs. The majority of these metabolites were N-dealkylated products of the parent drug and are considered to be pharmacologically active. It must also be remembered that a number of them, for example nordiazepam and nortriptyline, are available as therapeutic agents alone. Table 5 shows the results of the qualitative metabolite analysis; the data has been tabulated as a ratio of the percent positive responses of the parent to the percent positive responses of the metabolite. Only one case (sample 3) was this ratio unity. In some cases this ratio was greater than two. These metabolites may also aid in the qualitative identification of a particular therapeutic agent. It is also important to quantitate these pharmacologically active metabolites.

Although a number of the metabolites are available from commercial sources, for example methadone metabolite and benzoylecgonine can be purchased from Applied Science Incorporated and others can be obtained from pharmaceutical companies; some of them may only be obtainable by chemical synthesis.

From these qualitative data there are two major areas of concern. Firstly, the identification of opiate narcotics in blood samples and secondly, the identification of low concentrations of barbiturates. It is interesting to note that sample 8, a blood sample containing pentobarbital (weighed-in value 10 mg/L) caused little problem to the participants with 80% of the 70 laboratories responding positively identifying the barbiturate. This blood concentration of barbiturate is of course more typical of those encountered in fatal cases.

QUANTITATIVE DATA

As with the initial screening results the most common analytical techniques used for quantitation are 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 prior to the initial step in any extraction and separation procedure. Of the laboratories that indicated they quantitated drugs and/or metabolites by chromatographic techniques, the majority stated

that they performed such analyses using internal standards, for example, of the 48 laboratories who quantitated methaqualone by gas chromatography in sample 8, 38 used an internal standard and of the 41 laboratories who quantitated propoxyphene in sample 12, 35 used an internal standard. There was not therefore sufficient number of laboratories, who used other procedures, for comparitive purposes to arrive at a statistically valid conclusion concerning the use of internal standards in quantitative procedures.

The histograms for quantitative examinations are shown in Figures 1 to 38. These histograms represent the total quantitative data, there being no statistical difference in standard deviation and mean when individual procedures, such as GLC or HPLC were considered. A number of points are obvious from studying these figures:

1. The quantitation of blood ethanol was performed satisfactorily in all cases. The following is a tabulation of the data obtained by the responding laboratories.

SAMPLE NUMBER	WEIGHED-IN VALUE (mg/dL)	NO. OF LABS.	MEAN (mg/dL)	C.V. %
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

2. The quantitation of drugs and metabolites, other than ethanol, was not as satisfactory. In general, the coefficient of variations 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. The data obtained by the participants is tabulated below:

SAMPLE NUMBER	DRUG	WEIGHED-IN VALUE (mg/L)	NUMBER OF LABS.	MEAN (mg/L)	C.V.%	RANGE (mg/L)
1	Diazepam	1.0	55 (74%)	1.2	48	0.3 - 3.5
13		1.0	50 (83%)	1.04	48	0.2 - 2.6
1	Nordiazepam	1.5	35 (47%)	1.5	35	0.68 - 3.3
13		1.5	38 (63%)	1.49	50	0.3 - 3.5

The coefficient of variation for diazepam in sample 13 is the same as that for sample 1 although the mean was nearer the weighed-in value. The coefficient of variation for the quantitation of nordiazepam in sample 13 was higher than that in sample 1.

b. Propoxyphene and norpropoxyphene in sample 12 and 17. The data are tabulated below:

SAMPLE NUMBER	DRUG	WEIGHED-IN VALUE (mg/L)	NUMBER OF LABS.	MEAN (mg/L)	C.V.%	RANGE (mg/L)
12	Propoxyphene	5	42 (69%)	4.63	43	0.8 - 10
17		5	60 (92%)	4.7	46	0.4 - 10.2
12	Norpropoxyphene	4	36 (59%)	4.29	63	0.2 - 11
17		4	50 (76%)	4.9	71	0.2 - 13.8

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 from both parent and metabolite; the history for this sample indicated that the deceased had been prescribed Darvocet ®. However, the coefficient of variations for quantitation were similar, although an increasing number of laboratories responded.

c. Secobarbital in samples 4 and 20. The data are tabulated below:

SAMPLE NUMBER	DRUG	WEIGHED-IN VALUE (mg/L)	NUMBER OF LABS.	MEAN (mg/L)	C.V.%	RANGE (mg/L)
4	Secobarbital	2.5	23 (32%)	2.1	48	0.15 - 5
20		2.0	24 (44%)	2.4	43	1 - 4.4

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 was a liver homogenate that contained methaqualone, methaqualone metabolite 1 and pentobarbital and sample 18 was one that contained propoxyphene, norpropoxyphene, acetaminophen and ethanol. In general the coefficients of variation for the quantitative determination of these drugs in liver homogenate were similar to those for the same analyses in blood. However, when the analysis of acetaminophen in liver is considered there is a noticeable increase in the coefficient of variation over that obtained from the analysis of blood. For blood

the coefficient of variation was found to be 32%, whereas that for liver 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 saturation of carboxyhemoglobin in sample 2 was 60% and that in sample 14 was 30%. The coefficient of variation for the sample 60% saturated was 20% and that for the other 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 noticeable 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 for quantitation. Comparison with other proficiency testing programs, particularly the College of American Pathologists Toxicology Proficiency Program, however are 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 immunoassay techniques are applicable only to the analysis of plasma or serum samples and not the analysis of hemolyzed blood samples.

RECOMMENDATIONS

The Advisory Board and the Principal Investigators made the following recommendations to the National Institute of Justice:

1. That a continuing proficiency testing program, similar to this one, be established to form the basis of a continuing evaluation of performance in analytical forensic toxicology.
2. This continuing program should be for a time period of not less than 3 years, to include samples that replicate typical case samples seen in forensic toxicology laboratories and that a coding system be introduced by which laboratories remain anonymous, but which could also be used to note improvements in a laboratory performance. This coding system would also have the advantage of observing whether particular results bias the total data to the low or high end. This program would attempt to include all forensic toxicology laboratories, it would include agents other than drugs or metabolites and possibly include non-biological samples (for example a sample may be included that would replicate contents of a syringe). It is the unanimous recommendation of the Advisory Board that the present format should be continued; i.e. the program should be organized and administered by practicing forensic toxicologists with the advice and guidance of an Advisory Board consisting

of respected members of the profession.

3. An educational program be established to operate on a nationwide basis. This program would have several aspects to it, including the establishment of workshops, literature reviews and surveys and an analytical toxicology training program. Reference materials and methodologies used by the laboratory of the Principal Investigator would be made available upon request. In addition, consultant assistance would be available to the participants.
4. There is a need to evaluate modern analytical procedures for their application in forensic toxicology. These evaluations should be undertaken by qualified forensic toxicologists and will be made available in published reports to practicing toxicologists. This program would offer advice and guidance on analytical procedures to be used for the determination of drugs, their metabolites and other agents in biological fluids. The establishment of such a laboratory within the Forensic Science Service in the United Kingdom has been a success.
5. National Institute of Justice consider establishing a program by which metabolites of parent drugs be made available to practicing forensic toxicologists.
6. The Advisory Board recommends that the National Institute of Justice or other government agencies make this final report available for distribution.

LIST OF TABLES

Table 1: Drugs and Samples Included in the Project.
Table 2: Stability Studies in Samples (for Quantitation).
Table 3: Results of Qualitative Analyses.
Table 4: Results of Quantitative Analyses.
Table 5: Metabolite Analyses (Qualitative).

Table 1: Drugs and Samples to be included in the Project

Batch #	Sample #	Sample	Drug	Concentration
I	1	Blood	Diazepam	1.0 mg/L
			Nordiazepam	1.5 mg/L
			Ethanol	0.05 mg/dL
	2	Blood	Carboxyhemoglobin	60%
			Amitriptyline	0.5 mg/L
			Nortriptyline	0.75 mg/L
			Ethanol	0.3 mg/dL
	3 (Paired with #2)	Urine	Ethanol	0.4 mg/dL
			Amitriptyline	2.0 mg/L
			Nortriptyline	3.0 mg/L
	4	Blood	Ethanol	0.1 mg/dL
			Methanol	0.05 mg/dL
			Secobarbital	2.0 mg/L
	5	Urine	Morphine	2.0 mg/L
			Methadone	5.0 mg/L
			Methadone Metabolite	10.0 mg/L
II	6	Urine	Propoxyphene	20.0 mg/L
			Norpropoxyphene	30.0 mg/L
			Salicylate	100.0 mg/L
	7	Blood	Ethanol	0.1 mg/dL
			Flurazepam	0.8 mg/L
			Desalkylflurazepam	0.5 mg/L
	8	Blood	Methaqualone	15.0 mg/L
			Metabolite I	25.0 mg/L
			Pentobarbital	10.0 mg/L
	9 (Paired with #8)	Liver	Methaqualone	
			Metabolite I	
			Pentobarbital	
	10	Urine	Cocaine	20.0 mg/L
			Benzoylecgonine	50.0 mg/L
			Dextromethorphan	2.0 mg/L
III	11	Blood	Salicylate	300.0 mg/L
	12	Blood	Propoxyphene	5.0 mg/L
			Norpropoxyphene	4.0 mg/L
			Doxepin	0.4 mg/L
			Nordoxepin	0.6 mg/L

Table 1: Drugs and Samples to be included in the Project (cont.)

Batch #	Sample #	Sample	Drug	Concentration
	13	Blood	Diazepam	1.0 mg/L
			Nordiazepam	1.5 mg/L
			Morphine	0.05 mg/L
			Codeine	0.15 mg/L
	14	Blood	Phenobarbital	20.0 mg/L
			Carboxyhemoglobin	30%
	15	Urine	Meprobamate	75.0 mg/L
			Imipramine	2.0 mg/L
			Desipramine	3.0 mg/L
IV	16	Blood	Propoxyphene	325.0 mg/L
			Norpropoxyphene	
			Ethanol	0.1 mg/dL
			Acetaminophen	100.0 mg/L
	17 (Matched with #16, 18, 19)	Liver	Propoxyphene	
			Norpropoxyphene	
			Ethanol	
			Acetaminophen	
	18 (Matched with #16, 17, 19)	Urine	Propoxyphene	
			Norpropoxyphene	
			Ethanol	0.13 mg/dL
			Acetaminophen	250.0 mg/L
	19 (Matched with #16-18)	Gastric	Propoxyphene	10.0 mg/L
			Norpropoxyphene	25.0 mg/L
			Ethanol	100.0 mg/dL
			Acetaminophen	500.0 mg/L
	20	Blood	Ethanol	0.1 mg/dL
			Methanol	0.05 mg/dL
			Secobarbital	2.0 mg/L

Table 2: Stability Studies in Samples for Quantitation

SAMPLE NO.	DRUG	WEIGHED-IN VALUE	ANALYTICAL METHOD[1]	PERIOD OF ANALYSIS (MTHS)[2]	NO. OF ANALYSES	ANALYTICAL RESULTS MEAN	S.D.	C.V.%	RANGE
1-Blood	Diazepam	1.0 mg/L	GC-MS & GC-ECD	8	13	0.98	0.16	16.8	0.65-1.2
	Nordiazepam	1.5 mg/L	GC-MS & 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		60	4.10	6.9	55-66
	Amitriptyline	0.50 mg/L	GC-MS & GC-NPD	8	5	0.46	0.04	8.6	0.41-0.5
	Nortriptyline	0.75 mg/L	GC-MS & GC-NPD	8	5	0.66	0.12	17.7	0.55-0.8
	Ethanol	300 mg/dL	GC-FID		2	230			
3-Urine	Ethanol	400 mg/dL	GC-FID			324			
	Amitriptyline	2.0 mg/L	GC-MS & GC-NPD	8	5	2.23	0.14	6.3	2.03-2.38
	Nortriptyline	3.0 mg/L	GC-MS & GC-NPD	8	5	2.94	0.28	9.5	2.5-3.25
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 & HPLC	8	10	2.24	0.30	13.6	1.9-2.7

Table 2: Stability Studies in Samples for Quantitation (cont.)

SAMPLE NO.	DRUG	WEIGHED-IN VALUE	ANALYTICAL METHOD[1]	PERIOD OF ANALYSIS (MTHS)[2]	NO. OF ANALYSES	ANALYTICAL RESULTS MEAN	S.D.	C.V.%	RANGE
7-Blood	Ethanol	100 mg/dL	GC-FID		2	83			
	Flurazepam	0.80 mg/L	GC-MS & GC-ECD	5	12	0.91	0.14	15.9	0.7-1.1
	Desalkylflurazepam	0.50 mg/L	GC-MS & 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	7.00	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	302	18.20	6.0	279-328
12-Blood	Propoxyphene	5.0 mg/L	GC-NPD & GC-MS	2.5	9	5.20	0.80	15.20	4.3-6.9
	Norpropoxyphene	4.0 mg/L	GC-NPD & 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

Table 2: Stability Studies in Samples for Quantitation (cont.)

SAMPLE NO.	DRUG	WEIGHED-IN VALUE	ANALYTICAL METHOD[1]	PERIOD OF ANALYSIS (MTHS)[2]	NO. OF ANANLYSES	ANALYTICAL RESULTS MEAN	S.D.	C.V.%	RANGE
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	0.20	0	0	0.2
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 Contents	Propoxyphene	325 mg	GC-NPD	1	3	378		8.0	
	Acetaminophen	3250 mg	HPLC	1	8	2795	131	11.7	2462.5-3402.5
	Ethanol	150 mg/dL	GC-FID		2	160			
17-Blood	Propoxyphene	5.0 mg/L	GC-NPD	1	3	8.20		8.0	
	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		8.0	
	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			

Table 2: Stability Studies in Samples for Quantitation (cont.)

SAMPLE NO.	DRUG	WEIGHED-IN VALUE	ANALYTICAL METHOD[1]	PERIOD OF ANALYSIS (MTHS)[2]	NO. OF ANALYSES	ANALYTICAL RESULTS MEAN	S.D.	C.V.%	RANGE
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

[1] 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

[2] Period between first and last analysis

Table 3: Qualitative Analyses

Sample #	Analytes Present	Weighed-In Value	% Positive Responses
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)

Table 3: Qualitative Analyses (cont.)

Sample #	Analytes Present	Weighed-In Value	% Positive Responses
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	0.05 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)

Table 3: Qualitative Analyses (cont.)

Sample #	Analytes Present	Weighed-In Value	% Positive Responses
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

SAMPLE #	ANALYTE/METHOD	# LABS	MEAN	S.D.	C.V.%	RANGE
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	35			31-46
	Diazepam (mg/dL)					
	All Methods	55	1.2	0.57	8	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	1.1			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	2.0			1.71-2.2
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	277			250-295
	Carboxyhemoglobin (% sat.)					
	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	58			34.5-72

Table 4: Quantitative Analyses (cont.)

SAMPLE #	ANALYTE/METHOD	# LABS	MEAN	S.D.	C.V.%	RANGE
	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	0.45			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	0.76			0.36-1.07
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	91			65-104
	Methanol (mg/L)					
	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
7-Blood	Ethanol (mg/dL)					
	All Methods	69	82	8.5	10	60-104
	Gas Chromatography	64	82	8.5	10	60-104

Table 4: Quantitative Analyses (cont.)

SAMPLE #	ANALYTE/METHOD	# LABS	MEAN	S.D.	C.V.%	RANGE
7-Blood	Ethanol (mg/dL) cont.					
	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
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

Table 4: Quantitative Analyses (cont.)

SAMPLE #	ANALYTE/METHOD	# LABS	MEAN	S.D.	C.V.%	RANGE
	Pentobarbital (mg/L) cont.					
	Gas Chromatography Internal Standard	35	7.7	2.4	31	1.3-12.3
	U.V. Spectrophotometry	3				6.0-9.0
9-Liver Homogenate	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
	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
11-Blood	Salicylic Acid (mg/L)					
	All Methods	52	295	121	41	100-730
	Colorimetric	22	270	93	34	100-400
	UV	19	296	86	29	190-430
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	4.84	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

Table 4: Quantitative Analyses (cont.)

SAMPLE #	ANALYTE/METHOD	# LABS	MEAN	S.D.	C.V.%	RANGE
	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
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
14-Blood	Phenobarbital					
	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

Table 4: Quantitative Analyses (cont.)

SAMPLE #	ANALYTE/METHOD	# LABS	MEAN	S.D.	C.V.%	RANGE
	Carboxyhemoglobin					
	All Methods	51	29	11	38	13-50
	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
16-Gastric Contents	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
17-Blood	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
18-Liver Homogenate	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	105	15.1	14	76-134
19-Urine	Propoxyphene (mg/L)	35	11.2	4.0	35	3.0-20.8
	Norpropoxyphene (mg/L)	31	28.9	15.0	52	10.6-76.0
	Acetaminophen (mg/L)	28	649.0	256.0	40	286-1327
	Ethanol (mg/dL)	31	97.0	11.6	12	70-110
20-Blood	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

The data for samples 16 through 20 is for "All Methods." Some results were omitted from certain of these data. For details, please see the Interim Reports (Appendix C).

Table 5: Metabolite Analyses (Qualitative)

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

LIST OF FIGURES

Figure 1: Sample 1, Ethanol (All Methods).
Figure 2: Sample 1, Diazepam (All Methods).
Figure 3: Sample 1, Nordiazepam (All Methods).
Figure 4: Sample 2, Ethanol (All Methods).
Figure 5: Sample 2, Carboxyhemoglobin (All Methods).
Figure 6: Sample 2, Amitritpyline (All Methods).
Figure 7: Sample 2, Nortriptyline (All Methods).
Figure 8: Sample 4, Ethanol (All Methods).
Figure 9: Sample 4, Methanol (All Methods).
Figure 10: Sample 4, Secobarbital (All Methods).
Figure 11: Sample 7, Flurazepam (All Methods).
Figure 12: Sample 7, Desalkylflurazepam (All Methods).
Figure 13: Sample 7, Ethanol (All Methods).
Figure 14: Sample 8, Methaqualone (All Methods).
Figure 15: Sample 8, Pentobarbital (All Methods).
Figure 16: Sample 9, Methaqualone (All Methods).
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Figure 18: Sample 11, Salicylic Acid (All Methods).
Figure 19: Sample 12, Propoxyphene (All Methods).
Figure 20: Sample 12, Norpropoxyphene (All Methods).
Figure 21: Sample 12, Doxepin (All Methods).
Figure 22: Sample 13, Diazepam (All Methods).
Figure 23: Sample 13, Nordiazepam (All Methods).
Figure 24: Sample 13, Codeine (All Methods).
Figure 25: Sample 14, Phenobarbital (All Methods).
Figure 26: Sample 14, Carboxyhemoglobin (All Methods).
Figure 27: Sample 17, Propoxyphene (All Methods).
Figure 28: Sample 17, Norpropoxyphene (All Methods).
Figure 29: Sample 17, Acetaminophen (All Methods).
Figure 30: Sample 17, Ethanol (All Methods).
Figure 31: Sample 18, Propoxyphene (All Methods).
Figure 32: Sample 18, Norpropoxyphene (All Methods).
Figure 33: Sample 18, Acetaminophen (All Methods).
Figure 34: Sample 19, Propoxyphene (All Methods).

LIST OF FIGURES (cont).

Figure 35: Sample 19, Norpropoxyphene (All Methods).
Figure 36: Sample 19, Acetaminophen (All Methods).
Figure 37: Sample 20, Secobarbital (All Methods).
Figure 38: Sample 20, Morphine (All Methods).
Figure 39: Sample 20, Codeine (All Methods).

FIGURE 1 SAMPLE 1, ETHANOL (ALL METHODS)

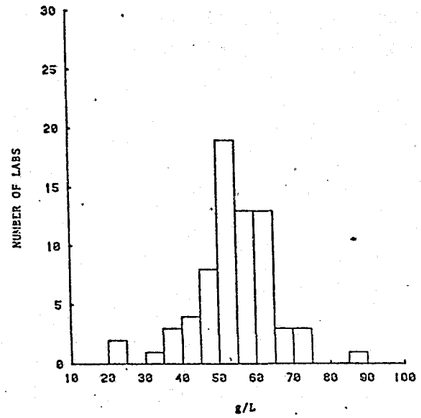


FIGURE 2 SAMPLE 1, DIAZEPAM (ALL METHODS)

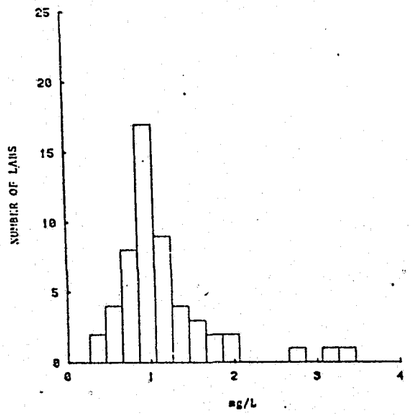


FIGURE 3 SAMPLE 1, NORDIAZEPAM (ALL METHODS)

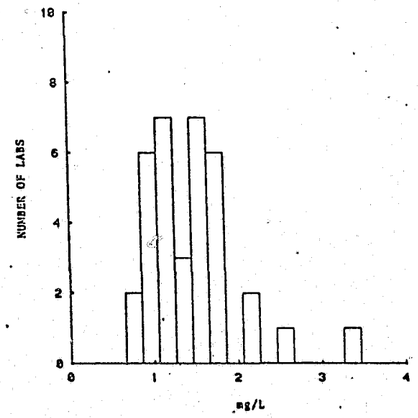


FIGURE 4 SAMPLE 2, ETHANOL (ALL METHODS)

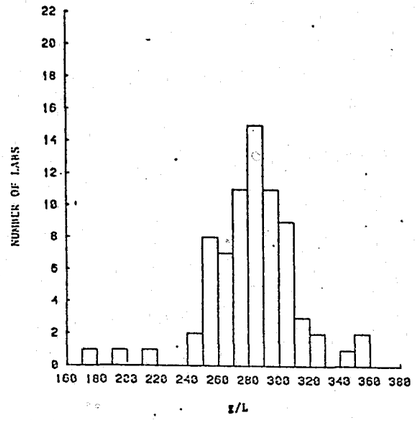


FIGURE 5 SAMPLE 2, CARBOXYHEMOGLOBIN (ALL METHODS)

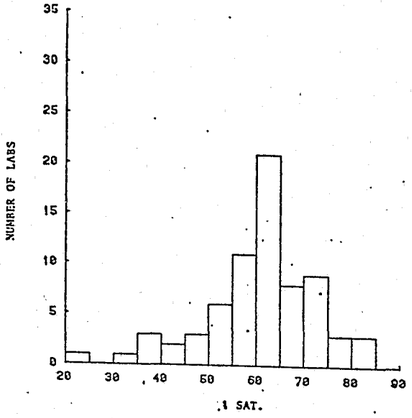


FIGURE 6 SAMPLE 2, AMITRIPTYLINE (ALL METHODS)

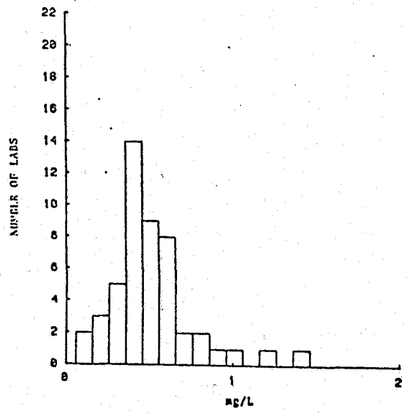


FIGURE 7 SAMPLE 2 NORTRIPTYLINE (ALL METHODS)

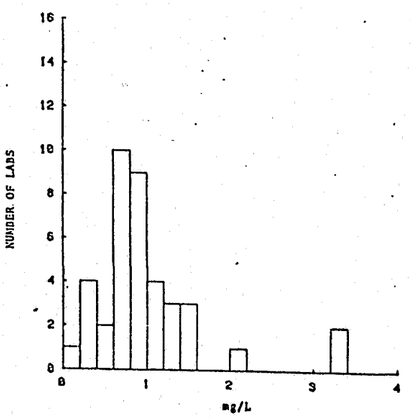


FIGURE 8 SAMPLE 4, ETHANOL (ALL METHODS)

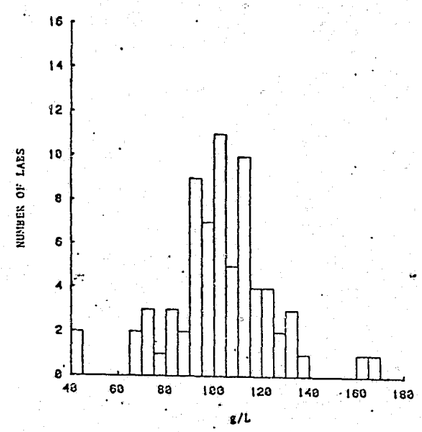


FIGURE 9 SAMPLE 4, METHANOL (ALL METHODS)

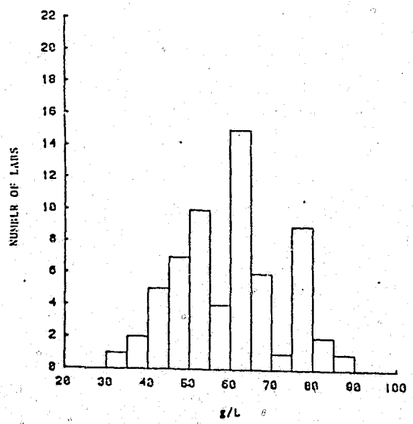


FIGURE 10 SAMPLE 4, SECOBARBITAL (ALL METHODS)

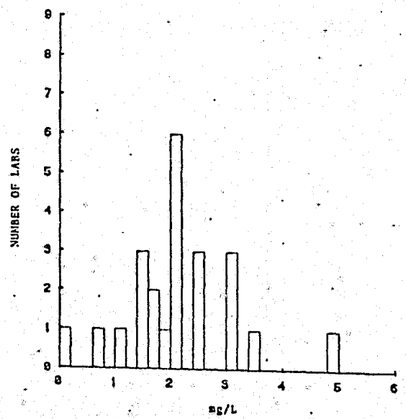


FIGURE 11 SAMPLE 7, FLURAZEPAM (ALL METHODS)

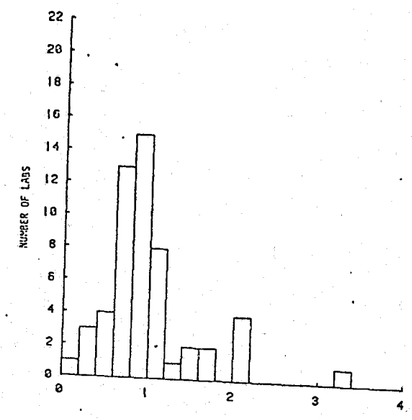


FIGURE 12 SAMPLE 7, DESALKYLFLURAZEPAM (ALL METHODS)

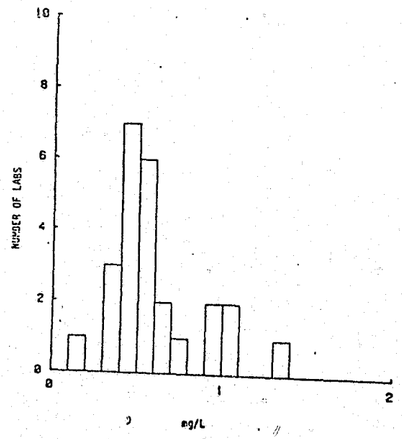


FIGURE 13 SAMPLE 7, ETHANOL (ALL METHODS)

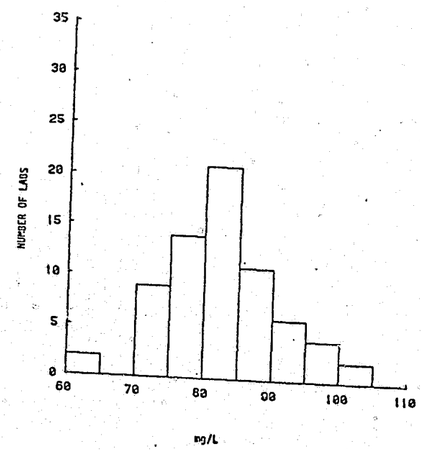


FIGURE14 SAMPLE 8, METHAQUALONE (ALL METHODS)

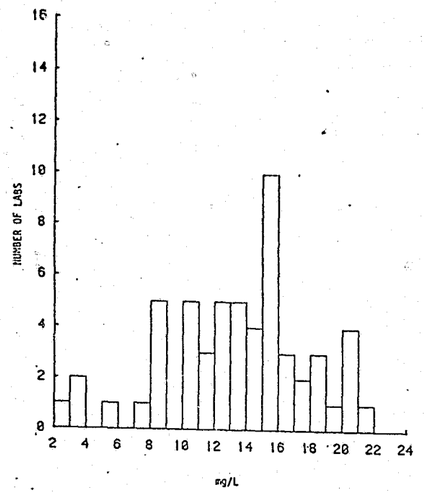


FIGURE15 SAMPLE 8, PENTOBARBITAL (ALL METHODS)

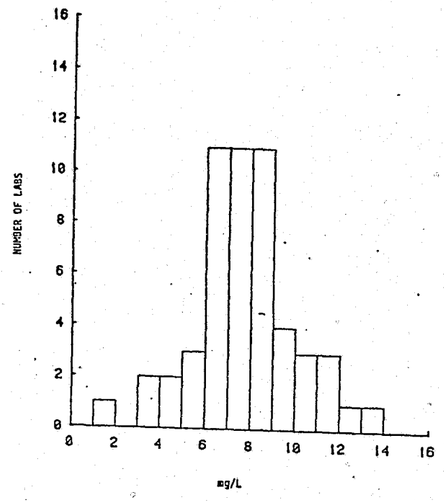


FIGURE16 SAMPLE 9, METHAQUALONE (ALL METHODS)

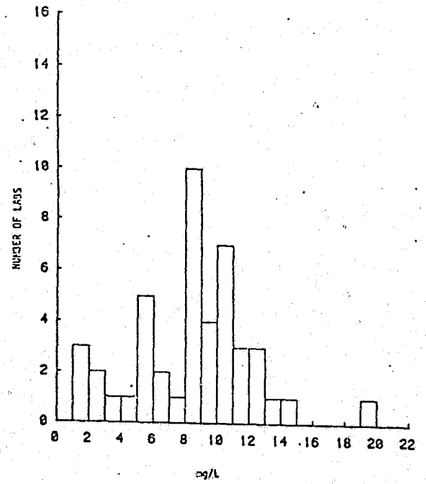


FIGURE17 SAMPLE 9, PENTOBARBITAL (ALL METHODS)

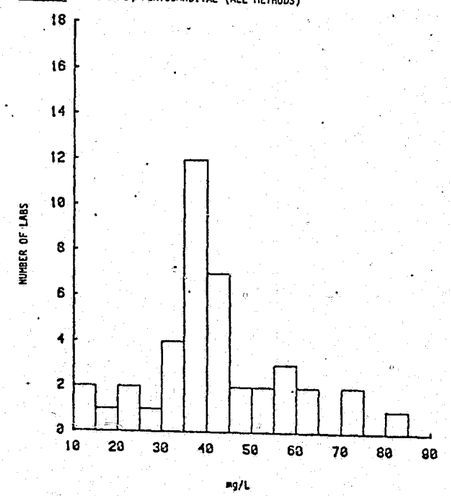
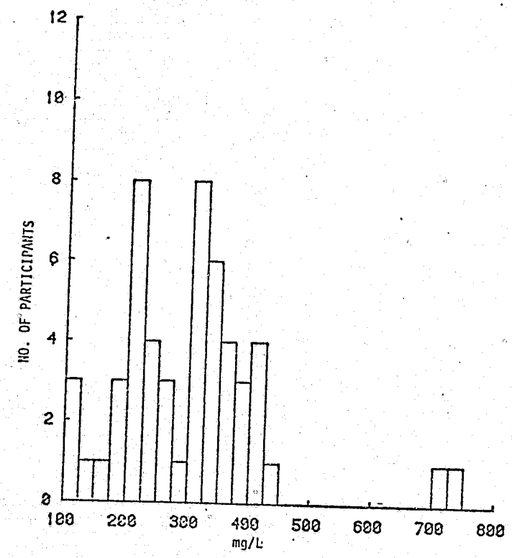


Figure 18: SALICYLIC ACID (ALL METHODS), Sample 11.



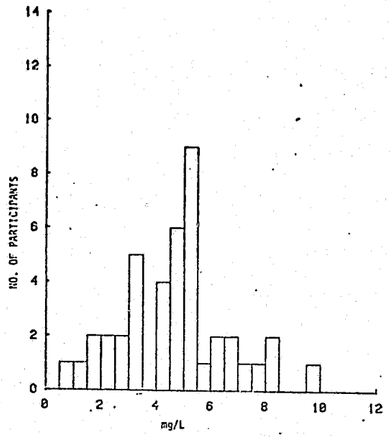


Figure 19: Sample 12, PROPOXYPHENE (ALL METHODS)

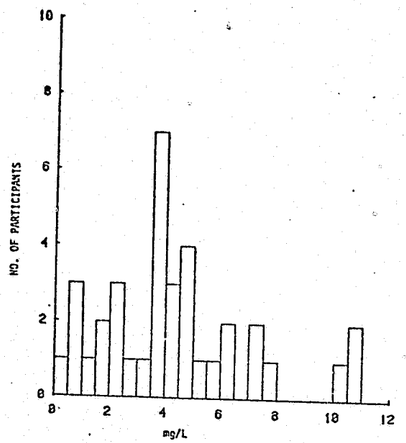


Figure 20: Sample 12, NORPROPOXYPHENE (ALL METHODS)

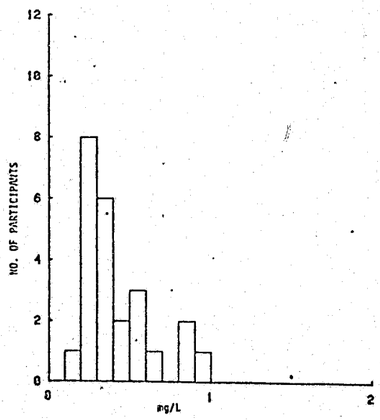


Figure 21: Sample 12, DOXEPIN (ALL METHODS)

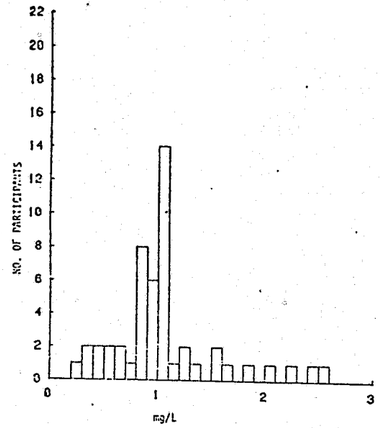


Figure 22: Sample 13, DIAZEPAM (ALL METHODS)

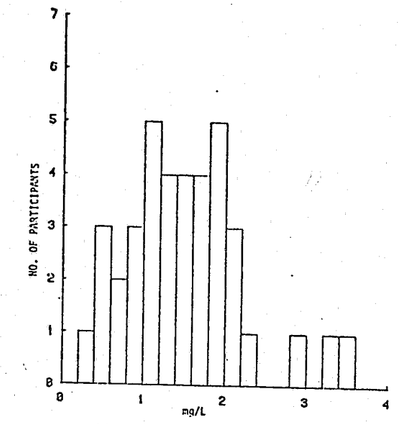


Figure 23: Sample 13, NORDIAZEPAM (ALL METHODS)

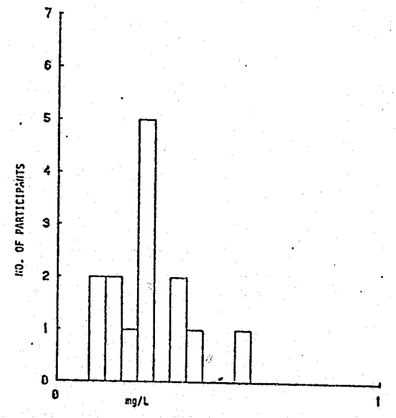


Figure 24: Sample 13, CODEINE (ALL METHODS)

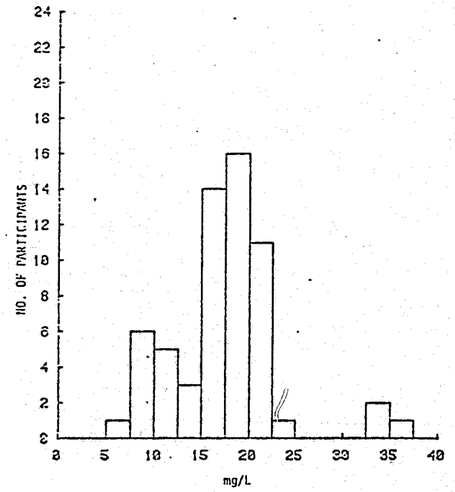


Figure 25: Sample 14, PHENOBARBITAL (ALL METHODS)

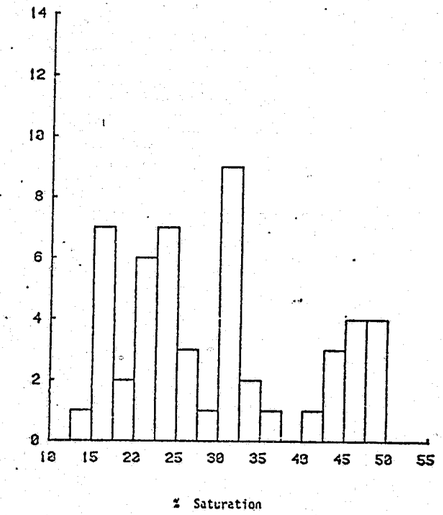


Figure 26: Sample 14, CARBOXYHEMOGLOBIN (ALL METHODS)

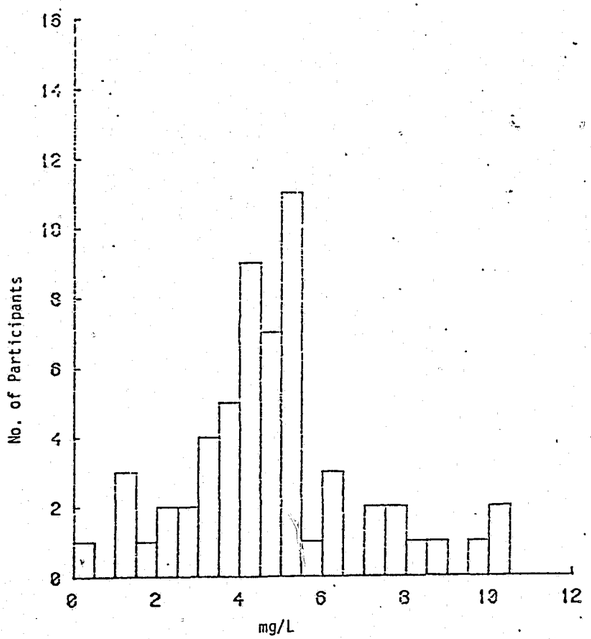


Figure 27: Sample 17, PROPOXYPHENE (ALL METHODS)

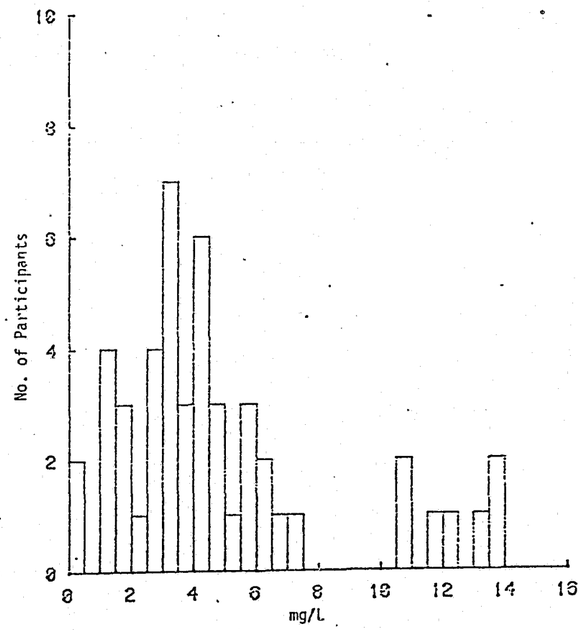


Figure 28: Sample 17, NORPROPOXYPHENE (ALL METHODS)

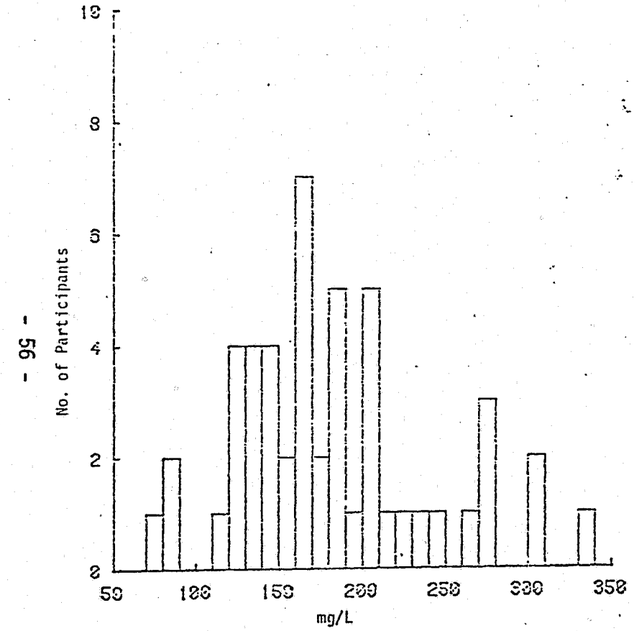


Figure 29: Sample 17, ACETAMINOPHEN (ALL METHODS)

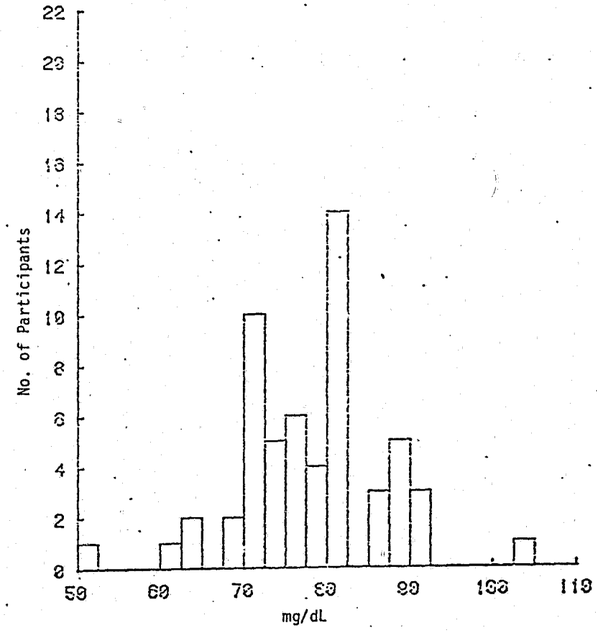


Figure 30: Sample 17, ETHANOL (ALL METHODS)

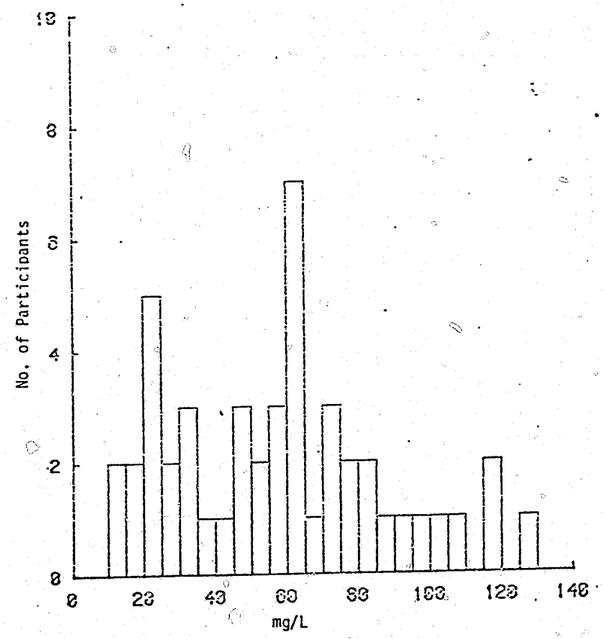


Figure 31: Sample 18, PROPOXYPHENE (ALL METHODS)

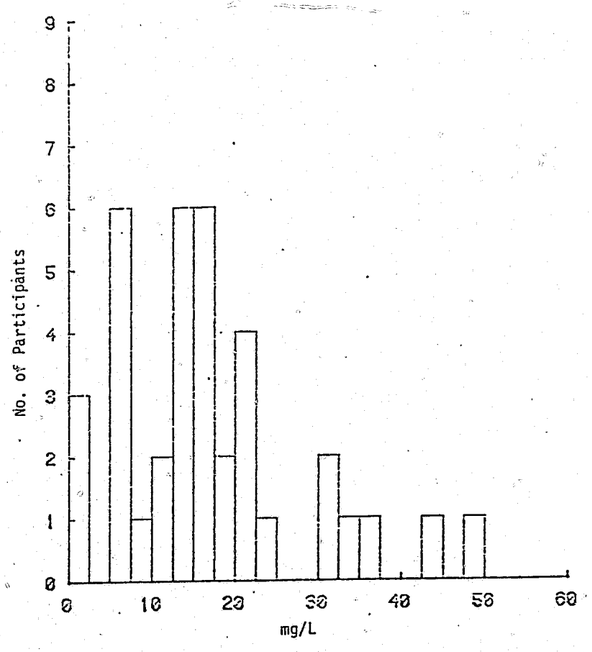


Figure 32: Sample 18, NORPROPOXYPHENE (ALL METHODS)

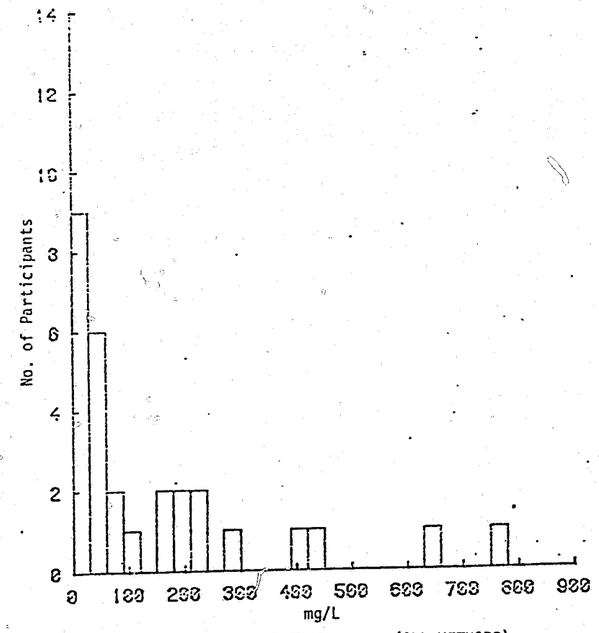


Figure 33: Sample 18, ACETAMINOPHEN (ALL METHODS)

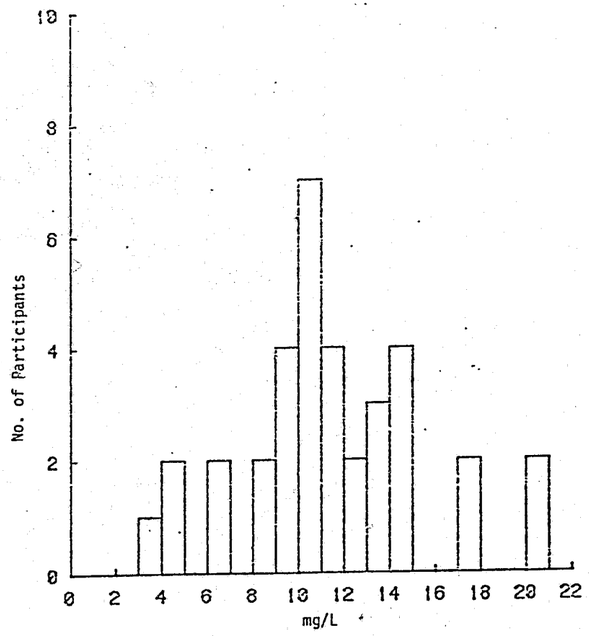


Figure 34: Sample 19, PROPOXYPHENE (ALL METHODS)

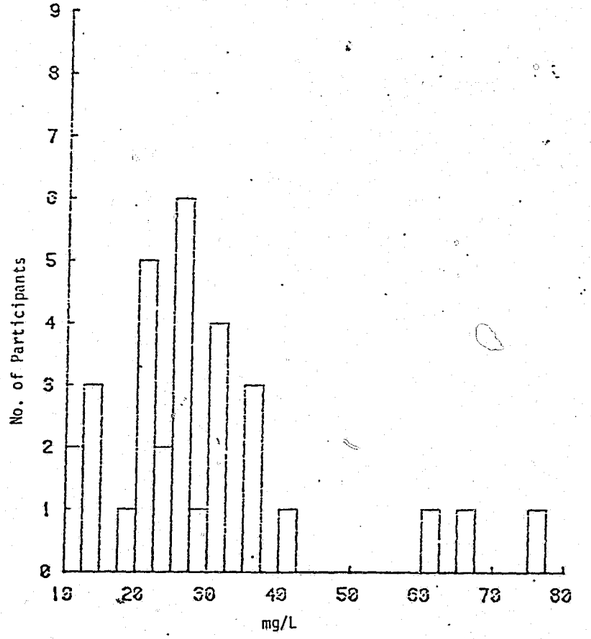


Figure 35: Sample 19, NORPROPOXYPHENE (ALL METHODS)

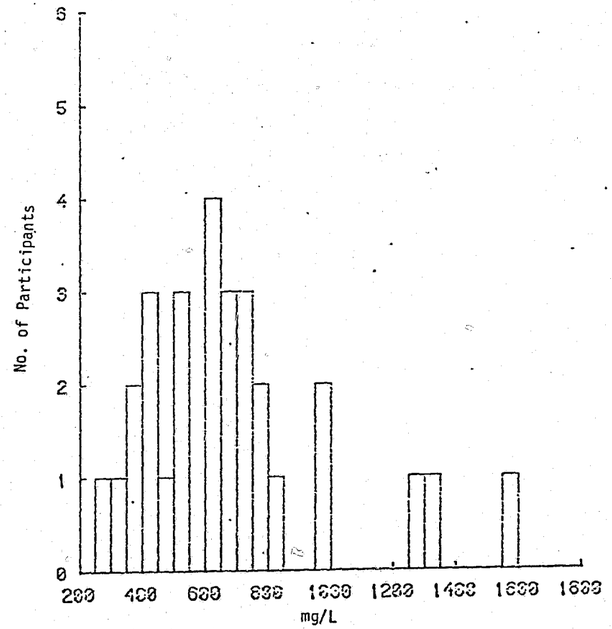


Figure 36: Sample 19, ACETAMINOPHEN (ALL METHODS)

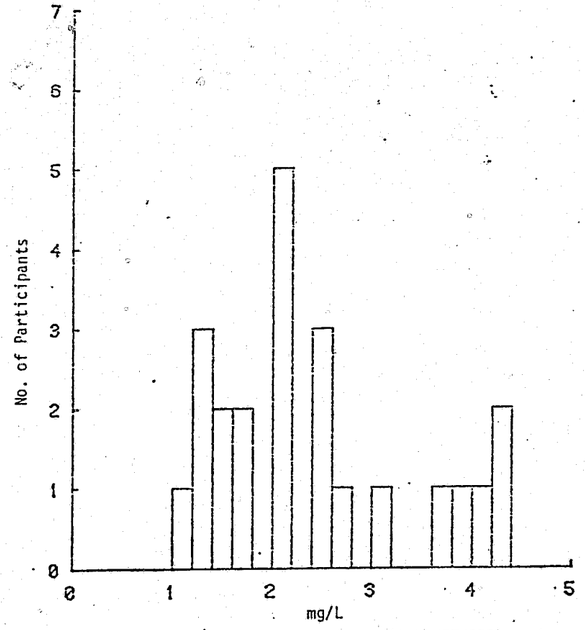


Figure 37: Sample 20, SECOBARBITAL (ALL METHODS)

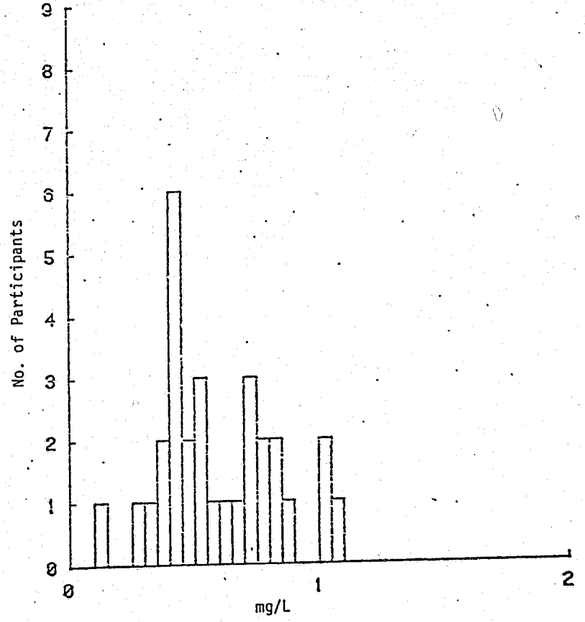


Figure 38: Sample 20, MORPHINE (ALL METHODS)

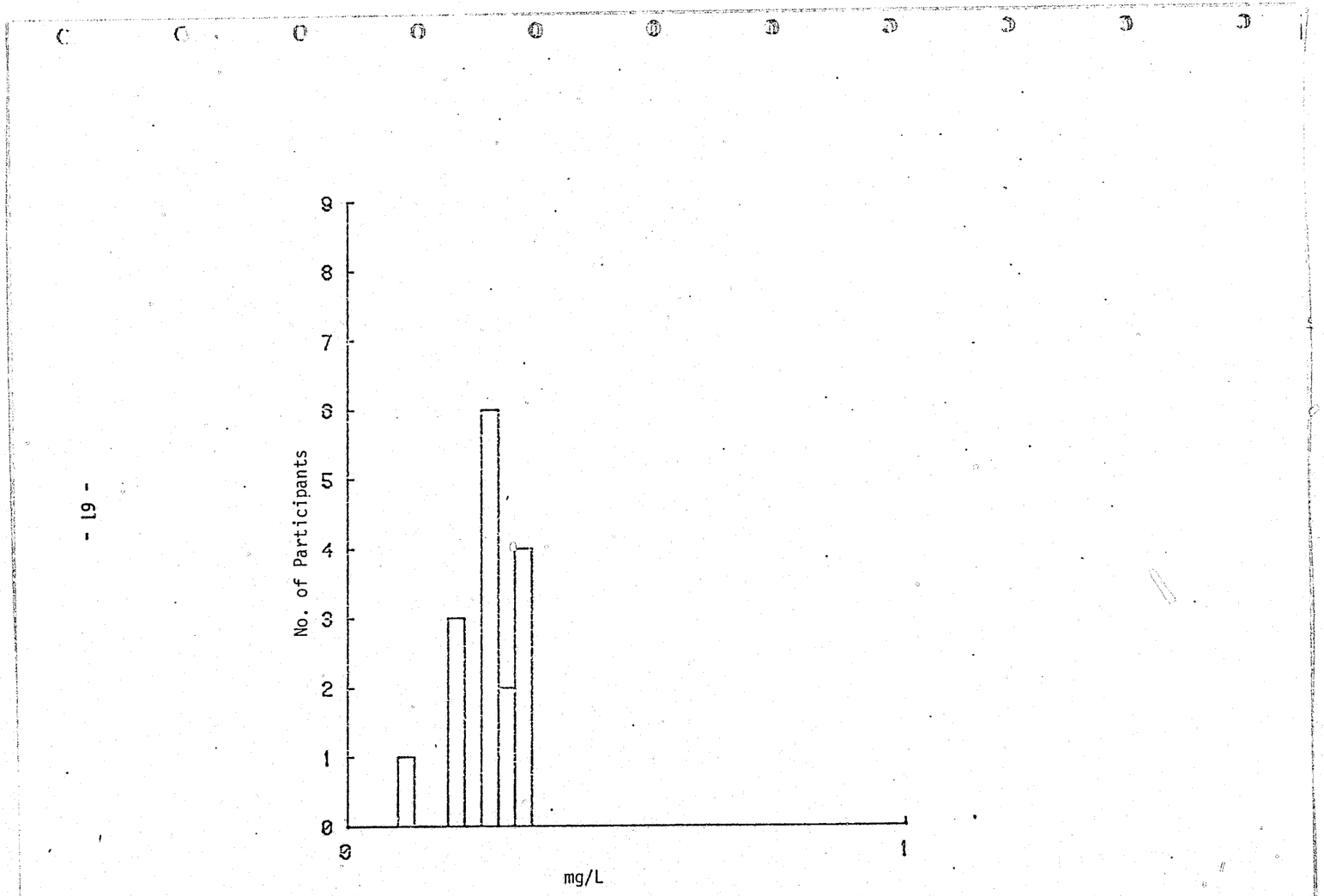


Figure 39: Sample 20, CODEINE (ALL METHODS)

APPENDIX A

Copy of Letter Sent

CENTER FOR HUMAN TOXICOLOGY
UNIVERSITY OF UTAH • SALT LAKE CITY, UTAH 84112 (801) 581-5117

Dear

The National Institute of Justice has recently awarded a grant to the Center for Human Toxicology to study a Proficiency Testing Program in Forensic Toxicology.

This research is not intended to evaluate externally any individual toxicologist or forensic toxicology laboratory. The project is designed to reach conclusions within a year concerning the feasibility of such a program. To achieve this, a total of four batches of five samples (total number = 20) will be sent to each participant over a period of approximately seven months. Each laboratory will be given a total of at least ten working days to analyze the samples and return the results. An appropriate "case history" will accompany each sample, and details of the analyses required and any background information that is available. The samples will be specimens that are familiar to forensic toxicologists and will include whole blood, urine, gastric contents, and homogenized tissue samples. The last specimen will be sent only if prior analysis of aliquots is shown to be statistically valid. Only drugs and metabolites that are routinely encountered by forensic toxicologists will be included.

When the results are returned they will be analyzed statistically and reports sent to each participant. The format will be similar to that used by the College of American Pathologists; i.e., they will be tabulated and presented as histograms around a mean.

Results will be forwarded on a "double-blind" basis to the Center for Human Toxicology; that is, they will be sent initially to a disinterested party who will open the stamped addressed envelope and forward the enclosed unaddressed envelope to the Center for Human Toxicology. It is, as yet, undecided as to whether participants will be listed in the final report to NIJ. This decision rests with the Advisory Board.

Throughout the entire project the Principal Investigator will be assisted by an Advisory Board consisting of several experienced and respected forensic toxicologists. This Board will have the final decision on the types of specimens and drugs to be included and will review and critique the final report in draft before submission to NIJ. This final report will provide a series of recommendations to the National Institute on, in addition to other things, the continuation of proficiency testing among forensic toxicologists.

In order for this research to succeed it is essential that all those involved in forensic toxicology participate, and we would strongly encourage you to do so. We realize that this will add an extra burden of work to many of you, particularly those who already have a heavy case load, but we are sure that the results of this research will benefit all of us involved in analytical forensic toxicology.

A questionnaire and a return envelope are enclosed. We would very much appreciate your returning the completed questionnaire even if you do not intend, for whatever reason, to participate in the project.

Thank you for your cooperation, and if I can be of any further assistance please feel free to call.

Yours sincerely,

Michael A. Peat
Principal Investigator

MAP:amh
Enc.

APPENDIX B

Reporting Forms

Sample #	Case History	Sample	Drug Code	Drug Name	Quantitation	Analytical Procedure
1	A 45 year old female, who had been prescribed Valium for the past year, was found dead by her husband upon returning from work. An autopsy was performed and a blood sample sent for toxicological analysis. Please screen sample and quantitate any drugs and/or metabolites detected.	Blood	/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
2 & 3	A 50 year old male was found dead in his car in a locked garage. A piece of pipe led from the exhaust into the car. The deceased was a heavy drinker and had, in the past, been treated for depression. Please screen the blood and urine samples. Quantitate any drugs and/or metabolites detected in the blood sample only.	Blood	/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
		Urine	/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
4	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(s) detected.	Blood	/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
5	A 25 year old male was found dead with stab wounds. He had a history of drug abuse and had been under treatment at a methadone maintenance clinic, although he had not been seen by the staff for three weeks. The pathologist requested a urine drug screen. No screen for volatiles is required.	Urine	/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			
			/__/__/			

REPORT ALL QUANTITATIONS IN MICROGRAMS/ML EXCEPT FOR VOLATILES. PLEASE REPORT THESE AS mg/dl.

Sample Number	Case History	Sample	Drug Code	Drug Name	Quantitation	Analytical Procedure	I.S.	E.S.
6	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.	Urine						
7	A 30-year-old female was found dead in bed by her roommate. An empty Dalmane bottle was found. Please screen and quantitate any drugs and/or metabolites detected.	Blood						
8 & 9	A 25-year-old male was found dead in a hotel room. A collection of drug paraphernalia was also found. Please screen the blood sample and quantitate any drugs and/or metabolites in this sample and in the liver homogenate. Cause of death: pending toxicology.	Blood						
		Liver						
10	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.	Urine						

REPORT ALL QUANTITATIONS IN MILLIGRAMS/L EXCEPT VOLATILES. PLEASE REPORT THEM AS MILLIGRAMS/DL. PLEASE CHECK WHETHER AN INTERNAL OR EXTERNAL STANDARD WAS USED FOR QUANTITATION, WHEN APPROPRIATE.

Sample Number	Case History	Sample	Drug Code	Drug Name	Quantitation	Analytical Procedure	I.S.	E.S.
11	A 6 year old child was admitted to a hospital suffering from acidosis. His mother indicated that a number of aspirin tablets were missing. Although the child was correctly treated he died twenty four hours after admission. An autopsy was performed and a blood sample taken. Please determine the salicylate concentration and screen the specimen for other drugs. Determine the concentrations of any other drugs and/or metabolites detected.	Blood	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
12	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.	Blood	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
13	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 tranquillizers for anxiety. Please screen the blood sample and determine the concentration of any drugs and/or metabolites detected. Cause of death: pending toxicology.	Blood	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
14	An industrial worker was found dead near a carbon monoxide generator. The deceased was a known epileptic. An autopsy revealed signs of recent seizure activity. Please screen the blood sample for drugs and quantitate any drugs and/or metabolites detected.	Blood	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
15	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. And do NOT screen for volatiles.	Urine	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					

REPORT ALL QUANTITATIONS IN MILLIGRAMS/L EXCEPT VOLATILES. PLEASE REPORT THEM AS MILLIGRAMS/DL. PLEASE CHECK WHETHER AN INTERNAL OR EXTERNAL STANDARD WAS USED FOR QUANTITATION, WHEN APPROPRIATE.

Sample Number	Case History	Sample	Drug Code	Drug Name	Quantitation	Analytical Procedure	I.S.	E.S.
16	A 38 year old male suffered a lower back injury in an industrial accident and was subsequently unemployable. He was prescribed Darvocet-N-100 for chronic pain. He became despondent and was found dead in bed at home one morning. Suicidal drug overdose was suspected. Please screen the blood sample and determine the concentrations of any drugs and/or metabolites in each of the specimens submitted.	Gastric Contents (total weight 2500 G)	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
17		Blood	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
18		Liver	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
19		Urine	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
20	A young man was brought comatose to a hospital E.R. 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.	Blood	/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					
			/_/_/					

REPORT ALL QUANTITATIONS IN MILLIGRAMS/L EXCEPT VOLATILES. REPORT GASTRIC CONTENTS VALUE AS TOTAL WEIGHT OF DRUG(S) PRESENT. REPORT VOLATILES AS MILLIGRAMS/DL. PLEASE CHECK WHETHER AN INTERNAL OR EXTERNAL STANDARD WAS USED FOR QUANTITATION, WHEN APPROPRIATE.

APPENDIX C

Interim Reports

PROFICIENCY TESTING PROGRAM

INTERIM REPORT SAMPLES 1-5

INTRODUCTION

101 batches of samples were shipped on January 5, 1981. No reports of breakages were received, although one participant reported that sample #4 leaked in transit. 74 replies (postmarked by Jan. 23, 1981) were received. An additional 8 replies have since been received, these are not included in the report.

A number of participants reported similar comments, these concerned:
1) The stability of the ethanol in samples 1,3, and 4. A stability study is presently in progress at the Center For Human Toxicology to clarify this.
2) The presence of chloroform and other organic solvents. This was due to the fact that some of the samples, prior to shipment, had been stored in solvent bottles. Although these had been thoroughly washed, traces of organic solvent must still have been present.
3) Odor and decomposition. Although the blood was stabilized with oxalate/fluoride, it is possible that insufficient was added, greater amounts will be added to future samples.

All of the blood samples (#1,3, and 4) were prepared from bovine blood by disolving appropriate amounts of the drug, or a salt of the drug in water, 0.05M sodium hydroxide or methanol. These solutions were used to "spike" the blood sample.

Most participants completed the result forms appropriately, however, in a number of instances, respondents did not state whether they used an internal or external standard for quantitations by chromatography. For this reason the tabulations for "gas chromatography" and "gas chromatography-internal standard" overlap.

If there are any questions concerning the data in this report, please feel free to call. There are limited amounts of samples 1 to 5 available for repeat analysis if required.

SAMPLE 1

History

A 49 year-old female, who had been prescribed Valium for the past year, was found dead by her husband when he returned from work. An autopsy was performed, and a blood sample sent for toxicological analysis. Please screen sample and quantitate any drugs and/or metabolites detected.

QUALITATIVE IDENTIFICATION: 74 LABORATORIES RESPONDING

Analytes Present	Weighed-In Value	% Positive Responses
Ethanol	50.0 mg/dL	95 (70/74)
Diazepam	1.0 mg/L	84 (62/74)
Nordiazepam	1.5 mg/L	68 (50/74)

5% (4/74) reported positive benzodiazepines by ultraviolet spectrophotometry.

QUANTITATIVE DETERMINATION: HISTOGRAMS FOR RESULTS BY ALL METHODS ARE SHOWN IN FIGURES 1-3.

Analyte/Method	# Labs	Mean	S.D.	C.V.	Range
Ethanol					
All Methods	70	53	11	21	20-90
Gas Chromatography	77	54	10	19	20-90
Gas Chromatography Internal Standard	46	55	8	15	30-71
Enzymatic	3	35			31-46
Diazepam					
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	1.1			0.9-1.3
Nordiazepam					
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	2.0			1.71-2.2

* One result was omitted.

Results from the laboratories of Advisory Board members and those of routine analysis at the Center For Human Toxicology were not included.

Gas chromatography-chemical ionization mass spectrometry (GC-CIMS) (1) with deuterated internal standards was used to determine the concentrations of diazepam and nordiazepam in the sample. These analyses were performed during the week of shipment. The sample was stored at 4°C after preparation. The mean values (n=2) were 0.96 mg/L and 1.4 mg/L respectively.

COMMENTS

The coefficients of variation show a large interlaboratory variation. 84% of laboratories responding identified diazepam and 68% the normetabolite, even though the history indicated use of Valium.

CONTINUED

1 OF 2

By far the most common analytical procedure used to quantitate the three drugs was gas chromatography. Of the 70 laboratories determining the ethanol concentration, 66% (46/70) indicated that they used an internal standard. Their results were not statistically different from the total results. Enzymatic methods for the determination of ethanol were used by only 3 laboratories. Although gas chromatography, with a variety of detectors (flame ionization, nitrogen phosphorus and electron capture), was used widely by responding laboratories to quantitate diazepam and nordiazepam, only 55% (30/55) indicated that they used an internal standard for the diasepam assay and 75% (27/36) for the nordiazepam quantitation. As with the ethanol determination, there was no significant statistical difference between these groups and the total results.

Numerous groups have published gas chromatographic procedures (2-4) for the quantitation of benzodiazepines in biological fluids, and those using the more sensitive and specific electron capture detector (2,3) do not require an evaporation step. The chromatography of the normetabolites and other polar metabolites, with certain liquid phases, may, however, be inadequate for accurate determination. Recent work at the Center For Human Toxicology has shown that a 3% SP-2250 packing from Supelco Corporation is one of the more reliable liquid phases for these quantitations. In addition, a number of workers have used high pressure liquid chromatography (3,5) to quantitate the benzodiazepines, and it may well be that this could become an equally, or even a more satisfactory procedure.

REFERENCES

1. Diazepam and its major metabolite, N-desmethyldiazepam in GC/MS assays for abused drugs in body fluids. NIDA Research Monograph 32, 1980, Eds. R.L. Foltz, A.F. Fentiman, R.B. Foltz.

2. D.M. Rutherford. J. of Chromat. 137:439, 1977.

3. M.A. Peat and L.Kopjak. J. of For. Sci. 24:46, 1979.

4. R.C. Baselt, C.B. Stewart and S.J. French. J. of Anal. Tox. 1:10, 1977.

5. N.Stronjy, C.V. Puglisi, and J.A.F. de Silva. Anal. Letter 135:B11, 1978.

SAMPLES 2 and 3

History

A 50 year-old male was found dead in his car in a locked garage. A piece of pipe led from the exhaust into the car. The deceased was a heavy drinker and had, in the past, been treated for depression. Please screen the blood and urine samples. Quantitate any drugs and/or metabolites detected in the blood sample only.

QUALITATIVE IDENTIFICATION: SAMPLE 2 BLOOD: 74 LABORATORIES RESPONDING

Analyte	Weighed-In Value	% Positive Responses
Ethanol	300 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)

SAMPLE 3 URINE: 74 LABORATORIES RESPONDING

Analyte	Weighed-In Value	% Positive Responses
Amitriptyline	2.0	80 (59/74)
Nortriptyline	3.0	80 (59/74)

Caffeine and nicotine were also reported as being present in the urine sample.

QUANTITATIVE DETERMINATIONS: SAMPLE 2 BLOOD: HISTOGRAMS ARE SHOWN IN FIGURES 4-6

Analyte/Method	# Labs	Mean	S.D.	C.V.	Range
Ethanol					
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	277			250-295
Carboxyhemoglobin					
All Methods	71	60	12	20	20-85
Co-Oximeter	17	63	7	11	50.3-81.8
Visible Spectrophotometry	26	61	11	18	35-85
Diffusion/Palladium Chloride	15	56	17	30	20-75
Gas Chromatography	6	58			34.5-72
Amitriptyline					
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	0.45			0.2-0.67

Nortriptyline					
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	0.76			0.36-1.07

* One result was omitted from the gas chromatographic and high pressure liquid chromatographic data and two from the total data.

Results from the laboratories of the Advisory Board members and those of routine analysis at the Center For Human Toxicology were not included.

GC-CIMS (6) was used to determine the concentration of the tricyclic antidepressants in the blood and urine samples. These analyses were performed during the week of shipment, the samples had been stored at 4°C. The results (n=2) were as follows: amitriptyline, blood 0.47 mg/L, urine 2.4 mg/L; and nortriptyline, blood 0.78 mg/L, urine 2.9 mg/L.

COMMENTS:

Generally the quantitative results for carboxyhemoglobin were accurate. Use of the Co-oximeter and visible spectrophotometry for carboxyhemoglobin determination resulted in lower coefficients of variation than the diffusion procedures. Although only 50% of the laboratories reported ethanol as present in Sample 3, this caused no concern as all respondents reported ethanol in the blood sample. The reason for this discrepancy is, without a doubt, the fact that a large number of forensic toxicology laboratories do not routinely screen urine for volatiles.

The tricyclic antidepressant blood concentrations represent toxicity. The coefficients of variation for the quantitations show a large interlaboratory variation. With regard to the qualitative identification, all laboratories reporting a positive result identified the drug and metabolite correctly.

Recently several reviews (7-10) have been published on the analysis of tricyclic antidepressants in biological fluids, particularly serum and plasma. Gas chromatographic procedures with flame ionization and nitrogen phosphorous detectors are available for screening biological samples for the tricyclic antidepressants (11,12). They can also be quantitated using identical or similar procedures (11,13). In the past few years, HPLC has become a popular technique for quantitating the tricyclic antidepressants (14-16), particularly in serum and plasma samples.

REFERENCES

6. D.M. Chinn, T.A. Jennison et al. Clin Chem 26:1201, 1980.
7. R.N. Gupta and G. Molnor. Biopharm. and Drug Disposition 1: 259, 1980.
8. R.N. Gupta and G. Molnor. Drug Metab. Rev. 9:79, 1979.
9. W. Reiss, S. Brechbuhler and J.P. Dubois. Prog. Drug Metab. 3:115, 1979.
10. B.A. Scoggins, K.P. Maguire et al. Clin- Chem. 26:5, 1980.
11. E.H. Forester, D. Hatchett and J.C. Garriott. J. of Anal. Tox. 2:50, 1978.
12. W.O. Pierce, T.C. Lamoreaux et al. J. of Anal. Tox. 2:26, 1978.
13. L. Kopjak, B.S. Finkle, T.C. Lamoreaux et al. J. of Anal. Tox. 3:155, 1979.
14. F.L. Vandemark, R.F. Adams and G.J. Schmidt. Clin. Chem 24:87, 1978.
15. J.C. Kraak and P. Bijster. J. Chromat. 143:499, 1977.
16. B.Mellström and R. Braithwaite. J. Chromat. 157:379, 1978.

SAMPLE 4

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(s) detected.

QUALITATIVE IDENTIFICATION: 73 LABORATORIES RESPONDING

Analyte Present	Weighed-In Value	% Positive Responses
Ethanol	100 mg/dL	97 (71/73)
Methanol	50 mg/dL	92 (67/73)
Secobarbital	2.5 mg/L	33 (24/73)

Pentobarbital was identified by a single particpant.

QUANTITATIVE DETERMINATIONS: HISTOGRAMS ARE SHOWN IN FIGURES 8-10.

Analyte/Method	# Labs	Mean	S.D.	C.V.	Range
Ethanol					
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	91			65-104
Methanol					
All Methods*	63	59	13	22	30-87
Gas Chroamtography*	62	59	13	22	30-87
Gas Chromatography Internal Standard	36	59	13	22	30-87
Secobarbital					
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

*Three results were omitted from these data.

Results from the laboratories of the Advisory Board and those of routine analysis at the Center For Human Toxicology were not included.

The sample was also analyzed for secobarbital during the week of shipment, by GC-CIMS using amobarbital as internal standard. The sample had been stored at 4°C since preparation. The mean blood concentration (n=4) was found to be 2.6 mg/L.

COMMENTS:

The coefficients of variation for the quantitation of ethanol and methanol show interlaboratory variation in these assays. This variation in ethanol determination was not seen in Sample 3. Although exact details of the analytical methods used were not requested, it is possible that those laboratories using a Carbopack-Carbowax column for volatiles would find this packing more satisfactory for quantitation than those who used one of the Poropak series. Of the four participants who used enzymatic methods for the determination of ethanol, only one detected methanol by gas chromatography. It is also important to realize that the use of enzymatic procedures alone will cause problems when isopropranol cases are encountered.

Only 23 laboratories reported secobarbital present in the blood sample. Although a blood concentration of 2.5 mg/L is lower than that regarded as toxic, and customarily encountered in fatal

barbiturate cases, it is higher than that achieved following typical hypnotic dose of the drug. This concentration is detectable by gas chromatography with flame ionization detectors (17), immunoassay methods (18) and high pressure liquid chromatography (19). However, some of the U.V. procedures used for screening autopsy specimens for barbiturates may not have the required sensitivity to detect less than 3 mg/L.

A number of laboratories reported performing carboxyhemoglobin analyses, a test which is consistent with the history. All results were less than 10% saturation. Only one false positive (pentobarbital) was reported.

REFERENCES

17. E.H. Foerster, J.Dempsey and J.C. Garriott. J. of Anal. Tox. 3:87, 1979

18. Roche, Abuscreen RIA

19. R.F. Adams, G.T. Schmidt and F.L. Vandemark. J. of Chromat. 145:275, 1978.

SAMPLE 5

History

A 25 year-old male was found dead with stab wounds. He had a history of drug abuse and had been under treatment at a methadone maintenance clinic, although he had not been seen by the staff for three weeks. The pathologist requested a urine drug screen. No screen for volatiles is required.

QUALITATIVE IDENTIFICATION: 74 LABORATORIES RESPONDING

Analyte Present	Weighed-In Value	% Positive Responses
Morphine	2 mg/L	88 (65/74)
Methadone	5 mg/L	96 (71/74)
Methadone Metabolite	10 mg/L	68 (50/74)

This sample was prepared at the Center For Human Toxicology from a urine that was known to be drug free. However, twenty-four participants reported quinine, three reported codeine and one each reported acetaminophen, quinidine, meperidine and flurazepam. Caffeine and theobromine were also reported as positive; these may have been present from previous coffee and tea ingestion.

Two laboratories, using immunoassay procedures only, reported positive opiates. A number of laboratories reported the presence of morphine glucuronide, which was not added to the urine sample. Presumably, these identifications were based on the presence of morphine after acid or enzymatic hydrolysis.

COMMENTS:

A number of different analytical procedures were used to identify morphine, methadone and its metabolite, including thin layer chromatography, gas liquid chromatography and immunoassay. 88 percent or more of the participants identified morphine and methadone correctly, although 9 participants (12%) failed to detect morphine. Quinine was a major misidentification at 24 laboratories; thin layer chromatography being the principal method of identification. This is surprising, considering that the same "blank urine" was used to prepare Sample 3, and no reports of positive quinine were received on this sample. In addition, analysis at the Center by gas chromatography-electron impact mass spectroscopy did not detect the presence of quinine.

Identification of basic drugs by thin layer chromatography alone is not recommended, if possible other analytical procedures should be used to confirm the initial thin layer results. Moffat and Smalldon (20) reported on the discriminating power of thin layer and paper chromatographic systems for basic drugs. They found that the maximum combined discriminating power achieved with two systems approached 0.93, whereas for an ideal system it should approach unity. Values of 0.97 have been reported by the same group for two gas chromatographic systems.

REFERENCES

20. A.C. Moffat and K.W. Smalldon. J. of Chromat. 90:9 1974.

FIGURE 1 SAMPLE 1, ETHANOL (ALL METHODS)

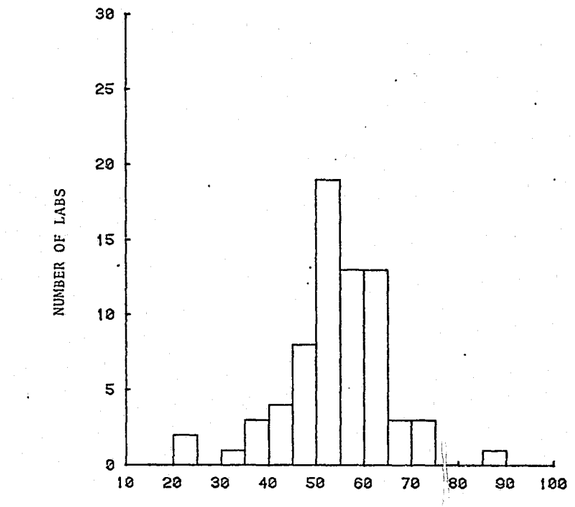


FIGURE 2 SAMPLE 1, DIAZEPAM (ALL METHODS)

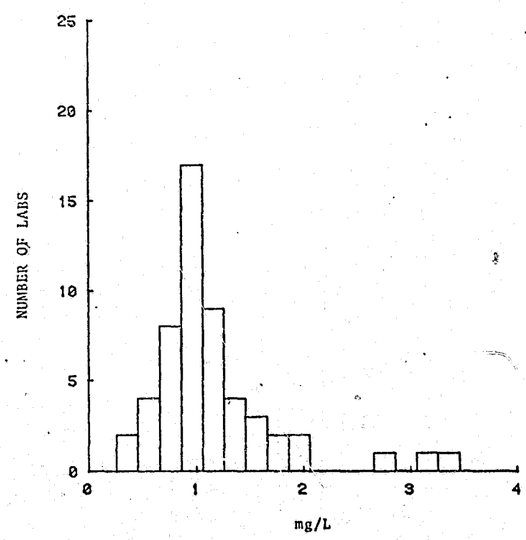


FIGURE 3 SAMPLE 1, NORDIAZEPAM (ALL METHODS)

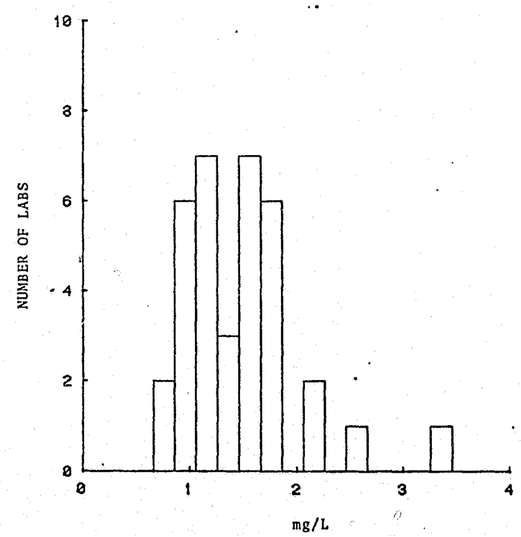


FIGURE 4 SAMPLE 2, ETHANOL (ALL METHODS)

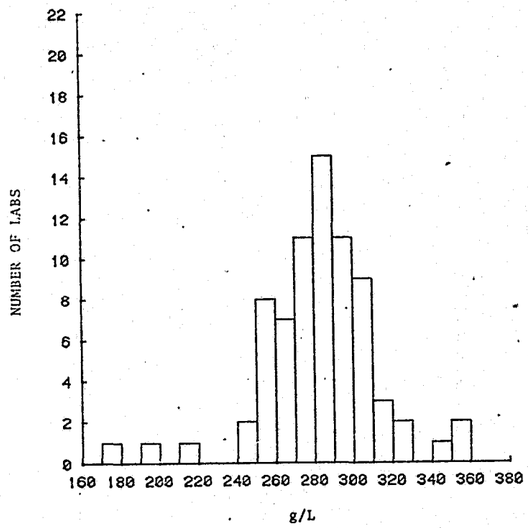


FIGURE 5 SAMPLE 2, CARBOXYHEMOGLOBIN (ALL METHODS)

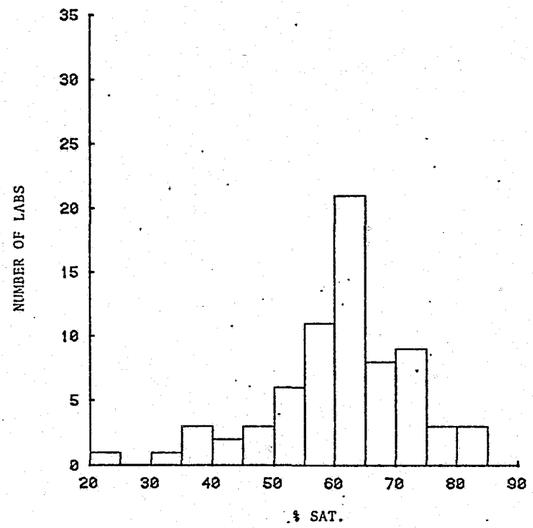


FIGURE 6 SAMPLE 2, AMITRIPTYLINE (ALL METHODS)

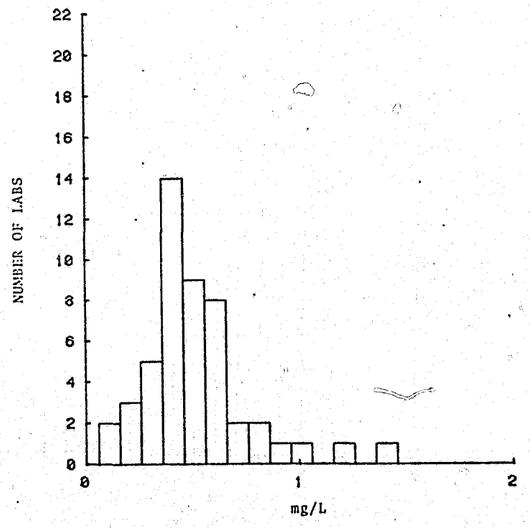


FIGURE 7 SAMPLE 2 NORTRIPTYLINE (ALL METHODS)

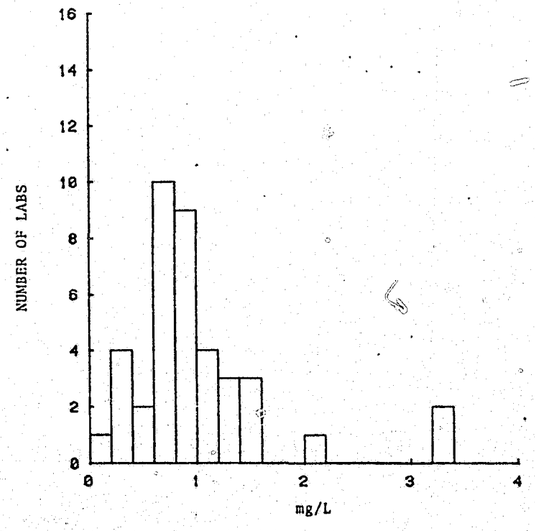


FIGURE 8 SAMPLE 4, ETHANOL (ALL METHODS)

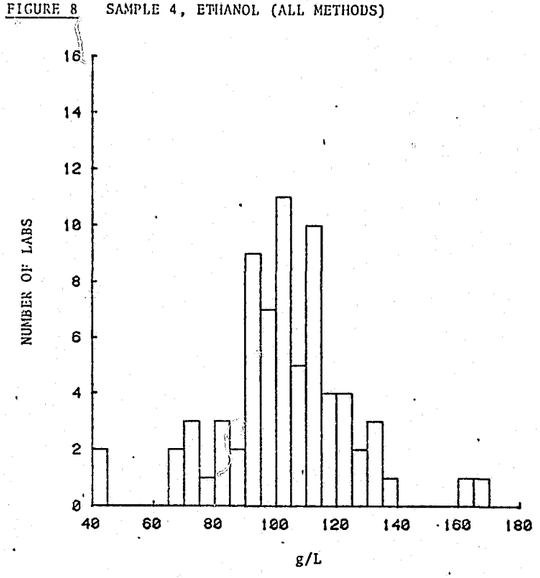


FIGURE 9 SAMPLE 4, METHANOL (ALL METHODS)

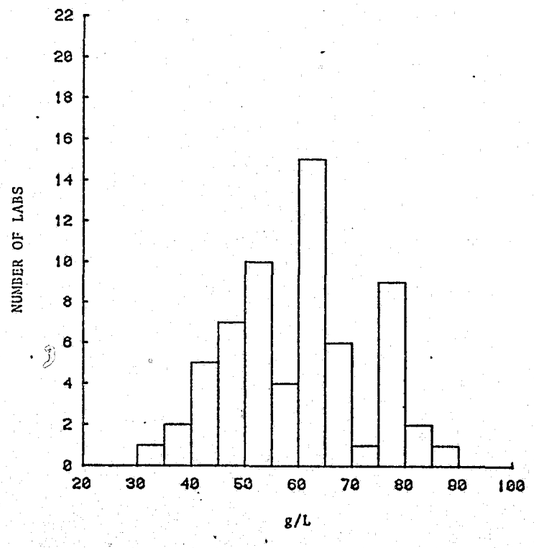
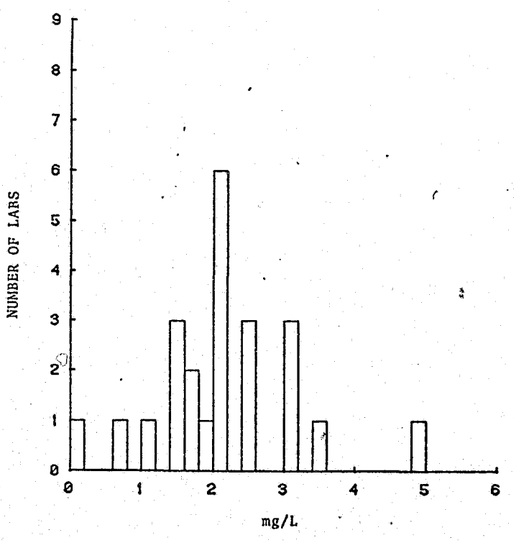


FIGURE 10 SAMPLE 4, SECOBARBITAL (ALL METHODS)



PROFICIENCY TESTING PROGRAM

INTERIM REPORT SAMPLES 6-10

INTRODUCTION

102 batches of samples were shipped on March 10, 1981. Several reports of breakages and spilled samples were received. Duplicate samples were shipped to these participants. 74 replies (postmarked by April 3, 1981) were received. For this shipment the blood was stabilized with larger amounts of oxalate fluoride than were used for the first shipment. No reports of odor or decomposition were received.

Both blood samples (#7 and #8) were prepared from bovine blood by dissolving appropriate amounts of the drug, or a salt of the drug, in water or ethanol. These solutions were used to "spike" the blood sample.

The liver homogenate was prepared from rat livers. The rats were given increasing daily doses of methaqualone and pentobarbital over a period of 30 days and were killed on day 31 by repetitive injections of pentobarbital. The pentobarbital was dissolved in saline and the methaqualone in a dilute ethanol solution or a methyl cellulose suspension.

If there are any questions concerning the data in this report, please feel free to call. There are limited amounts of samples 6-10 available for repeat analysis if required.

SAMPLE 6

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.

QUALITATIVE IDENTIFICATION: 74 LABORATORIES RESPONDING

Analytes Present	Weighed-In Value	% Positive Responses
Propoxyphene	20 mg/L	96 (71/74)
Norpropoxyphene	30 mg/L	84 (62/74)
Salicylate	100 mg/L	38 (28/74)

This sample was repared at the Center for Human Toxicology from a urine that was known to be drug-free; nonetheless, two laboratories reported acetaminophen and acetylsalicylic acid; there were single lab reports for each of the following drugs: amobarbital, secobarbital, phenylbutazone, theophylline, phenobarbital, primadone, and phenytoin. Caffeine and nicotine were also reported as positive. One laboratory reported negative salicylate.

COMMENTS

A combination of analytical techniques was used to identify the propoxyphene and norpropoxyphene, including thin-layer chromatography, gas chromatography, gas chromatography-mass spectrometry and EMIT. Although fewer laboratories reported norpropoxyphene as present, a number of participants used either EMIT (18/74) or U.V. spectrophotometry (5/74) for identification. Both of these procedures will not distinguish parent drug from metabolite. Although thin-layer chromatography was used by a large number of laboratories (39/74)

for the analysis of propoxyphene, the majority of these (31/39) used other chromatographic procedures or EMIT in addition, in order to identify the parent drug positively.

The screening of salicylate in urine requires a simple color test with 5% w/v ferric chloride (1). It is surprising, therefore, that only 28 laboratories (38%) reported a positive salicylate. The routine use of this spot test is recommended whenever a urine is screened.

Only a few false positives were reported. Two laboratories, however, reported acetylsalicylic acid as being present. In fact, "aspirin" is rarely detectable in the urine.

REFERENCE

1. Poison Detection in Human Organs, Third Edition, A. Curry, 1976.

SAMPLE 7

History

A 30-year-old female was found dead in bed by her roommate. An empty Dalmane bottle was found. Please screen and quantitate any drugs and/or metabolites detected.

QUALITATIVE IDENTIFICATION: 73 LABORATORIES RESPONDING

Analyte Present	Weighed-In Value	% Positive Responses
Ethanol	80 mg/dL	95 (69/73)
Flurazepam	0.8 mg/L	84 (61/73)
Desalkylflurazepam	0.5 mg/L	45 (33/73)

One report was received for each of the following drugs: codeine, methaqualone metabolite, diazepam, and carboxyhemoglobin (37% saturation). Two laboratories reported methaqualone. One participant identified "benzodiazepine metabolites" using EMIT.

QUANTITATIVE DETERMINATION: HISTROGRAMS ARE SHOWN IN FIGURES 1-3

Analyte/Method	# Labs	Mean	S.D.	C.V.%	Range
Ethanol					
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					
All Methods[1]	54	0.97	0.56	58	0.1-3.3
Gas Chromatography[1]	46	0.91	0.54	59	0.1-3.3

Analyte Method	# Labs	Mean	S.D.	C.V.%	Range
Flurazepam (cont.)					
Gas Chromatography[2] Internal Standard	40	0.93	0.56	60	0.1-3.3
High Performance Liquid Chromatography	5				0.65-2.2
Desalkylflurazepam					
All Methods[2]	26	0.61	0.27	44	0.18-1.4
Gas Chromatography[2]	21	0.59	0.28	47	0.18-1.4
Gas Chromatography[2] Internal Standard	19	0.60	0.29	48	0.18-1.4
High Performance Liquid Chromatography	4				0.41-0.75

[1]Two results were omitted from these data.

[2]One result was omitted from these data.

One laboratory reported total flurazepam and desalkylflurazepam by U.V. spectrophotometry (1.1 mg/L).

Results from the laboratories of the Advisory Board members were not included.

GC-ECD (2) was used to determine the concentration of flurazepam and its metabolites in the blood. The sample had been stored at 4°C since preparation. The results were as follows: Flurazem 0.99 mg/L (n = 8) and desalkylfurazepam 0.61 mg/L (n = 8).

COMMENTS

Generally, the quantitative results for ethanol were accurate, although 4 laboratories failed to identify the drug. The benzodiazepine blood concentrations are representative of those found in overdose cases. The coefficients of variation for the quantiations show a large interlaboratory variation. Of the 61 participants who identified flurazepam correctly, only 33 (54%) identified the metabolite. This metabolite would be of concern if the deceased survived for a long period after ingestion of flurazepam because the parent drug clears rapidly from blood, whereas the metabolite has a much longer half-life (3).

Analytical procedures that are used for the analysis of diazepam and nordiazepam can be adapted to screen and quantitate flurazepam and desalkylflurazepam. The latter has a retention time between that of diazepam and nordiazepam on the widely used OV-17 (or SP 2250) gas chromatographic systems. In addition to desalkylflurazepam, hydroxyethyl flurazepam may be detected in blood samples; however, its half-life is considerably shorter than that of the desalkyl metabolite.

REFERENCES

2. M.A. Peat and L. Kopjak. J. of For. Sci. 24:46, 1979.

3. S.A. Kaplan, J.A.F. deSilva et al. J. Pharm. Sci. 19 62:1932, 1973.

4. D.M. Rutherford. J. of Chromat. 137:439, 1977.

5. R.C. Baselt, C.B. Stewart and S.J. French. J. of Anal. Tox. 1:10, 1977.

6. N. Stronjy, C.V. Puglisi and J.A.F. deSilva. Anal Letter 135:B11, 1978.

SAMPLES 8 and 9

History

A 25-year-old male was found dead in a hotel room. A collection of drug paraphernalia was also found. Please screen the blood sample and quantitate any drugs and/or metabolites in this sample and in the liver homogenate. Cause of death: pending toxicology.

QUALITATIVE IDENTIFICATION: SAMPLE 8 BLOOD: 70 LABORATORIES RESPONDING

Analyte	Weighed-In Value	% Positive Responses
Methaqualone	15 mg/L	89 (62/70)
Methaqualone Metabolite	7 mg/L	41 (29/70)
Pentobarbital	10 mg/L	80 (56/70)

SAMPLE 9 LIVER HOMOGENATE: 68 LABORATORIES RESPONDING

Analyte	% Positive Responses
Methaqualone	84 (57/68)
Methaqualone Metabolite	34 (23/68)
Pentobarbital	76 (52/68)

One laboratory used the liver for screening purposes and another found the quantity of sample insufficient for methaqualone analysis.

QUANTITATIVE IDENTIFICATION: SAMPLE 8 BLOOD: HISTOGRAMS FOR METHAQUALONE AND PENTOBARBITAL ARE SHOWN IN FIGURES 4 AND 5.

Analyte/Method	# Labs	Mean	S.D.	C.V.%	Range
Methaqualone					
All Methods[1]	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
High Performance Liquid Chromatography[1]	3				12.5-16
Methaqualone Metabolite					
All Methods	10	7.5	4.0	53	1.87-14.1
Gas Chromatography	9				1.87-14.1

Analyte Method	# Labs	Mean	S.D.	C.V.%	Range
Pentobarbital					
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
U.V. Spectrophotometry	3				6.0-9.0

SAMPLE 9 LIVER-HOMOGENATE: HISTOGRAMS ARE SHOWN FOR METHAQUALONE AND PENTOBARBITAL IN FIGURES 6 and 7

Analyte Method	# Labs	Mean	S.D.	C.V.%	Range
Methaqualone					
All Methods[2]	45	8.3	3.7	45	1.5-20
Gas Chromatography[3]	39	8.2	3.7	45	1.5-20
Gas Chromatography Internal Standard[4]	32	7.9	3.3	42	1.5-14.5
High Performance Liquid Chromatography	4				8.6-11.3
Methaqualone Metabolite					
All Methods	7				2.7-12.03
Pentobarbital					
All Methods[3]	41	41.5	15	36	12-84.3
Gas Chromatography[5]	32	43	16	37	12-84.3
Gas Chromatography Internal Standard[5]	25	42	14.5	35	12-74

[1]Two Results were omitted from these data.

[2]Six results were omitted from these data.

[3]Five results were omitted from these data.

[4]One result was omitted from these data.

[5]Four results were omitted from these data.

Results from the laboratories of the Advisory Board were not included. The samples were analyzed at CHT by gas chromatography-chemical ionization mass spectrometry for methaqualone, and by HPLC for pentobarbital and methaqualone metabolite. The results were as follows:

Blood: Methaqualone 11 mg/L (n = 2), metabolite 5 mg/L (n = 3), and pentobarbital 8 mg/L (n = 2).

Liver Homogenate: Methaqualone 8 mg/L (n = 2), metabolite 6 mg/L (n = 3), and pentobarbital 48 mg/L (n = 3).

Five laboratories identified diazepam as positive in samples 8 and 9, two identified ethanol as being present in the blood sample, and others identified phenytoin, pentazocine, tripellenamine, nordiazepam, amobarbital, secobarbital, and glutethimide in sample 9. One laboratory identified a short-acting barbiturate in the liver homogenate.

COMMENTS

Most laboratories identified methaqualone and its metabolite correctly, even though a number of laboratories possibly do not have a pure standard of the metabolite. An appreciable number of laboratories (7%) identified diazepam; this benzodiazepine co-elutes with the methaqualone metabolite on many of the silicone gas chromatographic liquid phases. The presence of diazepam can be confirmed by either analyzing for the normetabolite which should also be present or by an alternative chromatographic technique, such as HPLC. The concentrations of methaqualone and metabolite present are detectable by routine screening procedures (7-8) for basic drugs and can be quantitated by similar methods.

Fewer laboratories reported pentobarbital as present in the blood or liver homogenate than expected. The concentrations present were detectable using all of the common screening procedures. As with the quantitation of methaqualone, there was a wide interlaboratory variation for the determination of blood and liver homogenate concentrations of pentobarbital.

REFERENCES

7. E.H. Forester, D. Hatchett and J.C. Garriott. J. of Anal. Tox. 3:155, 1979.

8. W.O. Pierce, T.C. Lamoreaux et al. J. of Anal. Tox 2:26, 1978.

SAMPLE 10

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.

QUALITATIVE IDENTIFICATION: 73 LABORATORIES RESPONDING

Analyte	Weighed-In Value	% Positive Responses
Cocaine	20 mg/L	92 (67/73)
Benzoylecgonine	50 mg/L	66 (48/73)
Dextromethorphan	2 mg/L	27 (20/73)

Three laboratories reported methaqualone, two reported methamphetamine, and one each reported nalorphine, ecgonine, amphetamine, methadone, and methadone metabolite.

COMMENTS

The majority of participants used a combination of chromatographic techniques and immunoassay to identify cocaine and its metabolite. Those laboratories which identified dextromethorphan used a combination of thin-layer and gas-liquid chromatography. Although the concentration of this drug is lower than that expected from an overdose, it is reasonable following therapeutic ingestion for cough suppression, and it should still be detected by those participants who use GLC and TLC techniques.

FIGURE 1 SAMPLE 7, FLURAZEPAM (ALL METHODS)

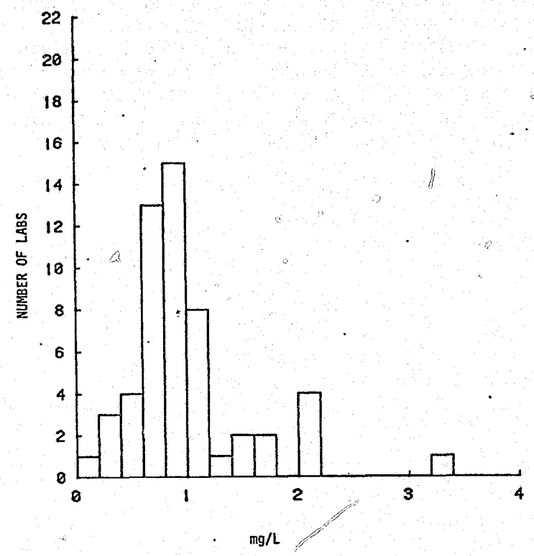


FIGURE 2 SAMPLE 7, DESALKYLFLURAZEPAM (ALL METHODS)

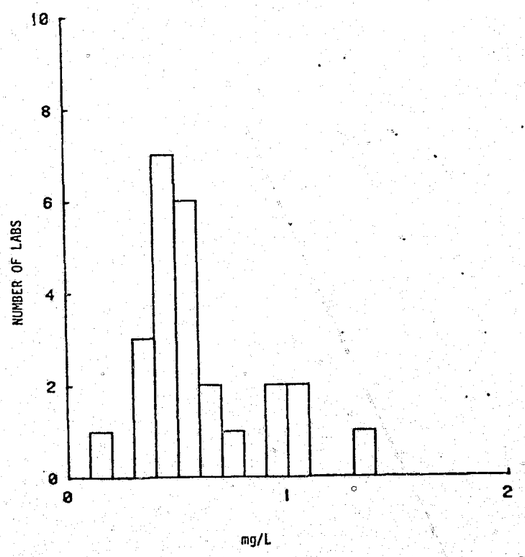


FIGURE 3 SAMPLE 7, ETHANOL (ALL METHODS)

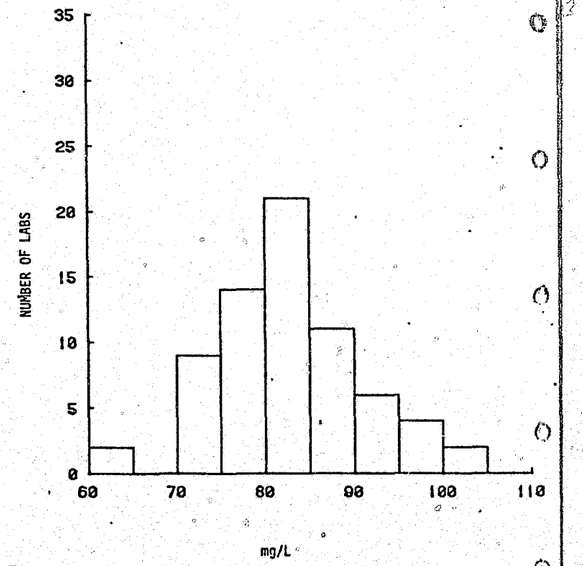


FIGURE 4 SAMPLE 8, METHAQUALONE (ALL METHODS)

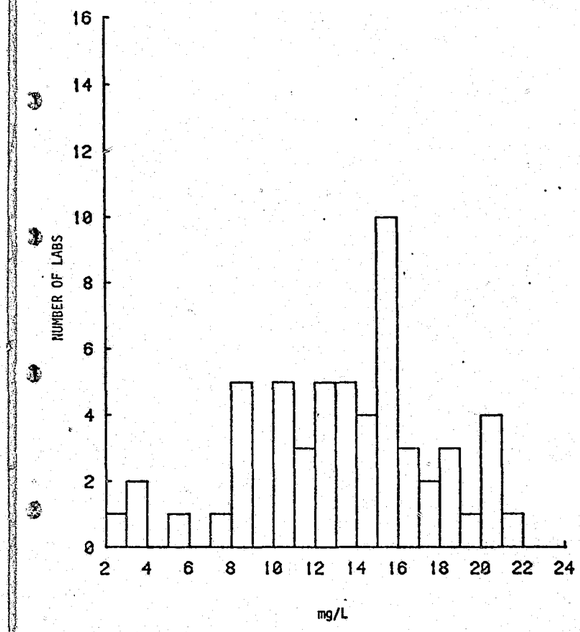


FIGURE 5 SAMPLE 8, PENTOBARBITAL (ALL METHODS)

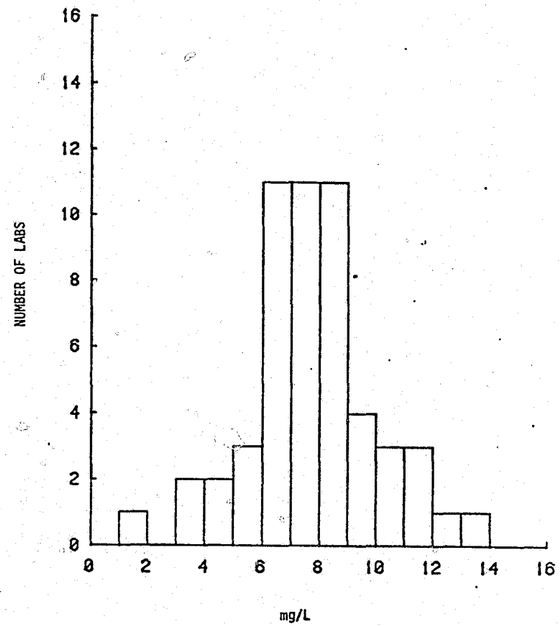


FIGURE 6 SAMPLE 9, METHAQUALONE (ALL METHODS)

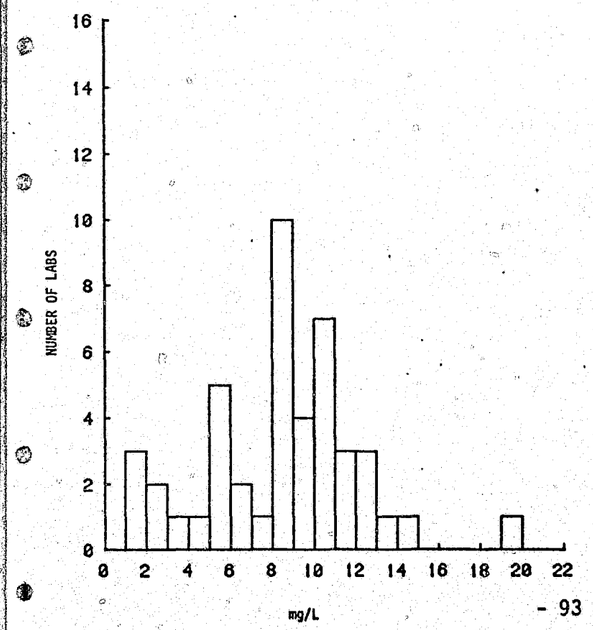
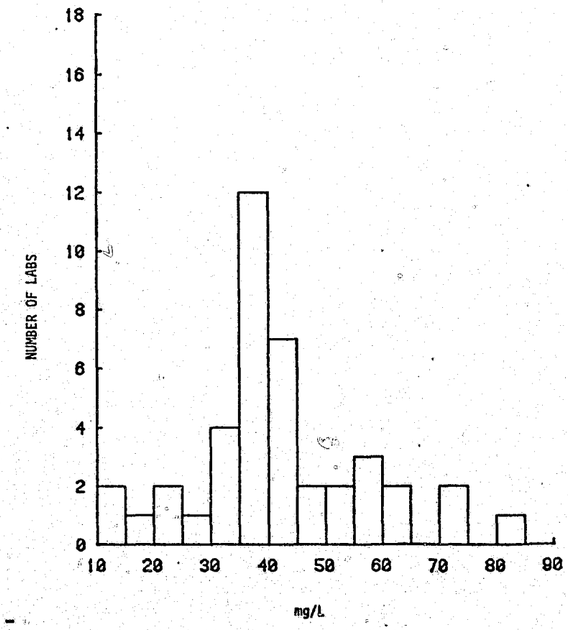


FIGURE 7 SAMPLE 9, PENTOBARBITAL (ALL METHODS)



PROFICIENCY TESTING PROGRAM

INTERIM REPORT SAMPLES 11-15

INTRODUCTION

103 batches of samples were shipped on May 5, 1981. A limited number of reports of breakage and spillage were received. Duplicate samples were shipped to these participants. 64 replies (postmarked by May 22, 1981) were received.

Blood samples (#11, 12, 13 and 14) were prepared from bovine blood by dissolving appropriate amounts of the drug or a salt of the drug in water. These solutions were used to 'spike' the blood sample. Urine sample # 15 was prepared by dissolving appropriate amounts of the drugs in water and using this solution to spike drug-free urine.

A small number of laboratories reported the presence of secobarbital in samples 11, 12 and 13, repeat analysis by RIA and HPLC at the Center also detected low levels (less than 0.5 mg/L). It is possible that these low levels resulted from contamination.

If there are any questions concerning the data in this report, please feel free to call. There are limited amounts of samples 11-15 available for repeat analysis if requried.

SAMPLE 11

History

A 6 year old child was admitted to a hospital suffering from acidosis. His mother indicated that a number of aspirin tablets were missing. Although the child was correctly treated he died twenty four hours after admission. An autopsy was performed and a blood sample taken. Please determine the salicylate concentration and screen the specimen for other drugs. Determine the concentrations of any other drugs and/or metabolites detected.

QUALITATIVE IDENTIFICATION: 62 LABORATORIES RESPONDING

Analytes Present	Weighed-In Value	% Positive Responses
Salicylic Acid	300 mg/L	98 (60/62)

Two laboratories of the 64 responding reported that the sample was incompatible for salicylate analysis by their techniques. Of the 62 laboratories responding, one reported a negative using the Dupont-ACA and two reported a positive without quantitation. In addition 2 laboratories reported acetaminophen, 2 acetylsalicylic acid, 3 methanol and one reported an ethanol concentration of less than 30 mg/dL.

QUANTITATIVE DETERMINATION: HISTOGRAM FOR SALICYLATE DETERMINATION IS SHOWN IN FIGURE 1.

Analyte/Method	# Labs	Mean	S.D.	C.V. %	Range
Salicylic acid					
All methods[1]	52	295	121	41	100-730
Colorimetric[2]	22	270	93	34	100-400
UV[3]	19	296	86	29	190-430

[1]Five results were omitted from these data.
[2]One result was omitted from these data.
[3]Two results were omitted from these data.

Results from the Advisory Board Members were not included in this analysis. A colorimetric method was used to quantitate the drug at the Center for Human Toxicology. The sample had been stored at 4°C since preparation. The mean salicylic acid concentration was 312 (n=3).

COMMENTS

Although the blood concentration of salicylate, in this case was low compared to those seen from suicidal overdoses, it is consistent with the described history. Generally, the quantitative results were accurate, inspection of Figure 1 shows that 85% fell within 1 standard deviation of the mean. Those results that were deleted from the data were all below 100. It is interesting to note that the histogram appears to demonstrate bimodal characteristics; there is no apparent explanation for this.

Comparison of the commonly used colorimetric and ultra-violet procedures failed to reveal any significant difference between them. Other analytical methodology used to quantitate the drug included fluorescence (n=4), gas chromatography (n=2), and high pressure liquid chromatography (n=1).

SAMPLE 12

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.

QUALITATIVE IDENTIFICATION: 61 LABORATORIES RESPONDING

Analytes Present	Weighed-In Value	% Positive Responses
Propoxyphene	5.0 mg/L	82 (50/61)
Norpropoxyphene	4.0 mg/L	69 (42/61)
Doxepin	0.4 mg/L	43 (26/61)
Nordoxepin	0.6 mg/L	21 (13/61)

Eight laboratories reported nortriptyline, 7 amitriptyline, 2 salicylate, 4 methanol, 1 phenobarbital, 1 acetaminophen and one a blood ethanol concentration of less tha 30 mg/dL.

QUANTITATIVE DETERMINATION: HISTOGRAMS FOR PROPOXYPHENE, NORPORPOXYPHENE AND DOXEPIN ARE SHOWN IN FIGURES 2-4

Analyte/Method	#Labs	Mean	S.D.	C.V. %	Range
Propoxyphene					
All methods[2]	42	4.63	2.0	43	0.8-10.0
Gas Chromatography[2]	41	4.64	2.0	44	0.8-10.0
Gas Chromatography Internal Standard[2]	35	4.84	1.9	39	1.0-10 0

Analyte/Method	#Labs	Mean	S.D.	C.V. %	Range
Norpropoxyphene					
All methods[1]	36	4.29	2.7	63	0.2-11.0
Gas chromatography[1]	35	4.29	2.7	63	0.2-11.0
Gas chromatography internal standard[1]	30	4.04	2.5	62	0.5-11.0
Doxepin					
All methods[2]	24	0.43	0.23	54	0.14-1.0
Gas chromatography[2]	21	0.46	0.24	52	0.14-1.0
Gas chromatography internal standard[2]	16	0.46	0.24	52	0.14-1.0
Nordoxepin					
All methods[2]	11	0.70	0.38	55	0.2-1.48

[1]two results were omitted from these data
[2]one result

Results from the laboratories of the Advisory Board Memebers were not included.

The sample was analyzed at CHT during the week of shipment and during the time of analysis by participants; propoxyphene and norpropoxyphene were determined by a combination of GC-CIMS and GC-NPD and doxepin and nordoxepin were determined by GC-CIMS (1). The results were as follows: propoxyphene 5.1 mg/L (n=8), norpropoxyphene 4.5 mg/L (n=4), doxepin 0.62 mg/L (n=8), nordoxepin 0.71 mg/L (n=5).

COMMENTS

The concentrations of propoxyphene, norpropoxyphene, doxepin and nordoxepin were representative of those encountered in cases of death resulting from the combined ingestion of propoxyphene and doxepin. 82% of laboratories responding identified propoxyphene and 69% norpropoxyphene, whereas, only 43% identified doxepin and 21% it's metabolite. A significant number of respondents reported nortriptyline (13%, 8/61) and amitriptyline (11%, 7/61). GLC was used by the majority of participants to screen and quantitate the particular drugs and metabolites in this case. Pierce et al (2) have reported the following relative retention times (to prazepam) for these compounds on the commonly used OV-17 and OV-1 systems:

	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

It is obvious, therefore, that caution should be exercised when identifying peaks that have retention times in this area. In addition the use of electron-impact mass spectrometry and identification of base peak could be confusing as propoxyphene, doxepin and amitriptyline all have a base peak of m/z 58 (3).

The coefficients of variation for the quantitation of the four drugs and metabolites show a large interlaboratory variation. The highest was that for norpropoxyphene (range 62-63%) which because of its chemical reactivity in alkali solution spontaneously rearranges to the amide. In fact, a more reliable quantitation is acheived by forcing this reaction to completion, and then chromatographing the amide (4). This would also assist in a positive identification of norpropoxyphene.

SAMPLE 13

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 tranquillizers for anxiety. Please screen the blood sample and determine the concentration of any drugs and/or metabolites detected. Cause of death: pending toxicology.

QUALITATIVE IDENTIFICATION: 60 LABORATORIES RESPONDING

Analyte Present	Weighed-In Value	% Positive Responses
Diazepam	1.0 mg/L	90 (54/60)
Nordiazepam	1.5 mg/L	73 (44/60)
Morphine	0.05 mg/L	25 (15/60)
Codeine	0.15 mg/L	25 (15/60)

One laboratory reported a total benzodiazepine by UV and another laboratory an opiate positive by RIA. Three participants reported methanol, 2 phenytoin, 1 chlordiazepoxide, 1 oxazepam, 1 amphetamine, 1 benzoylecgonine and 1 an ethanol concentration of less than 30 mg/dL.

QUANTITITAVE IDENTIFICATION: HISTOGRAMS FOR DIAZEPAM, NORDIAZEPAM AND CODEINE ARE SHOWN IN FIGURES 5 THROUGH 7.

Analyte/Method	#Labs	Mean	S.D.	C.V.%	Range
Diazepam					
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					
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					
All methods[1]	8	0.081	0.018	22	0.06-0.09
Codeine					
All methods[2]	14	0.28	0.13	46	0.10-0.60

[1] three results were deleted from these data
[2] one result was deleted from the data

Results from the laboratories of the Advisory Board Members were not included.

The samples were analyzed by GC-CIMS (5) for the opiate narcotics, and the benzodiazepines were quantitated by GC-ECD (6) at CHT. Prior to analysis the samples were kept at 4°C. The results were as follows: diazepam 0.94 mg/L (n=5), nordiazepam 1.46 mg/L (n=5), morphine 0.06 mg/L (n=2) codeine 0.20 mg/L (n=2).

COMMENTS

The concentrations of diazepam and nordiazepam included in this sample were the same as those in Sample 2. In general, the qualitative and quantitative results from Sample 13 were similar to those reported for Sample 2. The identification and quantitation of the benzodiazepines, in particular diazepam and nordiazepam, were discussed in the First Interim Report.

Low concentrations of morphine (0.05mg/L) and codeine (0.15 mg/L) were also included in this sample. Baselt (7) has reported that blood morphine concentrations range from 0.01-3.0 mg/L in heroin fatalities. Only 25% (15/60) of the respondents identified morphine and codeine as present and of those, 12 quantitated the morphine, whereas, the codeine was determined by all 15 participants. The most suitable screening technique for such low concentrations is radioimmunoassay. The commercially available I-125 kit cross reacts to morphine on approximately a 1:1 basis. A number of gas liquid chromatographic procedures are available for quantitating these opiate narcotics. Commonly, the silyl (8) or acetyl derivative (9) is formed for morphine and flame ionization detection used.

SAMPLE 14

An industrial worker was found dead near a carbon monoxide generator. The deceased was a known epileptic. An autopsy revealed signs of recent seizure activity. Please screen the blood sample for drugs and quantitate any drugs and or metabolites detected.

QUALITATIVE IDENTIFICATION: 63 LABORATORIES RESPONDING

Analyte Present	Weighed-in Value	% Positive Responses
Phenobarbital	20 mg/L	98 (62/63)
Carboxyhemoglobin	30 % saturation	91 (57/63)

Three participants reported the presence of methanol and one reported an ethanol concentration of less than 40 mg/dL.

QUANTITATIVE IDENTIFICATION: HISTOGRAMS FOR PHENOBARBITAL AND CARBOXY-HEMOGLOBIN ARE SHOWN IN FIGURES 8-9

Analyte/Method	#Labs	Mean	S.D.	C.V. %	Range
Phenobarbital					
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[1]	51	29	11	38	13-50
Co-oximeter[2]	12	34	13	38	16.2-48.4
Spectrophotometric[3]	18	29	9	31	15-47.4
Palladium chloride	11	27	12	44	13-42
Gas liquid chromatography	6				23-50

[1] four results were deleted from these data.
[2] one results was deleted from these data.
[3] two results were deleted from these data.

The results from the laboratory of the Advisory Board Members were not included in this analysis.

Analysis over the period of shipment etc. at CHT by spectrophotometric procedure showed a carboxyhemoglobin saturation of 30% (n=4). The phenobarbital concentration was found to be 17.8 mg/L (n=4) by HPLC.

COMMENTS

Generally, the qualitative and quantitative results for phenobarbital were accurate. It is surprising, considering the number of recent publications on HPLC of the anti-convulsants, that only 13% of the respondents used this technique to quantitate the phenobarbital.

Carboxyhemoglobin was also included in this sample. 91% of the respondents performed carboxyhemoglobin determinations, a test which is consistent with the history. The percent saturation was half that in Sample 2; however, the coefficients of variation, particularly that for the Co-Oximeter, were considerably higher. As with Sample 2, the use of palladium chloride diffusion methods resulted in the highest coefficient of variation.

SAMPLE 15

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.

QUALITATIVE IDENTIFICATION: 61 LABORATORIES RESPONDING

Analyte Present	Weighed-In Value	% Positive Responses
Meprobamate	75 mg/L	56 (34/61)
Imipramine	2 mg/L	87 (53/61)
Desipramine	3 mg/L	75 (46/61)

Three laboratories reported doxepin, 2 reported nordoxepin, and 1 each reported meperidine, normeperidine, amitriptyline, methaqualone and carisprodol.

COMMENTS

The majority of participants used a combination of chromatographic techniques to identify the three drugs included in this sample. Less than half those responding identified meprobamate; this drug is susceptible to thermal decomposition in the injection port of the gas chromatograph, and for this reason it is more reliable to use TLC as a screening technique; furfural:HCl (10) can be used as a relatively selective spray reagent for detection. The qualitative identification of the tricyclic antidepressants caused little problem to the majority of the participants, although, a small number misidentified them as other members of that group. For those using TLC as a screening technique, this is suprising as imiprimine and desipramine both react with FPN and H_2SO_4:-ethanol, two spray reagents commonly used to detect the phenothiazines.

The identification of the tricyclic antidepressants by GC and GC/MS was discussed under Sample 12.

REFERENCES

1) D.M. Chinn, T.A. Jennison, D.J. Crouch et al, Clin. Chem. 1980 26 p 1201

2) W.O. Pierce, T.C. Lamoreaux, F.M. Urry et al, J. Anal. Tox. 1978 2 P 26

3) B.S. Finkle, E.J. Cherry and D.M. Taylor, J. Chromat. Sci. 1971 9 P 393-419

4) R.L. Wolen, E.A. Ziege and C.M. Gruber, Clin. Pharmacol. & Therap. 1975 17 p 15-20

5) GC/MS Assays for Abused Drugs in Body Fluids, NIDA Research Monograph Series, #32, 1980, Eds. Foltz et al, p 110

6) M.A. Peat and L. Kopjak, J. of For Sci. 1979 24 p 46

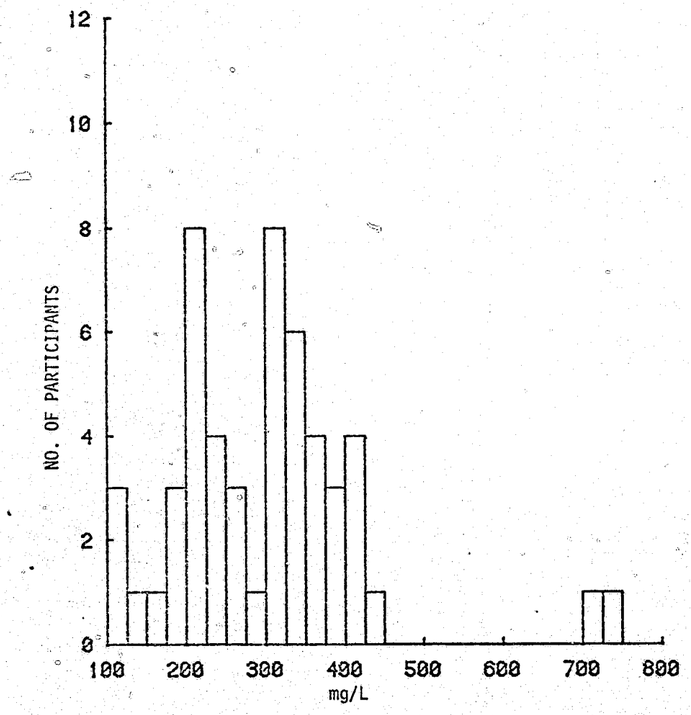
7) R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, Vol 1. Pub. Biomedical Publications, p 12.

8) G.R. Nakamura and E.L. Way, Anal. Chem. 1975 47 p 775

9) N.C. Jain, T.C. Sneath, R.D. Budd et al, Clin. Chem. 1975 21 p 1486

10) A.Curry, Poison Detection in Human Organs, 3rd Edition, Pub. Charles C. Thomas, p 269

FIGURE 1 SALICYLIC ACID (TOTAL)



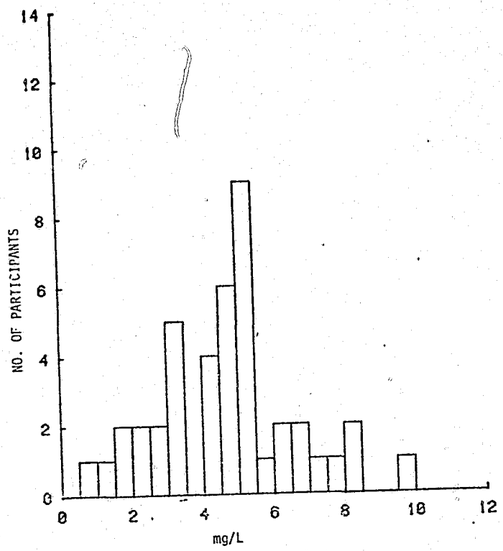


FIGURE 2: PROPOXYPHENE (TOTAL)

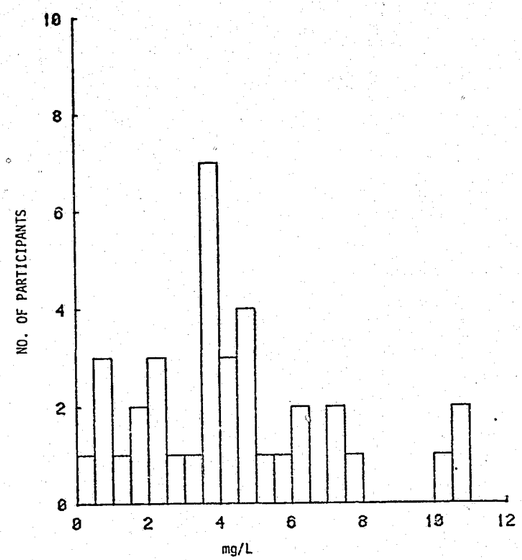


FIGURE 3: NORPROPOXYPHENE (TOTAL)

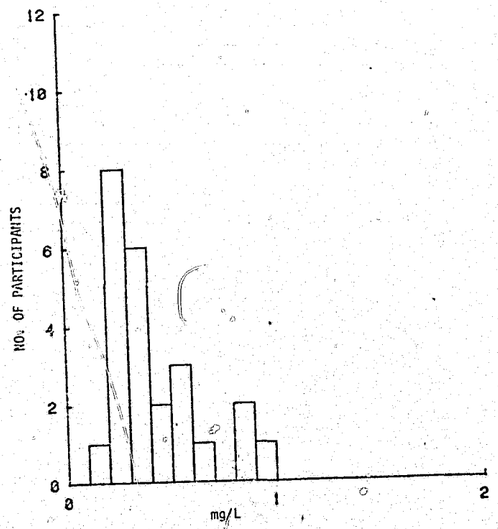


FIGURE 4: DOXEPIN (TOTAL)

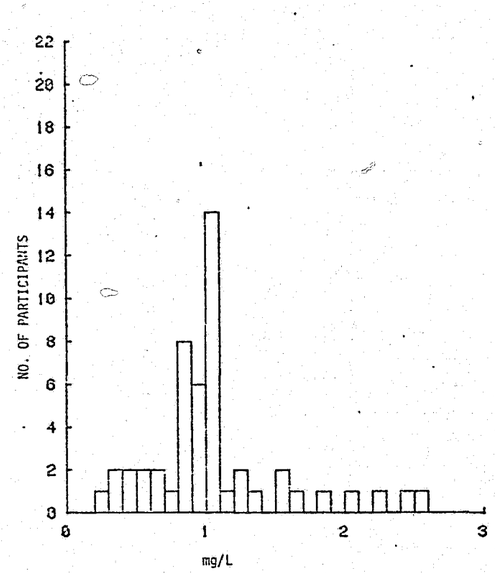


FIGURE 5: DIAZEPAM (TOTAL)

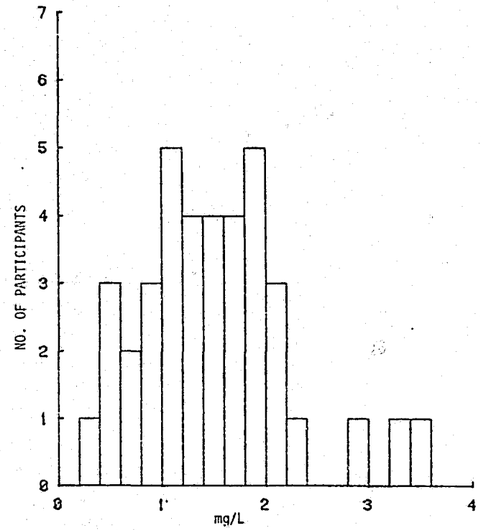


FIGURE 6: NORDIAZEPAM (TOTAL)

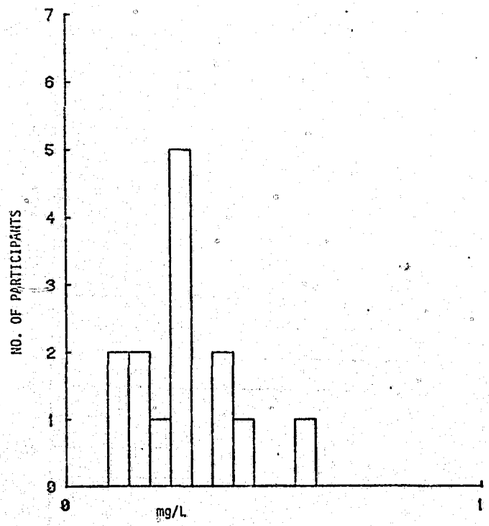


FIGURE 7: CODEINE (TOTAL)

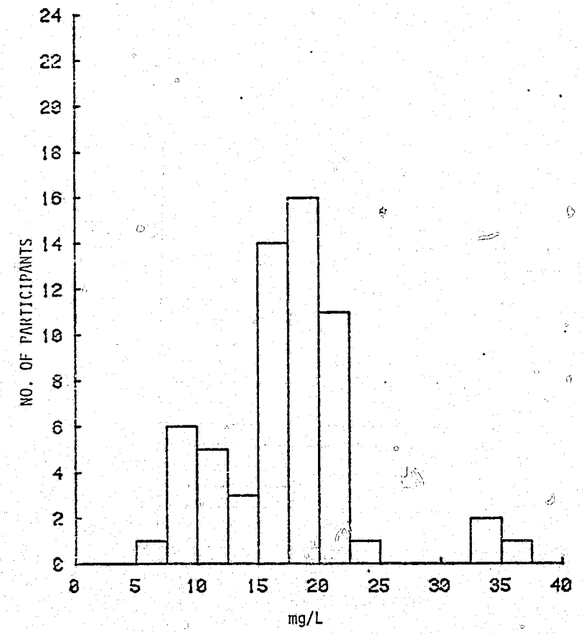


FIGURE 8: PHENOBARBITAL (TOTAL)

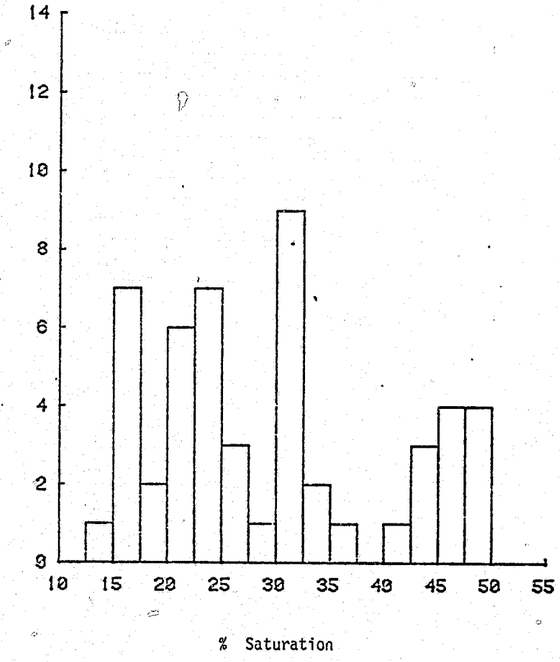


FIGURE 9: CARBOXYHEMOGLOBIN (TOTAL)

PROFICIENCY TESTING PROGRAM

INTERIM REPORT SAMPLES 16-20

INTRODUCTION

103 batches of samples were shipped on July 7, 1981. Only one report of breakage and spillage during shipment was received. Duplicate samples were mailed immediately to this participant. 66 replies (postmarked not later than July 24, 1981) were received.

Samples number 16,17,18 and 19 were prepared as a set from a single hypothetical case. Sample number 16, the "gastric contents" was prepared synthetically in the laboratory and had added to it an appropriate amount of Darvocet-N-100. Sample number 17 was prepared from bovine blood by dissolving appropriate amounts of the drug or a salt of the drug in water. These solutions were then used to "spike" the bovine blood. Sample number 18 was prepared by treating a population of rats with propoxyphene and acetaminophen chronically. The animals were eventually sacrificed: their livers were then removed, combined and homogenized. The urine sample number 19, was prepared by dissolving appropriate amounts of the drugs in water and using this solution to "spike" drug-free urine. An aqueous ethanol solution was added in appropriate volume to each of the samples to acheive the desired ethanol concentrations.

Blood Sample number 20 was from a separate hypothetical case and was prepared from bovine blood by dissolving appropriate amounts of the drugs or their salt forms in water. These aqueous solutions were used to "spike" the blood sample.

There were only six false positive laboratory reports for samples 16-19, that is from the first case. There were four false positive reports for sample number 20, that is the second case. There are a few remaining samples (16-20) in storage at the Center and are available for repeat analysis if required. If there are any questions concerning the data in this report, or if you wish additional samples, please feel free to call.

SAMPLES 16-19

Case History

A 38 year old male suffered a lower back injury in an industrial accident and was subsequently unemployable. He was prescribed Darvocet-N-100 for chronic pain. He became despondent and was found dead in bed at home one morning. Suicidal drug overdose was suspected. Please screen the blood sample and determine the concentratons of any drugs and/or metabolites in each of the specimens submitted.

Sample 16 - Gastric Contents

Qualitative Identification: 65 Laboratories Responding

Analytes Present	Weighed-In Values	% Positive Responses
Propoxyphene	325 mg total	69 (45/65)
Acetaminophen	3250 mg total	49 (32/65)
Ethanol	1.5% w/v	26 (17/65)

There were no false positive results reported for this sample. Ten laboratories reported only qualitative results on this sample. Several laboratories reported trace concetrations of norpropoxyphene. Although this was not confirmed by

analysis at the Center it is possible that some norpropoxyphene was produced in some of the samples by slow hydrolysis of the parent drug.

Quantitative Determination:

Analyte	# Labs	Mean	S.D.	C.V. %	Range
Propoxyphene	45	290.4 mg	198.2	68	35-900 mg total
Acetaminophen	32	3228 mg	1373	43	1400-7530 mg total
Ethanol	17	1303 mg/dL (1.3 % w/v)	187	14	1026-1800 mg/dL

Results from the advisory board members were not included in this analysis. Gas chromatography and GC-CIMS was used to quantitate the propoxyphene, a HPLC method was used for acetaminophen, and GC for the ethanol quantitation at the Center for Human Toxicology. The sample has been stored a 4°C since preparation.

Comments

Almost all of the reporting laboratories used gas chromatography for the propoxyphene and ethanol analyses and HPLC for the acetaminophen. Only two laboratories used ultra-violet spectrophotometric methods for propoxyphene, and only three laboratories used colorimetric procedures and one an ultra-violet spectrophotometric procedure for acetaminophen. Almost all of the laboratories that reported only qualitative results on this sample used thin layer chromatography. It is interesting to note that the ratio of acetaminophen to propoxyphene free base in Darvocet-N-100 is 10 fold and that the mean reported values for these two drugs (despite the very wide range) approximate the same ratio.

Sample 17 - Blood

Qualitative Identification: 65 Laboratories responding

Analytes Present	Weighed-In Values	% Positive Responses
Propoxyphene	5.0 mg/L	92 (60/65)
Norpropoxyphene	4.0 mg/L	77 (50/65)
Acetaminophen	200 mg/L	75 (49/65)
Ethanol	80 mg/dL	88 (57/65)

One laboratory reported the presence of a methadone metabolite and another laboratory reported the presence of a "cyclopropoxyphene". These were the only false positives reported for this sample.

Quantitative Determination: Histograms for propoxyphene, norpropoxyphene, acetaminophen an ethanol are shown in Figures 1-4.

Analyte	# Labs	Mean	S.D.	C.V. %	Range
Propoxyphene	60	4.7	2.2	46	0.4-10.2 mg/dL
Norpropoxyphene	50	4.9	3.5	71	0.2-13.8 mg/dL
Acetaminophen	49	179.3	57.9	32	76-332 mg/dL
Ethanol	57	78.0	8.2	10	60-105 mg/dL

Results from the advisory board members were not included in this analysis.

The sample was analyzed at CHT immediately following preparation, during the week of shipment and also during the time of analysis by participants. Propoxyphene and norpropoxyphene concentrations were determined by GC-CIMS and GC-NPD. The acetaminophen by HPLC and the ethanol by GC-FID. The results were as follows: propoxyphene 5.4 mg/L, norpropoxyphene 4.7 mg/L, acetaminophen 210 mg/L and ethanol 77 mg/dL.

Comments

The concentration of drugs and metabolites in this sample are typical of those encountered in fatalities resulting from the ingestion of Darvocet and alcohol. The weighed-in values of propoxyphene and norpropoxyphene in this sample were the same as those in sample 12. It is interesting that a greater percentage of laboratories identified and quantitated propoxyphene and its metabolite in this sample than did in sample 12. Although there was a 92% positive response on propoxyphene there was only about three quarters (77%) of the laboratories who quantitated the norproxyphene metabolite. In addition the coefficient of variation for the norproxyphene indicates a very large inter-laboratory variation and clearly the accurate quantitation of this metabolite still presents problems for many laboratories.

Sixty five laboratories out of sixty six returns responded by analyzing this sample. One sample was broken in transit and obviously that laboratory could not respond.

Sample 18 - Liver

Qualitative Identification: 62 Laboratories responding

Analyte Present	Weighed-In Values	% Positive Response
Propoxyphene	-	77 (48/62)
Norpropoxyphene	-	61 (38/62)
Acetaminophen	-	48 (30/62)
Ethanol	150 mg/dL	24 (15/62)

There was one false positive report made for each of the following drugs: a methadone metabolite (GC-MS), methaqualone (GC-MS), codeine, methamphetamine, and salicylate (UV spectrophotometric).

Quantitative Determination: Histograms for Propoxyphene, Norpropoxyphene, Acetaminophen and Ethanol are shown in Figure 5-8.

Analyte	# Labs	Mean	S.D.	C.V. %	Range
Propoxyphene	48	58.2	30.0	51.1	12.3-130.0 mg/kg
Norpropoxyphene	38	16.7	10.8	64.7	1.4-48.0 mg/kg
Acetaminophen	30	146	194.5	133	13.0-780 mg/kg
Ethanol	15	105	15.1	14	76-134 mg/dL

Results from the laboratories of the advisory board members were not included.

Repetative analysis for ethanol at CHT provided a mean concentration of 138 mg/dL as compared to a weighed-in target value of 150 mg/dL.

Comments

Gas chromatography with either FID or NPD detectors was used almost exclusively for the determination of propoxyphene and norpropoxyphene in this sample. Similarly, HPLC was the method of choice by almost all of the respondents for the assay of acetaminophen. Only one or two laboratories used either ultra-violet spectrophotometric procedures or a colorimetric method for acetaminophen. Many laboratories used thin layer chromatography or GC-MS to support the identification of the drugs in this sample. The tabulated statistical data and the histograms show an extremely broad range of results and interlaboratory variation that can not be attributed to diverse analytical techniques for propoxyphene, norpropoxyphene and acetaminophen. Although less than half of the responding laboratories reported concentrations of acetaminophen in this sample, the concentration values are extremely variable and almost defy statistical analysis.

Sample 19 - Urine

Qualitative Identification: 65 Laboratories responding

Analytes Present	Weighed-In Values	% Positive Response
Propoxyphene	10 mg/L	54 (35/65)
Norpropoxyphene	25 mg/L	48 (31/65)
Acetaminophen	500 mg/L	43 (28/65)
Ethanol	100 mg/dL	48 (31/65)

Three false positive results were reported: a methadone metabolite (GC, GC-MS), salicylate (spectrophotometric) and "cyclopropoxyphene".

Quantitative Determination: Histograms for Propoxyphene, Norpropoxyphene, Acetaminophen and Ethanol are shown in Figures 9-12.

Analyte	# Labs	Mean	S.D.	C.V.%	Range
Propoxyphene	35	11.2	4.0	35	3.0-20.8 mg/L
Norpropoxyphene	31	28.9	15.0	52.0	10.6-76.0 mg/L
Acetaminophen	28	649	256	40	286-1327 mg/L
Ethanol	31	97.0	11.6	12	70-110 mg/dL

Results from the laboratories of the advisory board members were not included.

Comments

Only 42 of the responding 65 laboratories provided quantitative results on this sample. The remaining 23 laboratories detected and identified the drugs qualitatively, generally by thin layer or gas chromatography. Only one laboratory did not analyze the urine sample. Although the range of concentrations for urine propoxyphene is very broad the distribtuion about the mean, as shown in Figure 9, is reasonable. In contrast, the urine norpropoxyphene and the urine acetaminophen shown in histogram Figures 10 and 11 is both extremely broad and nonuniform in distribution. This observation is typical for the concentrations of norpropoxyphene and acetaminophen in each of the samples (16-19) in this set. The interlaboratory variation for these analyses can not be attributed to the analytical technique (GLC, UV etc.) alone because almost all of the responding laboratories used the same instrumental techniques; however,

these quantitative analyses are obviously neither simple nor routine for most analytical toxicologists and some inspection of the total method, including extraction and internal standards, seems warranted.

Sample 20 - Blood

History

A young man was brought comatose to a hospital E.R. 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.

Qualitative Identification: 54 Laboratories responding

Analytes Present	Weighed-In Values	% Positive Responses
Secobarbital	2.0 mg/L	44 (24/54)
Morphine	0.5 mg/L	57 (31/54)
Codeine	0.2 mg/L	31 (17/54)

One laboratory only received this specimen in broken or leaking condition and was unable to complete the analysis. Two laboratories reported that there was insufficient sample for them to complete their analysis. Two laboratories reported concentrations of propoxyphene in this sample, determined by GC-NPD. There was also, one report of acetone, ethanol, and cyanide. It was suggested that the cyanide may have resulted from decomposition of the sample.

Quantitative Determination: Histograms for Secobarbital, Morphine and Codeine are shown in Figures 13-15.

Analyte	#Labs	Mean	S.D.	C.V. %	Range
Secobarbital	24	2.4	1.0	43	1.0-4.4 mg/L
Morphine	31	0.59	0.23	39	0.1-1.1 mg/L
Codeine	17	0.25	0.05	22	0.1-0.3 mg/L

One result was omitted from the secobarbital data; two results from the morphine data; and one result from the codeine data.

Results from the laboratories of the advisory board members were not includ. .

This sample was anlyzed at CHT several times during the week of shipment, during the time of analysis by participants, and since receipt of participants reports. The secobarbital was assayed by both HPLC and GC-NPD, the morphine and codeine by GC-CIMS. The mean values were as follows: secobarbital 1.8 mg/L, morphine 0.62 mg/L, and codeine 0.28 mg/L.

Comments

The concentration of codeine in this sample was the same as that in sample 13 but the morphine was increased in this sample by ten fold. The identification and quantitation of morphine and codeine in blood samples was discussed in the interim report dealing with sample 13. Only 54 laboratories responded with quantitative data on this sample, and only 17 of those laboratories provided codeine results. Although the concentration of morphine was increased

by ten fold over that in sample 13, the C.V. % was much greater (sample 13: 22 %, sample 20: 39%). In any event the range of results for morphine in blood is very broad, and that for codeine only slightly better. Of the quantitative methods used for morphine there were 14 spectrofluorometric, 5 GC, 4 GC-MS and 8 RIA. For codeine; 12 laboratories used GC and 5 GC-MS. RIA, GC and TLC were about evenly divided for qualitative identification. Nineteen of 24 reporting laboratories used GC for the secobarbital quantitation. There was 1 HPLC, 1 GC-MS and 3 UV Spec. Apart from 2 GC-MS and 2 RIA, TLC was used for qualitative confirmation. There was no discernible statistical differences between the results obtained by any particular method, even the 14 morphine results by spectroflurometry were widely distributed.

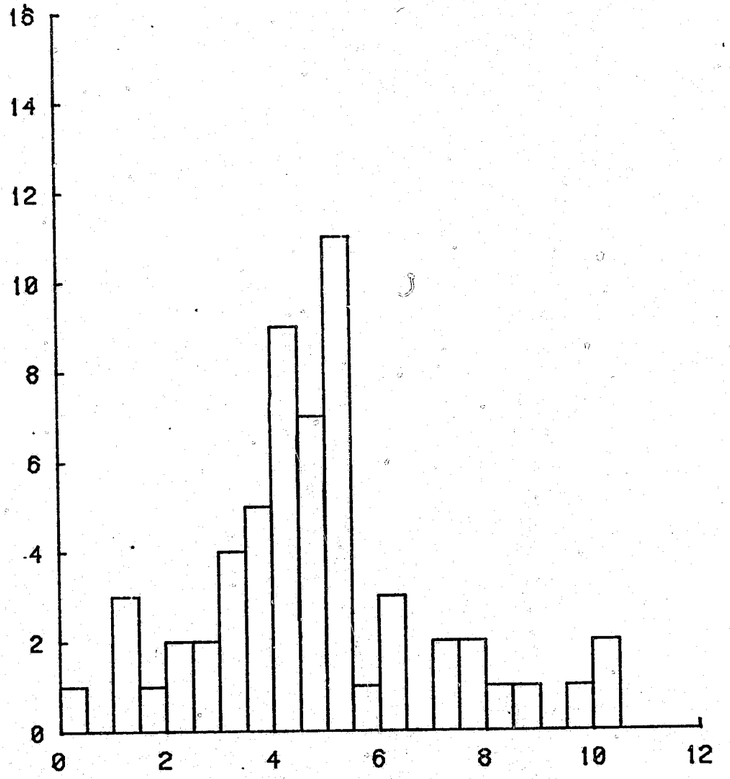


FIGURE 1 Blood-propoxyphene

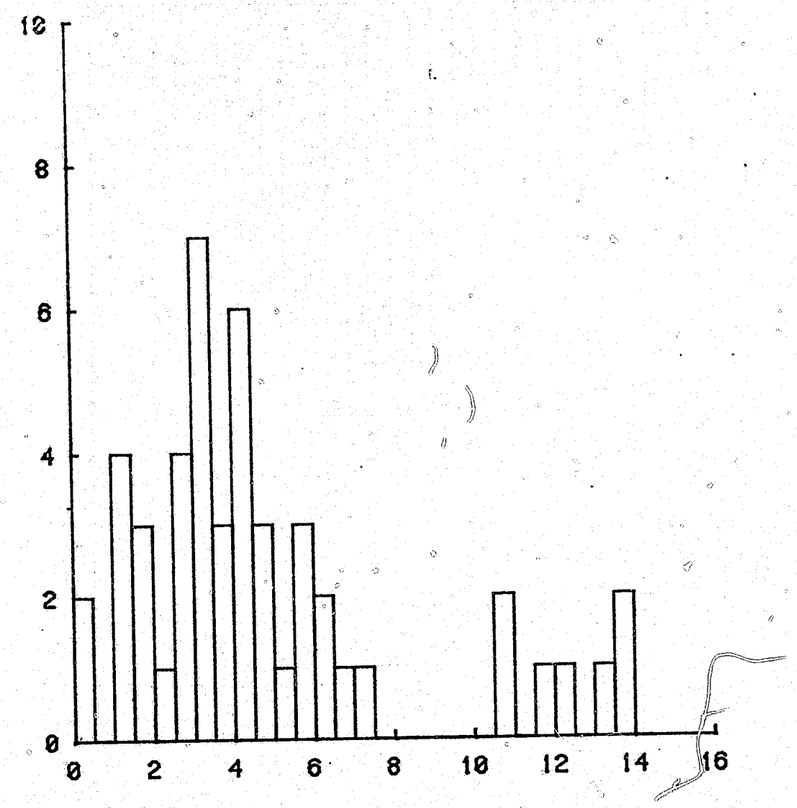


FIGURE 2 Blood-norpropoxyphene

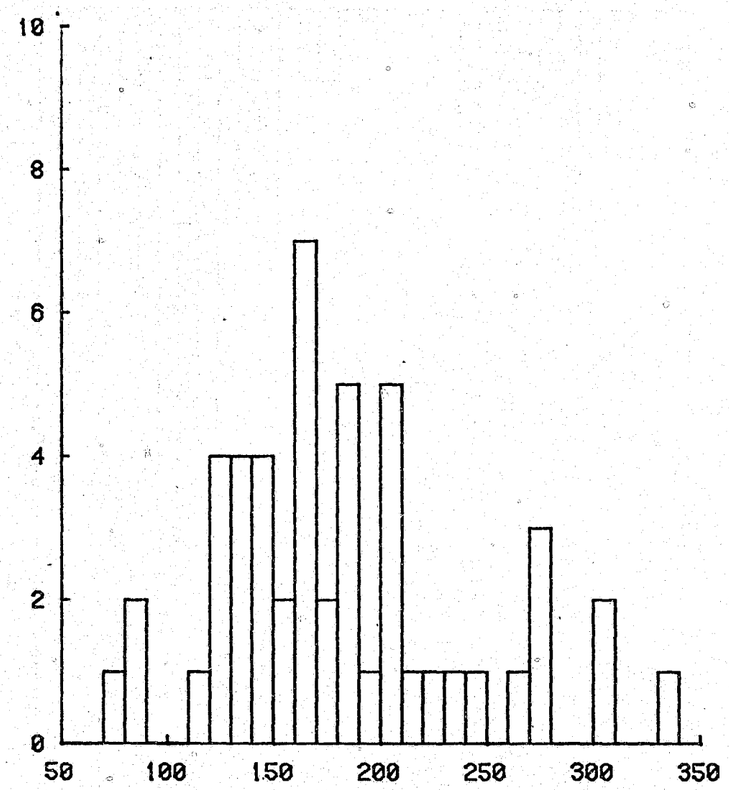


FIGURE 3 Blood-acetaminophen

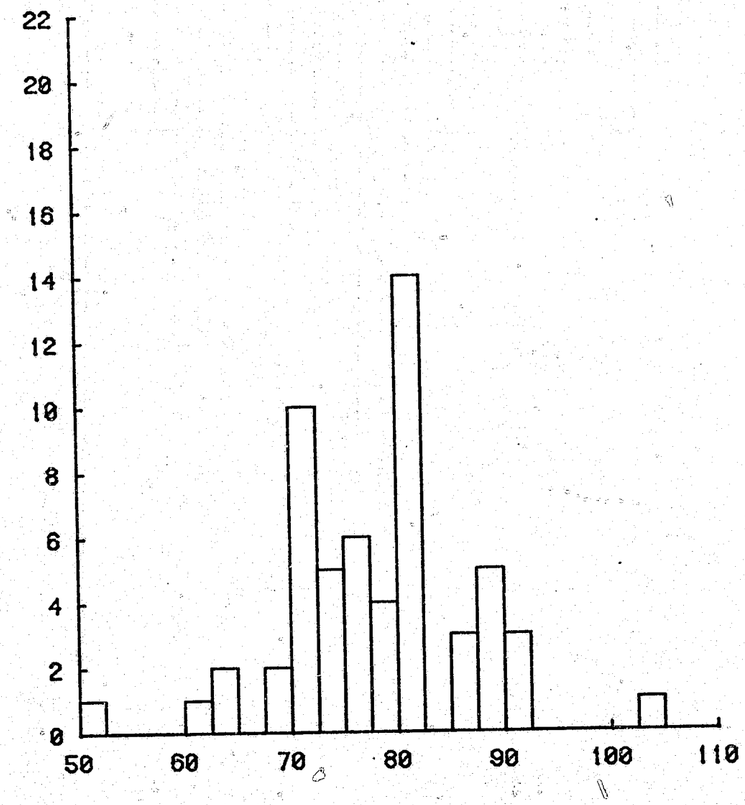


FIGURE 4 Blood-ethanol

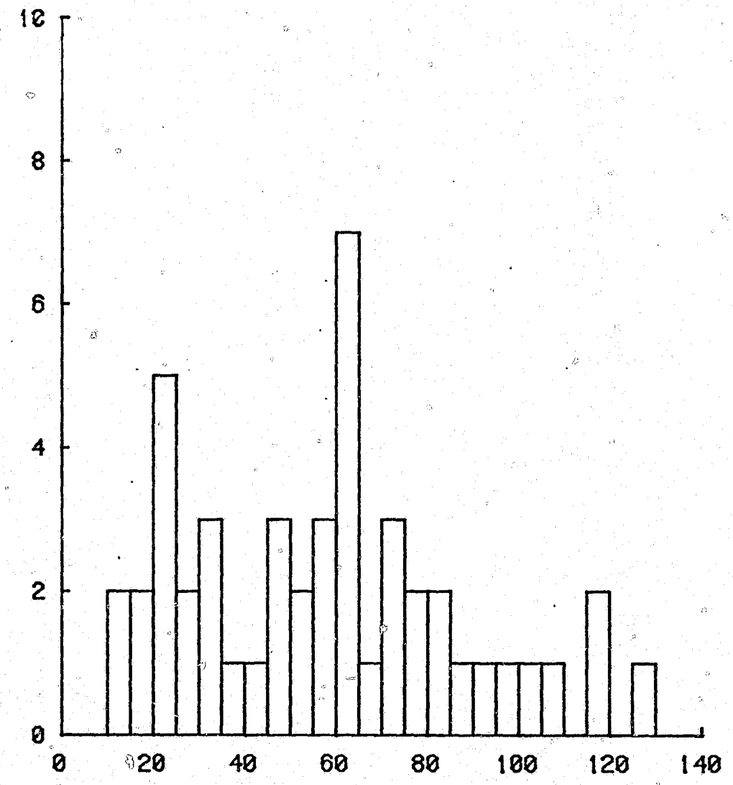


FIGURE 5 Liver-propoxyphene

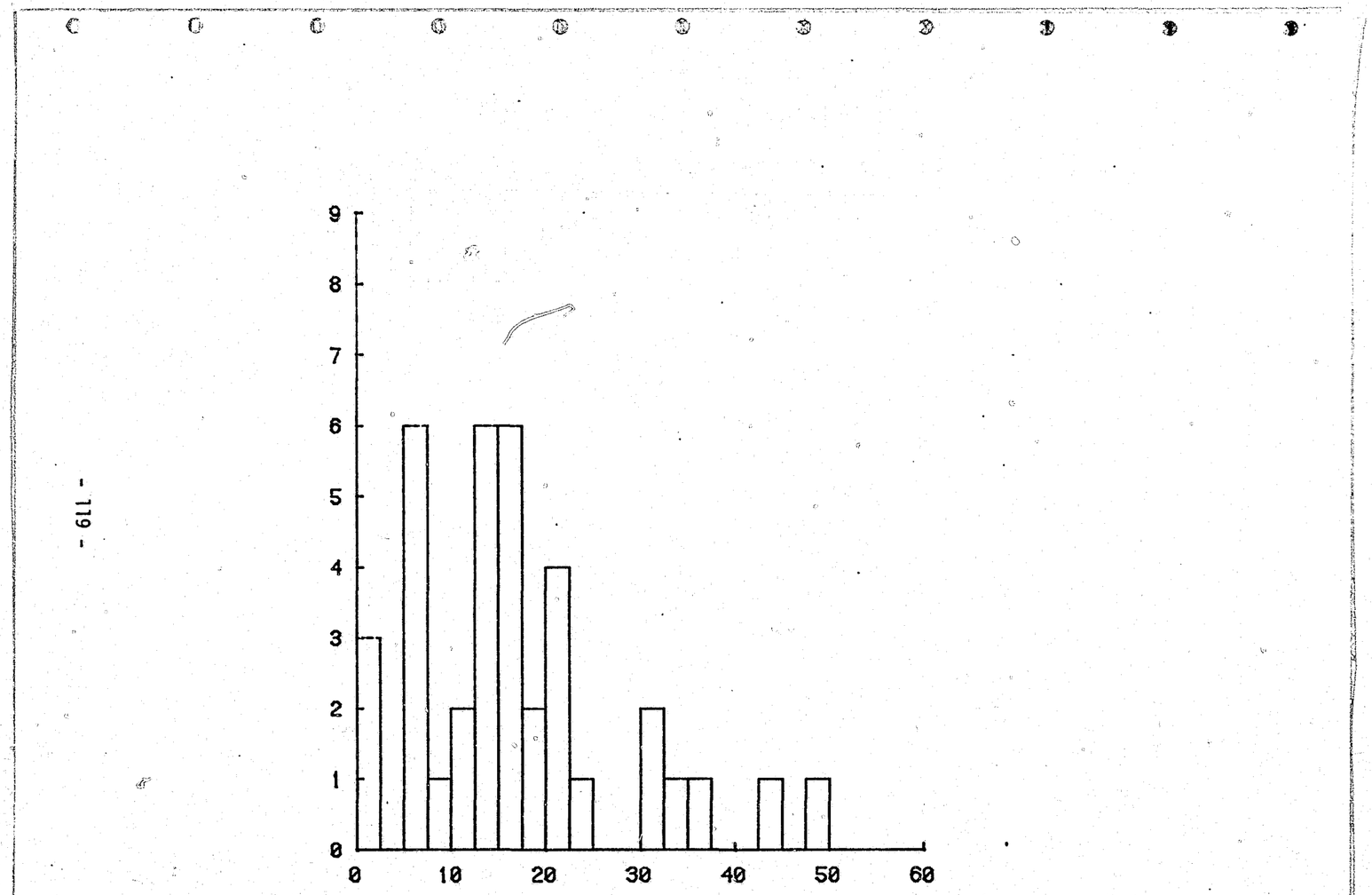


FIGURE 6 Liver-norpropoxyphene

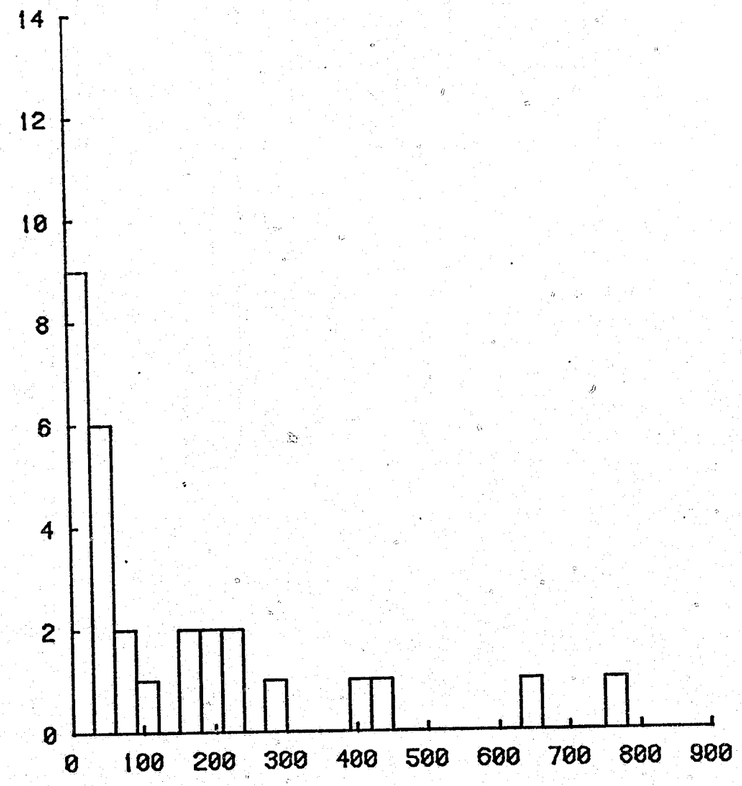


FIGURE 7 Liver-acetaminophen

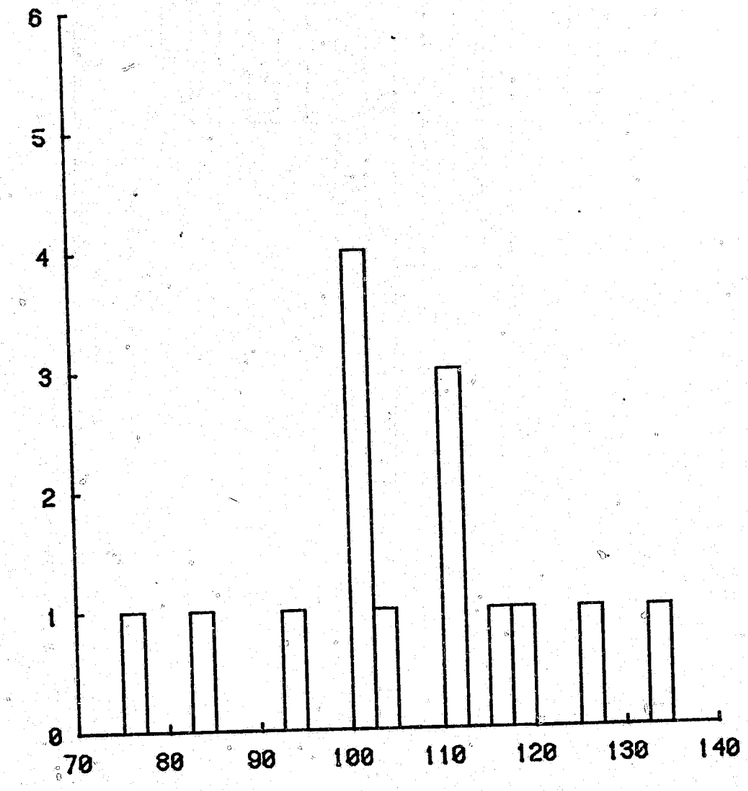


FIGURE 8 Liver-ethanol

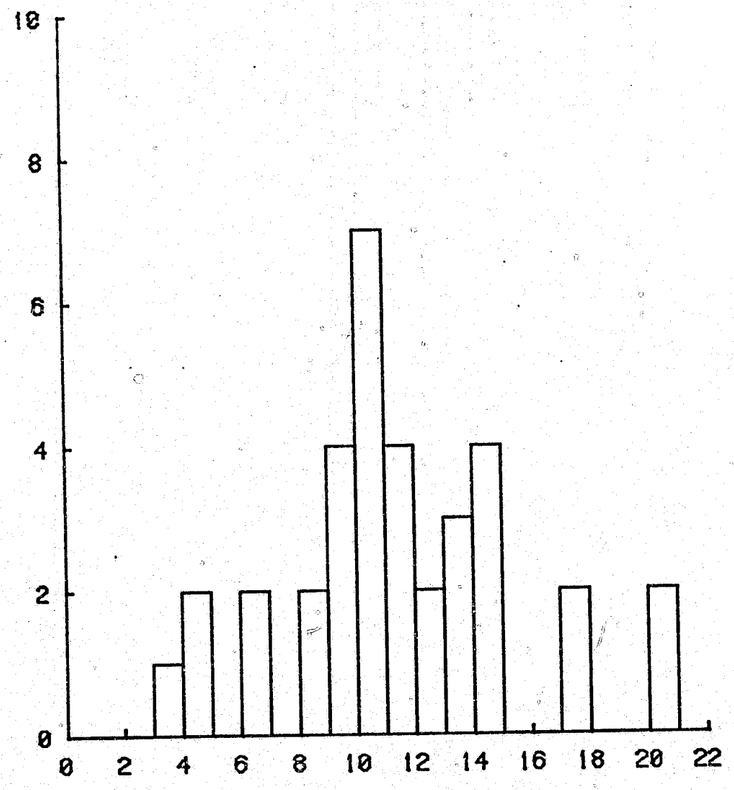


FIGURE 9 Urine-propoxyphene

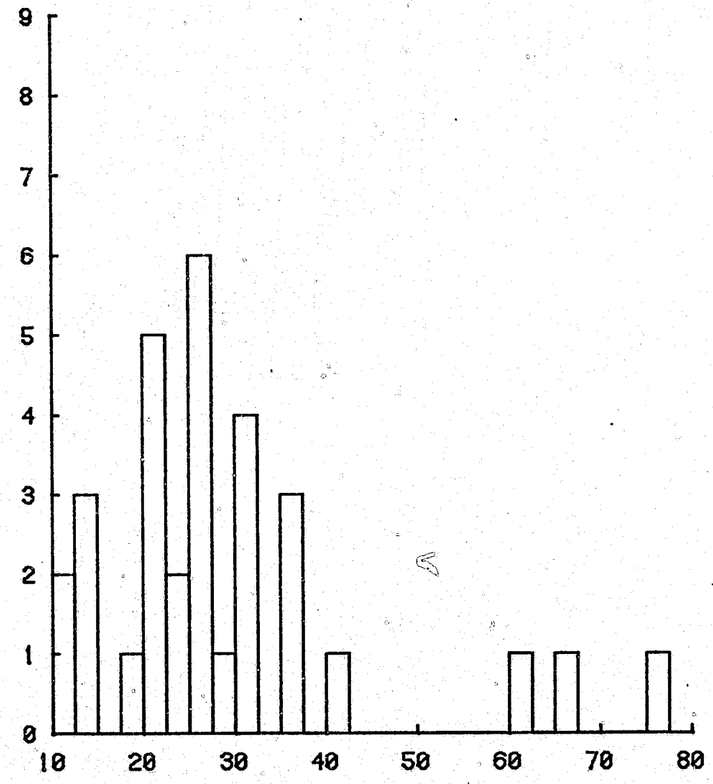


FIGURE 10 Urine-norpropoxyphene

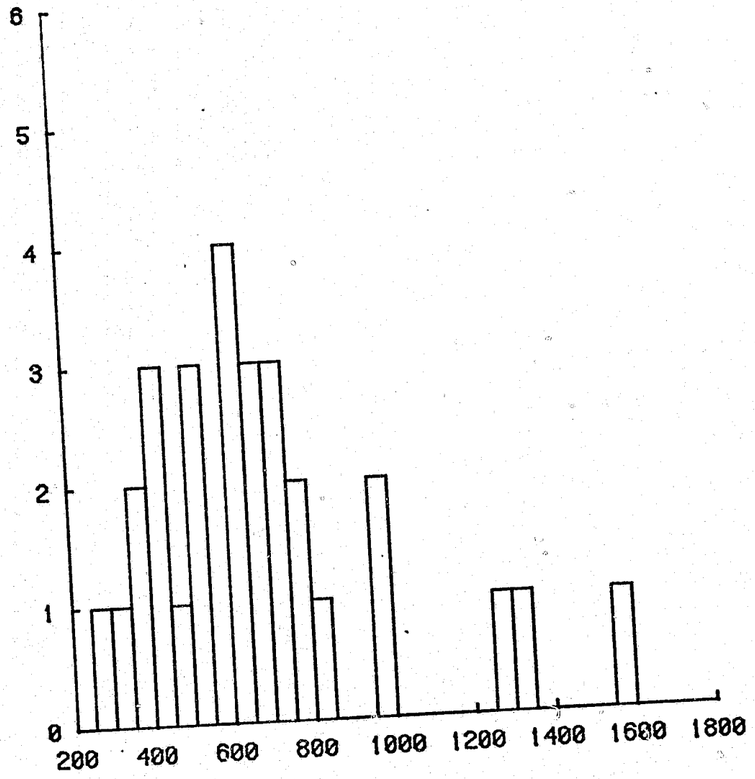


FIGURE 11 Urine-acetaminophen

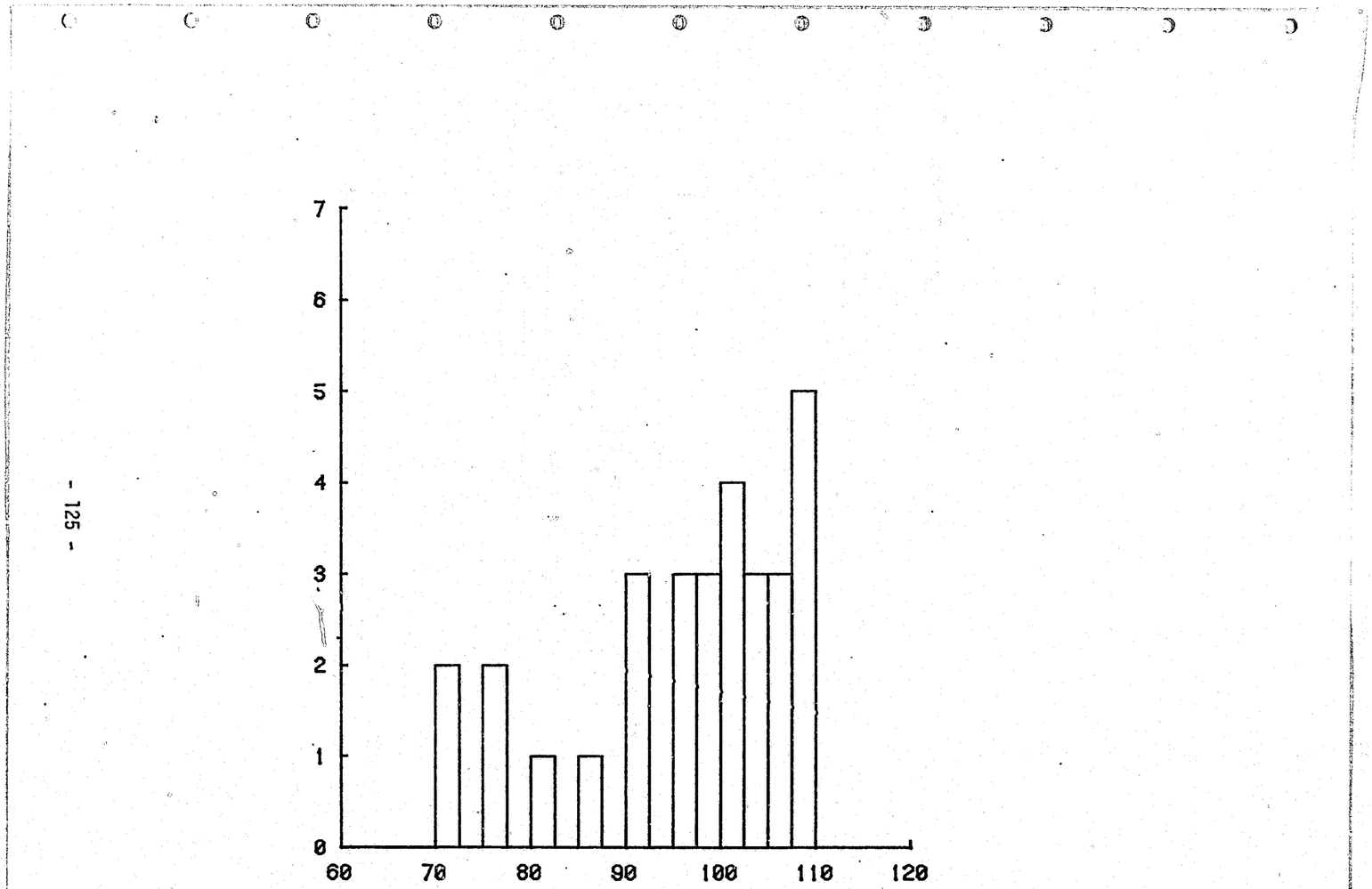


FIGURE 12 Urine-ethanol

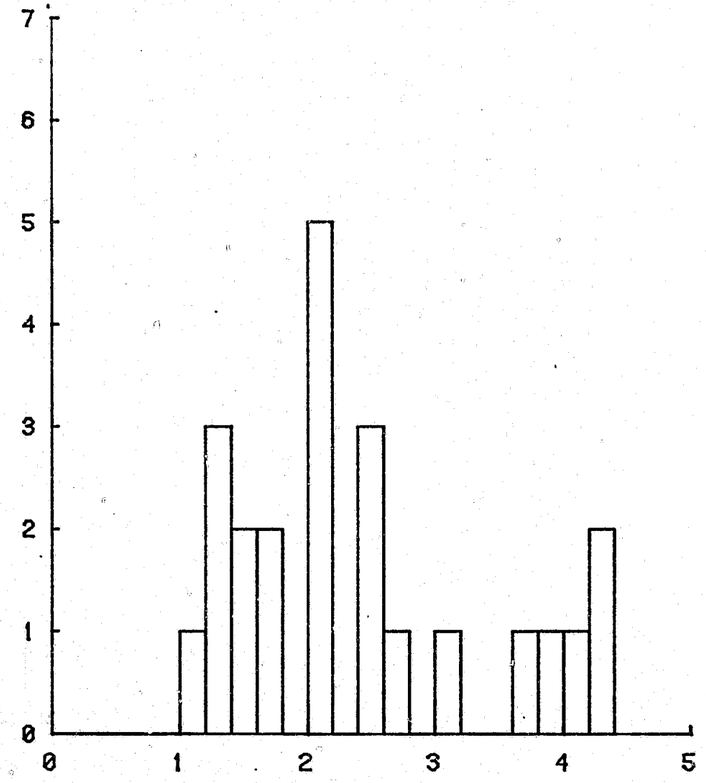


FIGURE 13 Sample 20-secobarbital

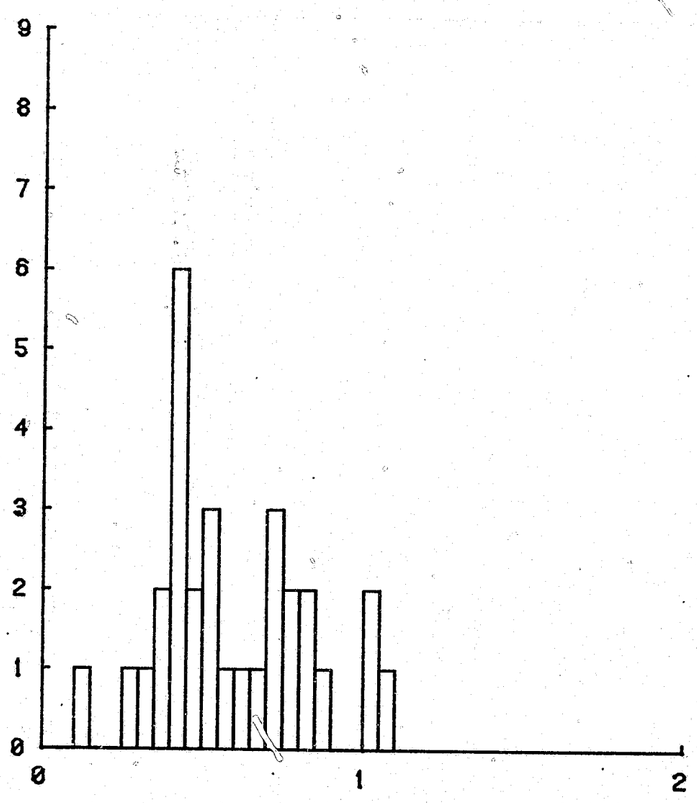


FIGURE 14 Sample #20-morphine

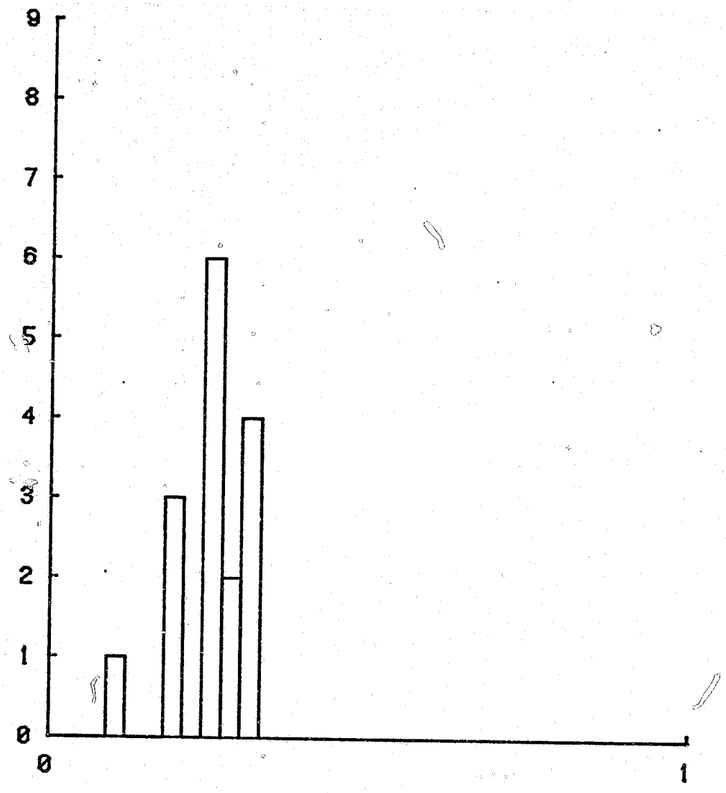


FIGURE 15 Sample #20-codeine

END