DRUG RESEARCH METHODOLOGY. VOLUME THREE. THE DETECTION AND QUANTITATION OF DRUGS OF INTEREST IN BODY FLUIDS FROM DRIVERS

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Prepared for
U.S. Department of Transportation
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16. Abstract

This report presents the findings of a workshop on the chemical analysis of human body fluids for drugs of interest in highway safety. A cross-disciplinary panel of experts reviewed the list of drugs of interest developed in a previous workshop and described an analytical perspective with which to address issues related to their detection and quantitation. Participants specified requirements in highway safety for the analysis of drugs and suggested approaches and techniques for determining their presence and amount in body fluids. Also summarized in this report are discussions of issues in the design of highway safety research involving drug analysis: the collection, handling, and storage of body fluid specimens; monitoring the quality of analytical results (proficiency testing of laboratories and quality control procedures); interpretation of analytical results, including collateral data needed for interpretation of findings; and the design of surveys of drug use among drivers. Recommendations developed by the panel are presented. An appendix that provides background information on the detection and quantitation of drugs in body fluids is included in the report.

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PREFACE

This report presents the results of one of a series of workshops on methodological issues in research on drugs and highway safety. The workshops addressed discrete--but interrelated--topics. The workshops were conducted by The University of Michigan Highway Safety Research Institute (HSRI) for the National Highway Traffic Safety Administration as part of a larger research program on drugs and driving.

A reader interested in the subject area will find the other workshop reports and technical reports produced under the research program of value. The workshop reports are:

- Drug Research Methodology. Volume One. The Alcohol-Highway Safety Experience And Its Applicability To Other Drugs.
- Drug Research Methodology. Volume Two. The Identification Of Drugs Of Interest In Highway Safety.
- Drug Research Methodology. Volume Three. The Detection And Quantitation Of Drugs Of Interest In Body Fluids From Drivers.
- Drug Research Methodology. Volume Four. Epidemiology In Drugs And Highway Safety: The Study Of Drug Use Among Drivers And Its Role In Traffic Crashes.
- Drug Research Methodology. Volume Five. Experimentation In Drugs And Highway Safety: The Study Of Drug Effects On Skills Related To Driving.

Other reports prepared under the HSRI project include an annotated bibliography of literature on drugs and driving and related topics:

- Joscelyn, K.B., and Donelson, A.C. 1979. Drugs And Driving: A Selected Bibliography. Supplement One. National Highway Traffic Safety Administration technical report DOT-HS-803-879;

as well as a comprehensive review of past, ongoing, and planned efforts

related to the study of and the response to the drug and driving problem:

- Joscelyn, K.B.; Donelson, A.C.; Jones, R.K.; McNair, J.W.; and Ruschmann, P.A. 1980. Drugs and Highway Safety 1980. National Highway Traffic Safety Administration contract no. DOT-HS-7-01530.

The latter report supported the preparation of a report to Congress by the Secretary of Transportation as requested in Section 212 of the Highway Safety Act of 1978. Both reports cited above developed from and extended similar work done under earlier contracts from NHTSA:

- Joscelyn, K.B., and Maickel, R.P. 1977. Drugs And Driving: A Research Review. National Highway Traffic Safety Administration technical report DOT-HS-802-189.

- Joscelyn, K.B., and Maickel, R.P. 1977. Drugs And Driving: A Selected Bibliography. National Highway Traffic Safety Administration technical report DOT-HS-802-188.

- Joscelyn, K.B., and Maickel, R.P., eds. 1977. Report On An International Symposium On Drugs And Driving. National Highway Traffic Safety Administration technical report DOT-HS-802-187.

- Joscelyn, K.B.; Jones, R.K.; Maickel, R.P.; and Donelson, A.C. 1979. Drugs And Driving: Information Needs And Research Requirements. National Highway Traffic Safety Administration technical report DOT-HS-804-774.

- Jones, R.K., and Joscelyn, K.B. 1979. Alcohol And Highway Safety 1978: A Review Of The State Of Knowledge. National Highway Traffic Safety Administration technical report DOT-HS-803-714.

- Jones, R.K., and Joscelyn, K.B. 1979. Alcohol And Highway Safety 1978: A Review Of The State Of Knowledge. Summary Volume. National Highway Traffic Safety Administration technical report DOT-HS-803-764.

- Jones, R.K.; Joscelyn, K.B.; and McNair, J.W. 1979. Designing A Health/Legal System: A Manual. The University of Michigan Highway Safety Research Institute report no. UM-HSRI-79-55.

These reports provide entry points to the literature on alcohol, other

drugs, and highway safety for readers desiring general reviews as well as information on specific topic areas. In addition, the reports can serve as sources for identifying both U.S. and foreign literature pertinent to each reader's needs.

ACKNOWLEDGMENT

This report results from a multidisciplinary effort involving many persons and reflects their able and welcome contributions. We thank all who assisted in its development, preparation, and production.

Special recognition is due to those who participated in the workshop and whose expert contributions form the basis for the report:

- Milton L. Bastos
- Fred B. Benjamin
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- Bryan S. Finkle
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- Charles W. Gorodetzky
- Richard L. Hawks
- Roger P. Maickel
- Arthur J. McBay
- Philip C. Reynolds
- Clifford B. Walberg
- Robert E. Willette

Each of the participants gave freely of their time, energy, and knowledge, often continuing work after long, formal sessions. Their willing support has made this report possible.

We gratefully acknowledge the staff of the Belmont Conference Center, who provided a warm and gracious setting and who maintained an atmosphere most conducive to productivity. We especially thank Mrs. Mary Force, director of the center, and Ann Higgins, our conference coordinator, both of whom assisted us through the vagaries of planning and arranging this workshop.

The technical approach of the project was designed by Kent B. Joscelyn and Roger P. Maickel. This report was drafted by Alan C. Donelson from notes and records of the workshop session. Early drafts of the report were reviewed by Stephen D. Benson, the NHTSA Contract Technical Manager, whose comments were insightful and helpful in preparing the report. A working draft was circulated among participants, who reviewed it for accuracy and comprehensiveness. The final text was then prepared, based on their comments.

Other HSRI personnel also made important contributions. This report

was edited by James E. Haney. Anne L. VanDerworp served as the production editor. Deborah M. Dunne produced the report. Draft versions of the report were produced by clerical staff of the Policy Analysis Division under the supervision of Jacqueline B. Royal.

We thank all who contributed.

Kent B. Joscelyn
Principal Investigator

Alan C. Donelson
Principal Investigator

CONTENTS

1.0 INTRODUCTION

This report presents the findings of a workshop on the chemical analysis of human body fluids for drugs of interest in highway safety. The workshop was held on 8-11 April 1978 at the Smithsonian Institution's Belmont Conference Center, Elkridge, Maryland. The workshop was one of a series conducted by the Policy Analysis Division of The University of Michigan Highway Safety Research Institute under the sponsorship of the U.S. Department of Transportation, National Highway Traffic Safety Administration contract no. DOT-HS-7-01530.

1.1 Background

The extent to which the use of drugs by drivers contributes to highway safety problems is unknown (Joscelyn and Maickel 1977a; Willette 1977; Organisation for Economic Co-operation and Development 1978; Seppala, Linnoila, and Mattila 1979; Joscelyn, Jones, Maickel, and Donelson 1979). (The work "drug" is used here and throughout this report in its most generic sense; that is, substances not usually considered drugs are included within its meaning, for example, carbon monoxide and organic toxicants. "Drugs of interest" are substances that have the potential to increase the likelihood of traffic crashes and concomitant losses.) Research has not established that any drug besides alcohol increases the probability of a traffic crash and associated losses. Although present knowledge about drugs and driving is limited, available evidence indicates that drugs alone or in combination with alcohol or other drugs **can** impair driving skills and **may** increase the likelihood of traffic crashes. Further inquiry in this area is warranted. Among the factors that limit the state of knowledge are problems and issues in major areas of drug and driving research.

In November 1976, The University of Michigan Highway Safety Research Institute (HSRI) received a contract entitled "Drug Research

Methodology" from the National Highway Traffic Safety Administration (NHTSA). Its general objectives are:

- to develop a greater understanding of the nature of the drugs and driving problem on the basis of existing literature; and
- to define directions for future research with greater precision than has been done in the past NHTSA-sponsored efforts.

The project emphasizes solutions to research issues in drugs and highway safety. The overall task is to identify and develop methodologies for research in drugs and driving. Specific objectives of this study are:

- to identify problem areas that should be addressed in drug methodology;
- to specify workable and detailed approaches that could be implemented with current technology; and
- to provide a listing of priority items of research that NHTSA could address in the foreseeable future.

To accomplish these objectives, an approach based on workshops was used to examine issues in four distinct but interrelated areas:

- The Identification of Drugs of Interest in Highway Safety;
- The Detection and Quantitation of Drugs of Interest in Body Fluids From Drivers;
- Epidemiology in Drugs and Highway Safety: The Study of Drug Use Among Drivers and its Role in Traffic Crashes; and
- Experimentation in Drugs and Highway Safety: The Study of Drug Effects on Skills Related to Driving.

The division of topics had advantages as well as a possible disadvantage. For example, on one hand, a tighter focus on specific issues could be achieved. On the other hand, for some topics the wisdom and expertise of participants in other workshops might be lost. To offset this disadvantage, summaries of earlier workshops were mailed to invitees, and

participants were later asked to comment on findings as well as issues in those areas.

These workshops, conducted in the spring and summer of 1978, were highly productive and brought to focus other related issues. In 1978, a contract modification called for additional workshops within the scope of the original statement of work. In January 1978, a fifth workshop dealt with the alcohol and highway safety experience and its relevance to the study and control of the drug and driving problem. The remaining workshops will address other topics of priority interest to NHTSA.

These workshops constitute a series in which each is an integral part. Although the workshops were self-contained and are reported in separate volumes, in general the progression of topics has been systematic. An apparent exception is Workshop V, entitled "The Alcohol-Highway Safety Experience and Its Applicability to Other Drugs" and reported as Volume One. This deserves some explanation. References and comparisons to the study of and the response to the alcohol-crash problem occurred frequently during the first four workshops. In fact, public sensitivity to the alcohol-crash problem has itself led to an awareness that other drugs also have the potential to increase traffic-crash risk. Workshop V was therefore planned to examine the alcohol-highway safety experience in detail. As Volume One, the report on Workshop V serves as an introduction to the others, provides an historical perspective, and describes the relation of the alcohol and highway safety experience to other drugs. The workshop reports are designed to be read sequentially. A reader desiring information on a specific topic area, however, can refer to the particular volume of interest.

Another task under this contract is to update the literature review performed for NHTSA under contract DOT-HS-4-00994 (Joscelyn and Maickel 1977b). A report produced under this contract (Joscelyn and Donelson 1979) presents an annotated bibliography of recent literature on drugs and driving to supplement the parent volume. Another in this series of bibliographic reports is planned for publication in the summer of 1980.

The first workshop in this series, The Identification of Drugs of

Interest in Highway Safety, addressed the question of which drugs should be considered in the study of methodological and other issues. Its purpose was to identify drugs (1) that should be the focus of near-term NHTSA-sponsored research on drugs and driving, and (2) that should be the focus for discussing research issues in the other workshops. Two objectives of that workshop were:

- to develop a way to estimate the risk potential of drugs, based on an approach that formulates subjective judgments of experts and that synthesizes present knowledge in distinct fields related to drugs and driving; and
- to produce an initial rank ordering of identified drugs of interest, based on subjective estimates of their risk potential.

One output of Workshop I, the list of drugs of interest, became a basis for discussion in the second workshop, the subject of this report. The ranking identified drugs with greater perceived risk to highway safety, thus guiding the emphasis of discussion in this and the other workshops.

1.2 Purpose of Workshop II, The Detection and Quantitation of Drugs of Interest in Body Fluids

Requirements in drug and driving research include the capability to detect and quantitate drugs in body fluids. Reviewers have pointed out that most studies of drug use among drivers have been greatly handicapped by the lack--or nonuse--of adequate analytical methods (Joscelyn and Maickel 1977a; Willette 1977; Organisation for Economic Co-operation and Development 1978; Joscelyn et al. 1979). As a consequence, the value of data from past epidemiologic studies is very limited. Well-controlled surveys employing advanced techniques, many of which have been developed in the past decade, are essential for problem definition. Experimental studies of drug effects on measures of driving performance are also needed to correlate results of behavioral testing with body fluid concentrations of drugs and active metabolites (drug-like compounds produced in the body from the parent substance). These kinds of studies are rarely done, though specific and sensitive analytical

methods are available. The purpose of Workshop II, therefore, was to resolve issues in drugs and driving research related to the analysis for drugs in body fluids. In the context of epidemiologic and experimental research on drugs and driving, the general objectives of Workshop II were:

- to identify problem areas and research issues associated with the analysis of drugs in body fluids;
- to specify approaches to resolving identified analytical problems; and
- to suggest research needed to address methodological issues.

Participants recognized a third area of application involving methods of drug analysis: forensic toxicology (for example, the analysis of body fluids of fatally injured drivers and drivers suspected of driving under the influence of drugs other than alcohol). The concern in this workshop, however, was methodology for purposes of problem definition and risk identification. This focus helped simplify discussions of drug analysis, since analytical standards acceptable to the research and policy community may not be adequate to establish evidence beyond a reasonable doubt in the adjudication of impaired-driving cases.

Field surveys of drug use among drivers and laboratory-based studies of drug effects have different requirements for drug analysis. Specification of analytical requirements had to precede identification of techniques to satisfy them. Thus, specific objectives of Workshop II were:

- to outline analytical requirements for epidemiologic and experimental research in drugs and highway safety;
- to identify techniques and methods to detect and quantitate the drugs of interest; and
- to provide alternative approaches to complex problems.

The primary focus of the workshop was on analytical requirements and techniques and methods for drug analysis. But other topics were integrally involved, including:

- the concentrations of drugs in body fluid specimens expected to result from different patterns of use (chronic,

acute, abusive, etc.);

- the collection, preservation, handling, and storage of specimens for analysis;
- the pharmacokinetics (absorption, distribution, metabolism, and excretion) of the drugs of interest;
- the relationship between the concentration, for example, in blood, and the effects of drugs on the ability to drive safely.
- critical time periods between, for example, crash-involvement or arrest for impaired driving, and collection of blood specimens, beyond which results of analyses could not be considered representative of possible drug effects; and, more generally,
- the interpretation of analytical data.

To cover these topic areas, experts engaged in the development or application of methods and techniques of drug analysis, or both, were invited to participate in this workshop (see Appendix B, List of Participants). Participants represented many disciplines, including pharmacology; physiology; psychology; pharmacy; toxicology (analytical, clinical, and forensic), and analytical chemistry.

They held positions in research (basic, applied, and clinical) and in operational settings (for example, offices of medical examiner). Most members of the panel had direct experience in the type of analyses required for research in highway safety. Several had participated before in the study of drug use among crash-involved and impaired drivers, or in pharmacokinetic studies relating the concentration and effects of drugs in man. Other participants had conducted basic research in developing methods of drug analysis using state-of-the-art techniques, for example, for cannabinoids. The combined knowledge and expertise of participants allowed discussion of the full range of topics identified above.

The participants, both government and nongovernment, functioned as an interdisciplinary group in an informal workshop setting. A moderator with an extensive background in alcohol, other drugs, and highway safety functioned as "lowest common denominator" (1) to link panel members

from different areas of research, (2) to provide a ground for basic understanding in a many-disciplined group, and (3) to ensure that the workshop's product could be used by a lay audience.

1.3 Scope of Report

This report has six sections. The five that follow are briefly described below.

Section 2.0, Review and Analysis of Findings of Workshop I, summarizes comments by the panel members on the list of drugs of interest. An analytical perspective, developed by participants in Workshop II, is then presented.

Section 3.0, Requirements for the Analysis of Drugs in Body Fluids, outlines the basis for analytical approaches discussed later in the workshop.

Section 4.0, Approaches and Techniques to Detect and Quantitate the Drugs of Interest in Body Fluids, reports suggestions by the panel for determining the presence and amount of drugs, both known and unknown, in body fluids from drivers.

Section 5.0, Issues in the Design of Research Involving Analysis of Body Fluids for Drugs, pertains to problems peripheral to drug analysis per se, but which influence the validity and reliability of analytical results as applied in highway safety-related efforts.

Section 6.0 presents the conclusions and recommendations of the panel.

Appendix A provides background information useful to the reader unfamiliar with technical areas discussed at length during the workshop.

Appendix B is a list of workshop participants.

A list of references follows the text of this report.

2.0 REVIEW AND ANALYSIS OF FINDINGS FROM WORKSHOP I

The Drug Research Methodology project involves a series of workshops on distinct but interrelated areas of drug and driving research. Section 1.1 briefly describes efforts made to obtain input on issues from participants in other workshops. Thus, panel members of the second workshop were asked to review and comment on findings of Workshop I. Because participants were also asked to accept its findings as a basis for discussion, the list of drugs of interest and their order had special relevance to Workshop II.

2.1 Background

To give a frame of reference for comments on the rank-ordering of drugs of interest, the purpose, approach, and findings of Workshop I were briefly discussed. (For a more detailed discussion, the reader is referred to the report on Workshop I [Joscelyn and Donelson 1980].)

The purpose and objectives of Workshop I are outlined in Section 1.1. Because substances that can impair driving ability number in the thousands and because funding available for their study is limited, the number and type of drugs under consideration must also be limited, preferably to those of greatest interest in highway safety. Workshop I addressed in this issue.

In highway safety, the term **risk** has been defined as the likelihood or probability of a traffic crash and concomitant losses, such as loss of life or property, injury, medical costs, etc. Thus, for the purpose of Workshop I, a "drug of interest" was defined as one that has a **potential** to increase risk. Drugs of greater interest would be those that had the greater potential to increase the probability of a traffic crash and associated losses or, more simply, the greater **risk potential.**

Because a lack of data precludes an objective answer to the question of which drugs warrant further study in drug and driving research, the

panel of Workshop I developed a procedure by which to estimate the highway safety risk potential of drugs. Participants of that workshop identified drugs of interest in highway safety and, applying the procedure, rank-ordered the drugs of interest relative to alcohol. The rank order of drugs of interest developed in Workshop I is presented in Table 2-1. This table was the basis for comments by the panel in Workshop II.

2.2 Comments by Participants of Workshop II on the List of Drugs of Interest

Comments by participants of Workshop II on the list of drugs of interest were then solicited. In response to questions raised in discussion, the following points concerning Table 2-1 were made:

- The present ranking of drugs represents only one way of listing substances of interest. It contains, as one participant noted, a polyglot of names, including pharmacological classes, therapeutic groups, and single agents. Other schemes for ranking drugs of interest are possible.

- Ranked were drugs and classes of drugs listed in the second column; examples cited in the third column either identify specific agents mentioned or represent classes of drugs within a therapeutic grouping. Some drugs given as examples may rarely be used by drivers (e.g., hydralazine under antihypertensives).

- The literature on highway safety mentions some drugs and classes of drugs that were not, in the opinion of the panel, important. They were listed and ranked because the group wished to emphasize this opinion (e.g., antidiabetics, carbon monoxide, anticonvulsants).

- Not every drug or group of drugs listed was expected to be the focus of epidemiological or experimental studies. Rather, the list would serve as a guide for including substances in drug and driving research.

Two questions were posed to the panel of Workshop II:

1. Should any drug or class of drugs previously missed be added to the list of drugs of interest?

2. In light of present knowledge, does the rank of any drug

TABLE 2-1

A RANK ORDERING OF THE DRUGS OF INTEREST

RANK ORDER	DRUG OR DRUG GROUPING	EXAMPLES *
1	ethanol	alcoholic beverages
2	diazepam (Antianxiety Agent, Group I)	
3	cannabis sativa	marijuana, hashish
4	codeine (Narcotic Analgesic, Group I)	
5	Volatile Solvents	xylene, gasoline, toluene, butylnitrite, trichloroethylene
6	flurazepam (Sedative-hypnotic, Group I)	
7	d-propoxyphene (Narcotic Analgesic, Group I)	
8	Antihypertensives	reserpine, propranolol, hydralazine, methyldopa, digoxin
9	oxycodone (Narcotic Analgesic, Group II)	
9	Sedative-hypnotics, Group IIa	secobarbital, pentobarbital, amobarbital (inclusive)
10	chlordiazepoxide (Antianxiety Agent, Group I)	
11	Antihistamines, Group I (over-the-counter)	diphenhydramine, chlorpheniramine, methapyrilene, doxylamine

TABLE 2-1

A RANK ORDERING OF THE DRUGS OF INTEREST (Continued)

RANK ORDER	DRUG OR DRUG GROUPING	EXAMPLES *
12	pentazocine (Narcotic Analgesic, Group I)	
13	Narcotic Analgesics, Group II	methadone, pethidine, morphine, hydromorphone
14	Antipsychotics	chlorpromazine, prochlorperazine, chlorprothixene, haloperidol
15	Hallucinogens	LSD, DMT, mescaline, psilocybin
15	caffeine	caffeinated beverages, OTC stimulants
15	carbon monoxide	automobile emissions, cigarettes
15	glutethimide (Sedative-hypnotic, Group I)	
15	methaqualone (Sedative-hypnotic, Group I)	
16	nicotine	tobacco products
17	Anesthetics (outpatient therapy, dental surgery)	lidocaine, procaine, thiopental, methohexital, halothane, nitrous oxide
18	Sedative-hypnotics, Group IIb	other barbiturates, e.g., butabarbital, butalbital, mephobarbital, metharbital
19	heroin	

TABLE 2-I

A RANK ORDERING OF THE DRUGS OF INTEREST (Continued)

RANK ORDER	DRUG OR DRUG GROUPING	EXAMPLES *
20	Antihistamines, Group II (prescription)	diphenhydramine, pyrilamine, chlorpheniramine, pheniramine
20	Stimulants	d-amphetamine, methamphetamine, phenmetrazine, methylphenidate
20	ethchlorvynol (Sedative-hypnotic, Group I)	
20	chloral hydrate (Sedative-hypnotic, Group I)	
20	Antianxiety Agents, Group II	oxazepam, prazepam, lorazepam, hydroxyzine, meprobamate
21	Anticonvulsants	phenobarbital, phenytoin, primidone, carbamazepine, ethosuximide, trimethadione
22	cocaine	
23	Antidiabetics	insulin, phenformin, tolbutamide

* The examples listed in column two of this table arose from one or two sources. The agents either were mentioned in the course of discussion or were selected by HSRI staff following the workshop. Before completion of this report, workshop participants had the opportunity to review this table. Additions and deletions of drugs under Examples were made based on their comments. The purpose of including examples is to represent members or subclasses of drugs within each grouping ranked. Some drugs given as examples, therefore, may themselves be rarely used by drivers. The examples are intended to illustrate the groups of drugs evaluated by the panel, not necessarily to identify specific drugs of interest within each group.

or grouping of drugs appear significantly **out of order**?

In response to the first question, the panel mentioned nonnarcotic analgesics (acetaminophen, the salicylates); theophylline (an antiasthmatic drug); and antibiotics with significant and potentially impairing side effects (e.g., kanamycin). With regard to the second question, some participants thought the rank of volatile solvents too high; others thought the rank of cocaine and PCP too low relative to substances like caffeine and nicotine. Some members of the panel indicated that sedative drugs should rank above the higher ranked narcotic analgesics. One participant recommended that butabarbital be included among barbiturates in Group IIa. The reason cited was that butabarbital has been more frequently detected on the highways and in emergency room cases since the rescheduling of other barbiturates under the Controlled Substances Act.

2.3 The Drugs of Interest From an Analytical Perspective

From the standpoint of drug analysis, specific points concerning the rank order of drugs were considered largely irrelevant. The list of drugs of interest contains a very diverse group of substances. Most (but not all) drugs of interest could be detected by routine methods. There are some exceptions. For example, volatile solvents are not routinely screened, and lithium requires special instrumentation. Scopolamine and most hallucinogens are present in very low amounts, and, for most purposes, their analysis is not cost effective. Nevertheless, even the exceptions could be included in an analysis, given sufficient interest and support.

Two basic concerns in the analysis of body fluids for the drugs of interest were expressed. **One**, all highly ranked substances should be included in any analytical scheme. **Two**, given the cost associated with obtaining a specimen of body fluid, an effort should be made to analyze for as many other drugs of interest as possible. Given the diversity of substances listed, the panel was, in fact, asked to give opinions on methods that would cover essentially every drug that could conceivably affect driving ability. To address these concerns and to facilitate

discussion of analytical requirements and methods, the following approach was adopted.

The panel first identified broad groupings or classes to which the drugs of interest belong (Table 2-2). From this listing a set of analytical classes was derived (Table 2-3). The analytical classes, roughly sketched and for purpose of discussion only, comprise one of many possible schemes for analysis of these drugs. Participants recognized that many objections to the list of drug groups presented in these tables could be raised. For example, many of the groups overlap; central nervous system depressants include anesthetics, tranquilizers, and some analgesics like heroin and codeine. "Drugs of abuse" include depressants and stimulants. Unfortunately, any attempt to devise a consistent, nonoverlapping scheme for classifying drugs is difficult. Chemical, pharmacologic, and therapeutic classes invariably overlap, at least to some degree. **Tables 2-2 and 2-3, therefore, only represent a heuristic approach taken to further discussion and to simplify the task of developing an analytical scheme for the drugs of interest.**

Participants agreed that any attempt to detect the drugs of interest would lead to the detection of most drugs present in body fluids. The present state of the art in drug analysis would permit a general screening for drugs. The techniques selected, however, depend (1) on the type and amount of body substance available for analysis and (2) the level of sensitivity required for detection of each substance. Therefore, a discussion of analytical requirements followed.

TABLE 2-2

GROUPS OR CLASSES REPRESENTED BY THE DRUGS OF INTEREST

- Central Nervous System Depressants
- Tranquilizers (including antianxiety agents and major tranquilizers)
- Drugs of Abuse (including cannabis, hallucinogens, phencyclidine [PCP])
- Antihistamines
- Antihypertensives
- Analgesics
- Central Nervous System Stimulants
- Anticonvulsants
- Volatile Solvents
- Carbon Monoxide (CO)*
- Nicotine*
- Antidiabetics
- Anesthetics

* Single substances considered separately.

TABLE 2-3

AN ANALYTICAL GROUPING OF THE DRUGS OF INTEREST

- Alcohols
- Volatile Solvents, Some Anesthetics
- Weakly Acidic Drugs
- Neutral Drugs
- Basic Drugs
- Benzodiazepines*
- Carbon Monoxide**
- Cannabinoids**
- Miscellaneous***

*A chemical class of drugs that can be analyzed as a group

**Substances considered separately for analysis

***Drugs left out of the general scheme and requiring separate tests

3.0 REQUIREMENTS FOR THE ANALYSIS OF BODY FLUIDS FOR DRUGS OF INTEREST IN HIGHWAY SAFETY

In general, analytical requirements fall into two categories:

- requirements of the user of analytical services; and
- requirements of the analyst, in order to satisfy user demands.

These requirements are interactive. For example, if a user specifies a level of detection for a certain drug, an analyst may require a certain type of specimen needed for analysis, a given quantity of specimen, or both. The availability of one type of specimen may also direct the choice of an analytical technique by the analyst. (Here, "specimen" may refer to a portion of body fluids [such as blood or urine], to tissues, or to other body substances [such as breath]. Discussions in this workshop emphasized body fluids, although other types of specimens were also mentioned. The term **specimen** will be used in summarizing points applicable to most body substances.)

In this workshop, participants were presented with a list of drugs of interest. Once a group of drugs are selected for analysis, three questions remain:

1. What specimens must (or should) be analyzed?
2. What quantity of specimen can be expected (or is needed) for analysis?
3. What levels of detection (or limits of sensitivity) must be achieved?

The panel addressed these questions in the context of highway safety, playing the role of both **user** and **analyst.**

3.1 Type and Quantity of Specimens for Drug Analysis

The panel identified two basic criteria for specifying the type and quantity of specimens required for drug analysis: suitability and availability. The **suitability** of specimens depends on the use to which analytical findings will be put and thus depends also on the value or meaning of results describing the presence and amount of drugs. For example, in highway safety, a specimen in which the concentration of a drug can be related to its effects on behavior would be much more suitable than one in which the concentration of drug has little or no meaning. The ease of detection of drugs in specimens is another factor in considering their suitability for analysis. For example, it may not be feasible to analyze for certain drugs in some types of specimens due to endogenous substances that interfere with their analysis.

The **availability** of specimens for analysis depends on real-world constraints--physical, legal, ethical, and political. For example, fatally injured drivers may provide, upon autopsy, a wide range of specimens, but not breath and probably not saliva. Political—and legal—constraints may limit specimens from nonaccident-involved drivers to breath and saliva only. Legal constraints may also reduce the availability of specimens from injured drivers. Thus, the type of specimens available for analysis may differ, depending on the driving population under study.

In addressing this topic, the panel stressed its aim to produce a scientific--not policy-based—analysis. Various constraints may operate to render studies of drug use among drivers less than optimal. Other workshops more directly concerned could deal with these issues. The function of this panel was to specify for them the requirements for an adequate analysis for drugs. The **availability** of specimens was therefore defined as the type and amount of specimens that could reasonably be expected for an adequate analysis. For the driving populations described below the panel ranked different types of specimens in order of their suitability for drug analysis.

The panel recognized, as indicated above, that the availability of specimens is a function of the population under study. Five populations were defined for drug and driving research:

- fatally injured driving population;
- nonfatally injured driving population (e.g., seriously injured drivers requiring emergency medical treatment and/or hospitalization);
- impaired driving population (drivers arrested for impaired driving);
- driving population at risk;
- human subjects (in experimental studies, usually laboratory-based, that measure the effects of drugs on skills related to driving).

Epidemiology in drugs and highway safety involves one or more of the first four listed driving populations; the analysis for drugs would involve screening specimens for their presence, then identifying and quantitating drugs detected. In experimental research, the identity and approximate concentrations of drugs present in body fluids are known; the analysis required is much more specific and, in many ways, simpler.

In the opinion of the panel, blood and urine are the most suitable for analysis. **Blood properly obtained, handled, and stored is the specimen of choice in all five populations.** The presence of drugs in blood usually relates more directly to the presence of drugs at their site of action, for example, the brain. Urine, while better than no specimen at all, serves as a pool for excreted drugs and especially for their metabolites (chemical compounds produced from parent drugs by the metabolism in the body). Because drugs and metabolites accumulate in the urine over time, the relationship between their concentration in urine and their effects on behavior is, for most drugs, marginal at best and nonexistent at worst. The usefulness of both blood and urine specimens can be enhanced by obtaining two or more specimens at known intervals. The relative amount of drug in specimens obtained at known intervals can indicate--but only approximately--**when** the drug was taken. The availability of "timed" specimens, however, may be limited in research and forensic settings.

The analysis of urine specimens can **supplement** the analysis for drugs

in blood. The availability of urine may make some analyses more cost effective. For example, urine specimens are usually available in larger volumes; initial screening can be performed on urine, reserving blood, which is available in generally smaller quantities, for confirmatory and quantitative tests. This approach might be particularly useful for basic drugs that are present in very low concentrations in the blood. Urine does not replace blood as a specimen for analysis, but it can provide options in screening for drugs.

In addition, confirmation of a drug's presence in other specimens lends weight to results in blood. In the autopsy of fatally injured drivers, a range of specimens other than blood and urine may be made available for analysis. These are listed below:

- bile (partial autopsy);
- liver (full autopsy);
- gastric contents (full autopsy);
- brain;
- cerebrospinal fluid (CSF); and
- vitreous humor (the transparent, colorless substance that fills the eyeball between the retina and lens).

Participants differed slightly in their opinions about how useful or available these types of specimens would be in highway safety research. In some cases of traumatic death, some might be rendered useless by destruction or contamination. The cost of autopsies, which may be a factor in some studies, increase for specimens beyond blood and urine. In fact, certain specimen--for example--specimens of brain tissue, must be obtained by special arrangement with a coroner or medical examiner.

From drivers other than those fatally injured, blood, urine, saliva, and breath are usually the only specimens available for analysis. The panel stressed that a specimen of blood is the **minimum** required for meaningful results. Blood and urine are the only acceptable specimens for epidemiologic studies of drugs other than alcohol alone. Although the

analysis of saliva may, for some drugs, supplement that of blood and urine, in general, specimens of saliva and breath are not useful for purposes of applied research in drugs and driving. In particular, specimens of breath are not useful in an analysis for drugs other than alcohol. In the interest of obtaining as much data for the research dollar as possible, specimens of saliva may be collected in epidemiological studies for purposes of basic research. Specimens of breath might also be taken from subjects of experimental research for study of the detectability of drugs. But the panel strongly emphasized that the state of knowledge of pharmacokinetics, as well as the state of the art in drug analysis, precludes exclusive use of breath or saliva to determine the prevalence of drugs in driving populations, at least for the foreseeable future.

The panel then discussed the quantity and quality of specimens required for analysis. "Blood," operationally defined, is whole blood, preserved in vials containing 1% sodium fluoride, frozen (below -20 degrees Celsius) until analysis. An acceptable specimen is uncontaminated blood taken from the heart or abdominal cavity. A volume of 20 milliliters is the minimum required for analysis of blood when it is not known which drugs are present or in what concentrations drugs are present.

When urine specimens are obtained along with blood, all available urine is needed for analysis. From nonfatally injured drivers, two urine specimens at timed intervals--in each instance, the total available volume--is highly desirable. If saliva is collected, a 5-milliliter specimen is required for analysis.

As for blood, some participants recommended that both urine and saliva specimens be stored frozen below -20 degrees Celsius until analysis. Others thought that "deepfreezing" was not necessary if analyses were performed within two weeks of specimen collection. All recognized the lack of data on the stability of concentrations of drugs in stored specimens. Storage at temperatures below -20 degrees Celsius was considered a prudent if not essential requirement. In experimental research, the type and quantity of specimens required vary according to the purpose of each study and the particular drug involved.

Table 3-1 summarizes major points discussed above.

3.2 Limits of Detection and Quantitation for the Drugs of Interest

A "drug screen" is a method or group of methods whose primary purpose is to determine whether one or more detectable drugs is present in a specimen. These methods are often supplemented by others for the purpose of verifying positive findings or quantitating the amount of drug present. Each technique or method used in drug screening (and for confirmative, quantitative analyses) has an inherent **limit of sensitivity,** usually defined for each drug in terms of its concentration in the specimen analyzed. If a drug is present in too low a concentration, it will not be detected or its amount will not be accurately quantitated. An "acceptable" limit of detection depends on the purposes for which analyses are done.

The importance of specifying sensitivity limits for analytical methodology applied in drug and driving research was stressed. Findings of exploratory studies of drug use among drivers—fatally injured, injured, or impaired—are likely to guide further research on those drugs detected, in particular, experimental research to correlate drug concentrations in body fluids with drug effects on skills related to driving. Therefore, in discussing limits of detection for drugs in body fluids, the panel focused primarily on analytical requirements in epidemiologic research, and specifically on general guidelines for detection limits in drug screening.

Two general approaches to drug analysis, which represent extremes in detection limits for drugs, were first considered:

1. Routine screening for drugs present in large amounts.

   In many analytical laboratories only toxic concentrations of drugs are of interest. Examples are forensic laboratories that investigate cases of suspected poisonings and clinical laboratories dealing with possible drug overdose cases. Methods routinely used in these settings are usually designed to detect concentrations of drugs not resulting from therapeutic doses.

2. Specific analyses, using state-of-the-art methodology, to detect (and quantitate) drugs **known to be present** in

TABLE 3-1

TYPE AND AMOUNT OF SPECIMENS FOR THE DETECTION AND QUANTITATION OF DRUGS OF INTEREST IN HIGHWAY SAFETY

SUBJECT POPULATION	SPECIMEN	AMOUNT
Fatally injured drivers	Blood (minimum required)	20 milliliters
	Urine	All available
	Bile	
	Liver	
	Gastric contents	
	Brain	
	Cerebospinal fluid	
	Vitreous humor	
Injured drivers	Blood (minimum required)	20 milliliters
	Urine (2 specimens at timed intervals, if possible)	All available
	Saliva (for basic research on drug detectability only)	5 milliliters
Drivers arrested for impaired driving	Blood (minimum required for analysis for drugs other than alcohol alone)	20 milliliters
	Urine (2 specimens at timed intervals, if possible)	All available
	Saliva (for basic research on drug detectability only)	5 milliliters
Drivers at risk (control population)	Blood (minimum required)	20 milliliters
	Urine (2 specimens at timed intervals, if possible)	All available
	Saliva (for basic research on drug detectability only)	5 milliliters
Experimental subjects	Blood	(Amount of specimen will vary according to experimental objectives and type of drug under study.)
	Urine	
	Saliva	
	Breath	
	(for basic research on drug detectability only)	

specimens.

> In some applications, like therapeutic monitoring of drug concentrations in a patient or pharmacokinetic studies (investigations concerning the behavior of drugs in the body), the analyst will know what drug is present in a specimen. A specific, sensitive method can be used to detect only that drug at well below effective concentrations. This situation rarely arises in drug and driving research or in impaired-driving cases.

The panel concluded that analytical requirements for epidemiologic studies lie somewhere between the extremes of detection limits illustrated above.

Participants noted that therapeutic concentrations of some drugs can adversely affect driving performances, and that some other drugs may improve driving ability at these concentrations. The panel therefore decided that epidemiologic studies based on the analysis of driver body fluids should employ analytical methods to detect, at a minimum, "therapeutic concentrations" of the drugs of interest. It was noted that some drugs of interest have no accepted therapeutic use, for example, phencyclidine (PCP). For these drugs, an equivalent concentration, such as "minimum effective concentration," could be suggested.

Efforts were then made to define "therapeutic concentration" operationally. This task proved difficult. Therapeutic concentrations for most drugs are variable and dependent upon many factors. For example, intersubject and intrasubject variables produce a range of concentrations in blood (or serum) for a given therapeutic dose. Therapeutic drugs may be taken repeatedly over a period of time (chronically) or once as needed (acutely); concentrations of drugs in body fluids will usually reflect differences in their pattern of use. To specify one limit of detection for all drugs (e.g., 0.1 micrograms per milliliter, in blood [10^{-7} g/ml]) has other drawbacks. Therapeutic levels of drugs vary from milligrams per deciliter (mg/dl, mg%, 10^{-5} g/ml) to picograms per milliliter (10^{-12} g/ml), a ten million-fold range. Analytical methods are designed accordingly. Many highly ranked drugs of interest have average concentrations in blood below 0.1 micrograms per milliliter (e.g., diazepam [acute], cannabis,

flurazepam [measured as active metabolite]). If, as is probable, a specimen is obtained several hours following a therapeutic dose, even lower levels would be expected. Interestingly, for some drugs, ultrasensitive methods are now available for routine application that have limits of sensitivity well below therapeutic levels, even below the minimum level expected for drug effects.

To simplify matters for the purposes of any national study, the panel assumed one central laboratory (possibly replicated on a regional basis) equipped with state-of-the-art methodology. This laboratory would apply "reasonably available" instrumentation and techniques; the procedures and methods used would have "substantial scientific acceptance." Given these assumptions, the panel specified a limit of detection of **at least** 50 nanograms per milliliter of blood for the drugs of interest (a nanogram is 10^{-9}g). A "realistic, practical" lower limit for quantitation of positive findings is, in the opinion of participants, 0.1 microgram/ml of blood.

These limits are not necessarily possible with methods routinely applied today in other areas. They reflect the requirements of applied research in drugs and driving, taking into account the state of the art in drug analysis, the constraints of cost, and the type and amount of specimens required.

Finally, the panel advised great care in reporting findings of drugs in body fluids produced in this manner. Modern methods of analysis can detect substances long after they cease having an effect. Ultrasensitive screening methods—for example immunoassays--have limits of detection below concentrations where it is reasonable to quantitate positive findings. In reporting these cases, participants recommended that (1) the limits of sensitivity for detection and confirmation/quantitation be specified; and (2) positive findings in such instances be labelled "detected/not quantitated" for the purpose of drug prevalence studies. The panel also stressed that negative findings do not necessarily mean that drugs are not present, simply that they were not detected by the screening methods used. Negative findings should therefore be reported as "drug not detected" rather than "drug not present." Participants also recommended that drugs not detectable by screening protocol also be identified in reporting results

of analysis. **The panel stressed that careful, accurate, and precise reports of analytical results are necessary to avoid possible misinterpretations of their meaning.**

3.3 Summary

The panel specified requirements for the analysis of the drugs of interest in human body fluids. Participants outlined the type and amount of specimens available from various driving populations and experimental subjects and ranked them in order of suitability. In each group, blood is the specimen of choice, followed by urine. According to the panel, the level of detection for the drugs of interest should be at least 50 ng/ml of blood. The limits of sensitivity are thus approximate to the state of the art in drug analysis, given a central laboratory suitably equipped and adequately funded.

4.0 APPROACHES TO THE ANALYSIS OF BODY FLUIDS FOR DRUGS OF INTEREST IN HIGHWAY SAFETY

After specifying analytical requirements for drug and driving research, the panel identified:

- factors that influence the development of analytical schemes for drug detection and quantitation; and
- analytical techniques for the analyses of body fluids for drugs of interest in highway safety.

Discussion of these topics was technical in nature. An effort has been made to simplify their presentation in this section. In addition, for the reader desiring background information, Appendix A provides a brief description of analytical concepts and techniques.

To facilitate discussion, the panel assumed the following, hypothetical research setting:

- a central laboratory (perhaps replicated on a regional basis, but no more than two or three);
- methodology representing the present state of the art in drug analysis;
- 5000 cases or sets of specimens--1000 from the fatally injured driving population, 4000 from a control population;
- availability of blood and (possibly) urine specimens of the required volume and quality;
- required detection of the drugs of interest at "therapeutic concentrations" (50 nanograms per milliliter of blood or lower); and
- required quantitation of detected drugs down to (at least) therapeutic levels (about 0.1 micrograms per milliliter of blood).

The panel then discussed factors that influence the general approach to drug analysis. Next, participants outlined techniques to detect and quantitate the drugs of interest in body fluids.

4.1 Factors Affecting General Strategy for Analysis for the Drugs of Interest

Factors within a research setting influence the design of analytical schemes. Among these factors are **the range of drugs for analysis, the limits of detection required, the percentage of positive (or negative) findings,** and—a function of these factors—**cost.**

Two concerns surfaced in discussions of **the range of drugs for analysis.** One concern was that all highly ranked drugs of interest be analyzed as per assumed requirements. Some of these (e.g., marijuana and diazepam) have been missed in past field surveys. The approach suggested was (1) to select drugs or groups of drugs of higher rank order; (2) to specify techniques for them; and (3) to determine which other drugs would be detected by these techniques or could be detected for little added cost by additional methods.

The other concern dealt with the list of drugs of interest itself. To some participants it represented an exclusionary approach. In field surveys focused solely on these drugs, a substance that occurred in driving populations with great prevalence could be missed. More acceptable, at least in a toxicological sense, would be an approach based on the concept of the "general unknown." An alternative approach is to specify techniques to detect every substance possible, lest an important finding be lost. Here, cost becomes an obvious limiting factor. Thus, practical constraints alone force changes to narrow somewhat the scope of this latter, ideal approach.

The panel addressed both concerns. As noted before in this report, the list of drugs of interest—although it is drug-specific--is so broad in its grouping that it almost covers the "needle-in-the-haystack" approach. To select only the higher ranked drugs of interest (for example, rank orders 1 through 15, Table 1) need not limit the scope of inquiry. Most (but not all) drugs in body fluids would be detected by an analytical scheme designed for these substances. Such a scheme would, in addition, approximate "general screens" in routine practice in forensic and toxicologic laboratories.

The range of drugs tested does affect the overall cost of drug analysis. The greater the range, the greater the cost. Nevertheless, only by restricting screening to one or two groups of drugs (e.g., barbiturates, opiates) does the cost of analyses decrease significantly. In fact, this particular approach--screening only for one or more groups of drugs—was suggested as an alternative to screening for a broad range of drugs in surveys of drug use among drivers. Two reasons for **not** recommending that alternative are: (1) that there is a fixed (and substantial) cost associated with obtaining specimens; and (2) that, once specimens are obtained, it is pragmatic to obtain as much information per research dollar as feasible. A narrow analytical approach could not be defended in applied research on drugs and highway safety.

To detect and quantitate drugs of interest **at therapeutic levels** increases the time and cost of analyses. This requirement is rarely met by methods routinely applied in other areas, nor is it relevant for the most part. Nevertheless, the limits of sensitivity specified do not extend beyond the present state of the art. Hence, this factor was incorporated in the assumed research setting described above.

More significant in terms of both design and cost is the **percentage of specimens in which drugs are found.** Each positive finding requires additional effort by the analyst. To measure the amount of each identified substance may require an extra assay or a separate method. In a series of specimens with a low percentage of positive findings (e.g., less than 50%) rapid screening techniques with low rates of false negatives, followed by quantitation by the same or different technique, may be cost-effective. Immunoassay techniques are especially suited to this approach. On the other hand, higher percentages of positive findings may require methods that both detect and quantitate given drugs. These are, in general, more time-consuming and, therefore, more costly.

The range of drugs tested, if extended to include relatively common drugs like caffeine and nicotine, may drive the expected percentage of specimens positive for drugs to over fifty percent in some driving populations. In most screening systems, however, methods deal separately with different analytical classes of drugs. No single group of drugs is

expected to appear in fifty percent of the specimens. Therefore, a rational approach to drug analysis would include, where available, simple, rapid techniques designed to exclude from further analysis specimens negative for drugs.

4.2 Strategies and Techniques for Detection and Quantitation of the Drugs of Interest

Based on the assumptions and requirements noted above, participants outlined two general strategies in drug analysis and suggested techniques to detect and quantitate the drugs of interest.

In general, the methods based on these techniques are both qualitative and quantitative; that is, each technique may be used in screening, and for positive findings each can measure the amount of drugs detected. In screening methods based on **chromatography**, reference standards help identify and quantitate unknown drugs. Quantitation is made possible by adding known quantities of (internal) standards to each specimen. Findings in each run are compared to these standards. **Immunoassays** differ from methods based on chromatography. Immunoassays are usually highly specific for chemical classes of compounds, but within each class they may be highly nonspecific. For screening this nonspecificity can be of value. For example, several drugs within a class can be screened simultaneously. Metabolites present often cross-react and thereby enhance the sensitivity of the method. Confirmation and quantitation of positive findings by nonspecific immunoassay methods usually require chromatographic procedures to identify the drug and determine its amount. Thus, in Table 4-1, confirmatory/quantitative techniques are listed under immunoassays. The confirmatory/quantitative techniques may also be used in screening.

The second general approach mentioned is based on one technique, gas chromatography-mass spectrometry-computer (GC-MS-COM). GC-MS-COM is a powerful, sensitive analytical tool. It combines the ability of gas chromatography to separate components of complex extracts with a "universal" detector, the mass spectrometer, which is sensitive to the nanogram (10^{-9} g) level. A dedicated computer directs the acquisition of

TABLE 4-1

TECHNIQUES FOR ANALYSIS OF THE DRUGS OF INTEREST

ANALYTICAL CLASS	REPRESENTATIVE MEMBERS	TECHNIQUE(S)+
Alcohol and other volatiles	ethanol	GC
	methanol	
	isopropanol	
	acetone	
Benzodiazepines	diazepam*	GC-EC
	flurazepam*	Immunoassay
	chlordiazepoxide*	HPLC
Cannabinoids	Δ^9-THC	Immunoassay
		GC-MS
Volatile hydrocarbons	trichloroethylene	GC
	toluene	GC
Carbon monoxide		Chemical test
		GC (very low levels)
a. Weak acids	barbiturates	GC-FID
	phenytoin	GC-MS
	antidiabetics	
b. Neutrals	caffeine	GC-FID
	glutethimide	GC-NPD
	methaqualone	GC-MS
	ethchlorvynol	
	meprobamate	
	chloral hydrate*	
	phencyclidine [PCP]	
Bases	amphetamine derivitives	Immunoassay
		GC-FID
	cocaine	Immunoassay
		GC-MS
	codeine*	
	oxycodone	
	pentazocine	
	methadone*	Immunoassay
	meperidine*	GC-MS
	hydromorphone	GC-NPD
	morphine	
	propoxyphene*	GC-MS
	antihistamines*	GC-NPD
	tricyclic antidepressants*	
	phenothiazines*	
	nicotine	
	lidocaine*	

*Indicates important metabolites

+Abbreviations: GC=gas chromatography; GC-EC=gas chromatography-electron capture; GC-FID=gas chromatography-flame ionization detection; GC-NPD=gas chromatography-nitrogen phosphorous detection; GC-MS=gas chromatography-mass spectrometry; HPLC=high pressure liquid chromatography.

data, stores the information for retrieval, and facilitates later analysis. An analytical approach based on GC-MS-COM has advantages and disadvantages.

The **disadvantages** of this approach are the costs involved. The initial, or "start-up," cost of GC-MS-COM is high; assuming a central laboratory already so equipped avoids this hurdle and places the cost of drug analysis near that of more common screening methods. The instrument, however, is complex, tends to be relatively temperamental, and requires experienced operators. Maintenance costs may be higher than more conventional instrumentation. Further, its potential for application to general drug screening, though great, remains untested. Methods for GC-MS-COM are often more in a research category than in routine operation.

The **advantages** of GC-MS-COM in drug screening follow from its technical virtues. GC-MS-COM can integrate three stages of analysis--screening, confirmation, and quantitation—in a single run. It can also identify and quantitate a relatively large number of drugs in a single run, with a specificity and sensitivity superior to most other techniques. Computer techniques for controlling the MS greatly extend the capability of the system. For example, the data system makes possible "retrospective" assays by "extracted ion current profiles." This technique allows a selective look for specific drugs at specific retention times while maintaining the generality of continuous scanning. The use of GC-MS-COM also enhances the sensitivity of a screening procedure.

The proposed approach using GC-MS-COM, if developed, could reduce to three the seven analytical classes of the first approach:

1. alcohols, volatiles;
2. weak acids, neutrals, cannabis; and
3. bases, benzodiazepines.

Carbon monoxide was excluded in this design. Virtually all drugs would be detected at a level of 10 nanograms per milliliter of blood without a prohibitive increase in cost.

4.3 Summary

Participants discussed the analysis for drugs of interest in body fluids. Among factors that influence the analytical approach and design are the range of drugs tested, the required limits of detection, the percentage of specimens that contain drugs, and the cost of analysis. Based on analytical requirements set by the panel and on certain explicit assumptions, general approaches and techniques were suggested to detect and quantitate the drugs of interest. Similar to general toxicological screens now routinely used, one approach included a number of different techniques. The other approach, based entirely on the GC-MS-COM technique, has not been developed or tested. GC-MS-COM is also not widely available for the purpose of applied research studies in drugs and driving. Its potential as a powerful tool in the analysis of drugs warrants further research. The panel pointed out that many specific analytical schemes using methods based on state-of-the-art technology could be outlined. Reliance on a single technique (e.g., immunoassays, GC-MS) was neither necessary nor necessarily justified.

5.0 ISSUES IN THE DESIGN OF RESEARCH INVOLVING ANALYSIS OF BODY FLUIDS FOR DRUGS

The main topic of this workshop was the analysis of body fluids for the drugs of interest. But beyond analytical requirements, strategies, and techniques are issues that pertain to the design of studies involving drug analysis. Many of these topics concern the quality of analytical results as well as the overall quality of applied research in drugs and driving. Therefore, participants also discussed these issues. Because the role of drug analysis is so pivotal in surveys of drug use among drivers, the panel stressed the **context** of research to determine the prevalence of drugs in driving populations.

Five subsections organize and briefly summarize discussion of the following topics:

- collection, handling, and storage of specimens;
- enhancement and maintenance of the quality of analytical results;
- interpretation of analytical data;
- survey information of value in addition to analytical results; and
- issues in the design of epidemiological research in drugs and highway safety.

5.1 Collection, Handling, and Storage of Specimens

The quality of analytical results is a function of the quality of specimen analyzed. The quality of specimens--not to mention their availability—depends greatly on procedures for their collection, handling, and storage. Specimens of poor quality may be contaminated, or their volume insufficient for complete analysis. Variability in the quality of specimens also reduces the value of analytical data. The availability of

acceptable specimens depends on the care, competency, and attention to detail of persons responsible for their collection. Participants discussed measures and guidelines to ensure a quality of specimens acceptable for analysis of the drugs of interest.

In research to describe the association between drug use and driver fatalities, the cooperation of offices of medical examiners and coroners is essential, since these agencies are authorized to investigate the cause and manner of death. Good working relationships with pathologists who perform autopsies are important because these professonals direct others who may be responsible for collecting and handling specimens. The quality of specimens may also be a function of how well established a death investigation system is. Offices of medical examiners and coroners should agree to study requirements as a condition for participation in drug and driving research.

Persons responsible for obtaining specimens should be supplied with kits prepared in advance by researchers. Complete kits can reduce variability in the quality and quantity of specimens received. The kits should include all items necessary for collecting and handling specimens, even prepaid mailers for delivery of specimens to the site of their analysis. All procedures should be described clearly in detail. This approach does not eliminate all potential problems, but it can reduce them.

The quality of specimens also depends on **when** they were obtained. In the case of a driver fatality, the following two time periods are of concern:

1. The time period from the crash to when death occurred (or to when a specimen was obtained from a driver who later died from injury sustained in a traffic crash)

This first time period is of great concern because the concentration of drugs present at the instant of the crash best indicates their possible role in its occurrence. As the interval between crash and death lengthens, the amount of drugs in body fluids will increasingly differ from that at the moment of the crash. Intervening medical treatment may introduce other drugs into body fluids. The panel suggested **four hours** as a reasonable period beyond which time specimens should not be collected. For some drugs, for example, cannabinoids, concentrations in body fluids will greatly decrease even in two

hours. The interpretation of analytical data for each case should therefore take into account this time period as well as the particular drug detected. Finally, efforts should be made to determine whether drugs were administered after the crash as part of emergency medical treatment, prior to death of the driver.

2. The time period from death to when specimens are obtained.

This time period relates to the physical state of the specimen (degree of decomposition) and its effect on drug analysis. Badly decomposed specimens present great difficulty for analysis because substances that interfere with analysis greatly increase over time. Participants, however, saw little or no adverse effects on analytical results for concentrations of detected drugs, provided extremely long time periods were avoided, for example, five weeks. Nevertheless, this is an area of research where there is little information on the stability of drugs in such specimens, and more research is needed before definite conclusions are possible.

Properly stored, specimens may be kept for long periods prior to analysis. As discussed earlier in Section 3.1, the panel recommended that all specimens should be stored deeply frozen, below -20 degrees Celsius. Participants thought that unnecessary thawing and refreezing of specimens should be avoided and that specimens handled in this way could be stored up to six months prior to analysis.

5.2 Enhancement and Maintenance of the Quality of Analytical Results

For purposes of discussion, the panel assumed a central laboratory for drug analysis. Specimens from different points of collection would be sent singly or in batches to the central site. This approach avoids complications that arise when two or more sites perform tests for the same drug. For example, interlaboratory differences and intermethod comparisons do not become problems. At the same time, the quality of analytical findings can be better controlled. **Proficiency testing, quality control,** and **enhancement of analytical methods** are means to ensure the quality of analytical results.

Proficiency testing evaluates both the physical capability and the

analytical competence of a laboratory to perform analyses required of it. The panel stated that this kind of testing should be done before a laboratory is selected to perform analyses of drugs for a field survey and, if possible, before award of the contract itself.

Preselection testing of laboratories has drawbacks. It may be considered unfair to ask laboratories of the type desired to run analytical test specimens free of charge. Further, competent laboratories with satisfactory volumes of business may not be interested in a large contract (which may disrupt routine operation), much less in processing a handful of specimens for uncertain return. Nevertheless, the prospect of a multiyear contract with a steady supply of specimens for analysis may be attractive to some laboratories. These may opt to participate in proficiency testing for the opportunity to engage in such a project.

Participants specified the characteristics of a program to test proficiency:

- the program should be connected to a purely external, objective program already in existence and professionally recognized (e.g., the proficiency testing program of the College of American Pathologists [CAP]); and
- the program should **not** involve subcontracting to another laboratory to make up analytical samples for chosen laboratories.

Quality control procedures are attempts to maintain the proven level of performance by a laboratory chosen to perform drug analyses. External, postselection quality control programs should have characteristics similar to those listed above for proficiency testing. Any program of quality control must use a realistic approach. Blind, split specimens like those analyzed (e.g., drugs in whole blood) should be used. Test samples should be placed in the stream of samples flowing into analysis without the knowledge either of the analyst or of the person inserting the samples. The program of quality control should be ongoing throughout the analytical phase of the project.

Efforts to enhance techniques and methods employed by the chosen laboratory should also be made. For example, participants stressed the

need for reference standards. Procedures of quality control run in-house by the laboratory make use of reference compounds as internal standards in each run of a method. This ensures interassay comparability and provides a means to monitor test results. The National Institute on Drug Abuse has provided such standards to projects involving the analysis of drugs. The panel also noted the increasing need for standards of metabolites of the drugs of interest. Screening for many of these drugs (e.g., the benzodiazepines) may require detection and quantitation of their important metabolites.

5.3 Interpretation of Analytical Data

Participants often expressed concern about the interpretation of analytical data. Findings of the presence and amount of drugs in blood and other body fluids have, at best, uncertain meaning. For example, very little is known about the relationship between the level of drugs in blood and their effects on skills related to driving. When two or more drugs are present at the same time, the difficulty of interpretation is increased. The variability among individuals is well known. Wide ranges are found both for blood concentrations after the same dose of drug and for behavioral effects with similar blood concentrations. Even acute and chronic patterns of drug use cannot be distinguished with confidence; too many factors intervene for reliable interpretation. Because the present state of knowledge is so limited, participants urged great caution in interpreting analytical results. Such caution, they noted, is often not exercised.

Efforts to reduce problems arising from misinterpreting analytical results can be made. As discussed in Section 3.2, participants advised great care in reporting analytical results. Some modern analytical techniques can detect the presence of drugs long after their effect has ceased. In contrast, for some drugs, a metabolite is the only evidence of a parent drug's presence and ongoing effect. Some drugs act through metabolites, which must also be detected and quantitated for interpretation. Obviously, analytical findings should not be interpreted to mean more than they actually indicate.

5.4 Survey Information of Value in Addition to Analytical Results

In isolation, analytical data are difficult to interpret. Surveys can, however, obtain additional information that links such data with the real world. The panel suggested that this information be gathered and used in the interpretation of analytical results.

One participant, a member of a liaison committee of the California Association of Toxicologists (CAT), presented to the panel a form developed to elicit and record information deemed of value in studies of drugs and driving. The form had provided the basis for a drug and driving study performed by the CAT (Lundberg 1976; Lundberg, White, and Hoffmann 1979).

Figure 5-1 shows a copy of this form. Information obtained by use of this form includes:

- characteristics and observed behavior of the driver;
- patterns of self-reported and apparent drug use;
- accident and arrest data;
- comprehensive data on the analysis of drugs and the methods employed;
- analytical results; and
- characteristics of the laboratory that performed the analyses.

The form represents one effort to develop a standard base of information to aid in the interpretation of analytical results in traffic-related cases involving drugs.

5.5 Issues in the Design of Epidemiological Research in Drugs and Highway Safety

Participants also discussed issues related to the design of epidemiological research. Two topic areas were:

- alternative approaches to research on the prevalence of drugs in driving populations; and

FIGURE 5-1

A FORM FOR INFORMATION TO AID IN OBTAINING AND INTERPRETING ANALYTICAL DATA

California Association of Toxicologists - Liaison Committee

Drugs & Driving Study

Do Not Fill In
Study Case #_____

INSTRUCTIONS: Report all cases in which a sedative, hypnotic, stimulant, analgesic, narcotic, tranquilizer, antihistamine, or other related drug was suspected and/or identified in relation to real or potential vehicle driving performance. Report cases involving alcohol only when in combination with other drugs.

Age | Sex M F | Race | Chronic Drug User: Yes (0) No (1) Unknown (2) | If Yes, What Drugs? | Drug Prescribed? Yes No Unknown

Serious Diseases Present? If Yes, List: Yes No _____ Unknown _____

Suspected Acute Drug(s): | When Taken | Amount Taken | Yes No Unknown

Were Drugs in Possession at Time of Arrest or Accident? Yes No Unknown | If Yes, What Drugs? | How Much? | Apparent Prescription? Yes No Unknown

Date & Time Subject Apprehended:_____ | Yes No Unknown

Fatality of Subject: Yes No

Fatality of Others: Yes No If Yes, How Many?_____

Was Field Sobriety Test Performed? Yes No Unknown If Yes, State Type of Exam and Result.

Function of Subject at Time of Arrest or Accident? Driver Passenger Pedestrian Other (specify)_____

State of Subject at Time of Obtaining Specimen (Check those observed.)

__Normal __Belligerent __Hallucinating __Impaired Balance __Impaired Coordination
__Awake __Disoriented __Unconscious __Impaired Vision __Slowed Reaction Time
__Lethargic __Loquacious __Affectionate __Staggering __Coma
__Euphoric __Emotional __Slurred Speech __Odor Observed (specify)_____

Job Category of Observer: __Police Officer __Criminalist __Toxicologist __Physician __Other (specify)_____ Others (specify)_____

Driving Behavior Problem: Yes No

__Excessive Speed __Stop Light Violation __Weaving __Without Due Care __Driver under Influence
__Excessive Slowness __Stop Sign Violation __Other (specify)_____ __Right of Way

If Accident, __Injury to Subject __Single Vehicle __Off Roadway __Multiple Vehicle __Into Barrier

Testing Laboratory | Specimen # | Date & Time Specimen Obtained_____ | Date & Time Specimen Analyzed_____

DRUG FINDINGS:	B	U	T	P	A	NTF
ALCOHOL, ETHYL						
AMITRIPTYLINE						
AMPHETAMINE						
BARBITURATES,						
Long Acting						
Short Acting						
Specific:						
AMOBARBITAL						
BARBITAL						
BUTABARBITAL						
PENTOBARBITAL						
PHENOBARBITAL						
SECOBARBITAL						
BROMIDE						
CAFFEINE						
CARBON MONOXIDE						
CARISPRODOL						
CHLORAL HYDRATE						
CHLORDIAZEPOXIDE						
CHLORPHENIRAMINE						
CHLORPROPAMIDE						
COCAINE						
CODEINE						
DIAZEPAM						
DIPHENHYDRAMINE						
DIPHENYLHYDANTOIN						
ETHCHLORVYNOL						
ETHINAMATE						
FLURAZEPAM						
GLUTETHIMIDE						
MEPERIDINE						
MEPROBAMATE						
METHADONE						
METHAMPHETAMINE						
METHAQUALONE						
METHYPRYLON						
MORPHINE						
NICOTINE						
NORTRIPTYLINE						
OXAZEPAM						
OXYCODONE						
PENTAZOCINE						
PHENOTHIAZINE						
PHENYLBUTAZONE						
PRIMIDONE						
PROPOXYPHENE						
SCOPOLAMINE						
TOLBUTAMIDE						
TRIPELENNAMINE						
OTHERS (specify)						
_____						
_____						
_____						
_____						

B = BLOOD U = URINE T = TISSUE P = PRESENT A = ABSENT NTF = NOT TESTED FOR

1. Drug Found | Concentration in µg/ml. | Type Specimen

ANALYTICAL METHOD:

Isolation Technique: Resin column Yes No Resin Used_____ pH adjustment Yes No pH_____ Eluting Solvent_____
Extraction Yes No pH_____ Solvent Used_____ Other Techniques:_____

Identification Technique: UV GC TLC Other_____ Quantitation Technique: UV GC TLC Other_____

Confirmation or Verification Method(s): Yes No If Yes, What?_____

2. Drug Found | Concentration in µg/ml. | Type Specimen

ANALYTICAL METHOD:

Isolation Technique: Resin column Yes No Resin Used_____ pH adjustment Yes No pH_____ Eluting Solvent_____
Extraction Yes No pH_____ Solvent Used_____ Other Techniques:_____

Identification Technique: UV GC TLC Other_____ Quantitation Technique: UV GC TLC Other_____

Confirmation or Verification Method(s): Yes No If Yes, What?_____

Was Quality Control Program in Effect for Pertinent Drugs at Time of Analysis? Yes No Unknown. If Yes, Describe.

What Proficiency Testing Program(s) was engaged in by Testing Laboratory at Time of Analysis?

IF MORE THAN 2 DRUGS IDENTIFIED PER CASE, REPORT ADDITIONAL DRUGS FOUND ON BACK EXACTLY AS SHOWN ON FRONT FOR #1 & 2 DRUGS.

- generation of data on drug use by drivers from analyses ongoing in the field.

These are briefly summarized below.

5.5.1 Alternative Designs of Epidemiological Studies. The design of field surveys with limited scope was raised for discussion. This is similar in some respects to the analytical approach involving the analysis of a narrow range of drugs (Section 4.1). Some past studies, in the opinion of some participants, have apparently failed, partly due to attempts to analyze specimens for too many different drugs. As an alternative approach to "universal screening," a series of separate studies of single classes of drugs could be done. By restricting the scope of each survey, more complete analyses of each class of drugs might be possible.

Objections to the "limited" approach were similar to those listed earlier (Section 4.1). First, collecting specimens for analysis, be it for one drug or many, involves a substantial effort and cost. A more cost-effective approach would be including as many drugs of interest as possible for analysis. Second, a series of small-scale surveys is hard to justify if the main objective is to define the national drug and driving problem and to estimate its magnitude. Samples of drivers included in small-scale surveys would probably not be representative of the national driving populations to which they belong. The incidence of any one drug or class of drugs is probably low relative to alcohol, and patterns of drug use vary from region to region and even within regions of the country. To achieve representative samples, field surveys of drug use among drivers should be large-scale. Large-scale surveys are costly, requiring that as much data as possible are obtained for each specimen submitted for analysis. Study designs should be developed to gather as much relevant information as possible. Other alternative approaches to performing general drug screening that avoid the approach with narrowed scope described above were suggested. Possible objections to screening for a broad range of drugs of interest included:

- its cost;
- the unwillingness of analytical laboratories to undertake a project of short duration that disrupts laboratory routine; and
- the inability of laboratories to analyze specimens for all higher ranked drugs of interest.

Participants pointed out that an entire specimen need not be subjected to complete analysis. Specimens of blood and urine may be divided into smaller volumes. The splitting of specimens provides many options. Screens for different classes of drugs may be run in separate laboratories certified for them. A general screen for all drugs of interest may be run on a subset of the total sample. Specimens may even be stored for later analysis, although the concentration of some drugs may change upon prolonged storage. Each option, of course, requires that specimens be properly handled, preserved, and stored deeply frozen.

Participants also identified approaches to the design of field surveys that could better control the quality of specimens obtained. By carefully selecting areas for sampling driving populations (a "judgmental" approach), the aid and cooperation of competent medical examiners' and coroners' offices could be enlisted. The drawback of this approach is one shared by surveys based on judgment samples: the sample may or may not be representative of the driving population under study. An alternative to the judgmental approach is a design based on probability sampling. In that approach to sampling design, the possibility of selecting jurisdictions unable or unwilling to cooperate is greater, but a representative sample of drivers can be better ensured.

5.5.2 The Possible Use of Data on the Incidence of Drug Use among Drivers. Alternative ways of generating data on the prevalence of drugs other than alcohol in fatally injured drivers were also discussed. The testing for drugs in this driving population is ongoing at local, statewide, and multistate levels. The amount of existing data is unknown; the number and type of such efforts have not been identified or well

characterized. The quality of analytical results undoubtedly varies. Yet, given the paucity of data on the presence and amount of drugs in any driving population, even these findings would be of value for designing further research. Thus, participants raised the possibility of examining data produced by these local and state-level efforts.

The gathering of existing data might provide a basis for studying the prevalance of drug use among fatally injured drivers. First, however, information is needed on the sources of these data, on methods to collate available data, and on guidelines for their interpretation. Participants noted that the quality of results varies greatly, as does the range of drugs tested. Data must be very carefully selected. To garner data of value from case files (and to interpret it) requires substantial cooperation by each death investigation system. A careful critique of data must accompany the report of any such study; expert judgment is certainly needed. Participants characterized this approach as time-consuming, very expensive, and very difficult.

Efforts aimed at assembling new data produced on an ongoing basis may have more promise than gathering old data from files. This approach has several advantages:

- It may be less costly and more productive since data can be submitted to a central storage site when produced and information requirements can be established ahead of time.
- Analytical data would be more reliable and more comprehensive (1) because methodology used presently is superior to that used in past years and (2) because performance of laboratories engaged in drug analysis is improving due to licensure requirements and proficiency testing programs.
- Checking the source and quality of submitted data would be simpler since the information would be of a recent nature.

In short, this approach will result in a higher quality of data than now found in case files.

The gathering of data from operational agencies in the field will require substantial effort. Sources of reliable, well-characterized data

must still be identified; mechanisms to collect data must be designed and established; and the analytical methods and data must be compared. In particular, attempts to merge data bases, which this approach may require, could offer difficulty. Analytical approaches and methods employed by different laboratories may not be compatible. To integrate their findings of drugs in drivers may not be meaningful. Additional efforts could be made, however, to make data from different sources more comparable.

Ongoing work related to drugs and driving routinely done by operational agencies (for example, offices of medical examiners or coroners, police laboratories, state toxicology laboratories) can also be "enriched" to enhance the quality of data available for subsequent analyses. Problems faced by many analytical laboratories include lack of adequate funding. Discrete funding of laboratories willing to cooperate may lead to more complete data on drug-involved, traffic-related cases. Other forms of support are also possible. Assistance in designing local studies, recommendations concerning analytical methodology, and provision of reference standards are three ways to enhance the quality of analytical results. Over a period of time, a limited but sound data base could develop. This data base would not remove the need for large-scale surveys but would serve a role in exploratory research. Information obtained directly from local agencies would probably be more cost-effective and at least as productive as a series of small-scale surveys.

The panel concluded that both approaches to gathering data produced in the field were feasible. Captured data on the incidence of drugs in drivers would supplement limited information presently available. Assembled data would be an indicator of the magnitude of the problem. It could be used, for example, to identify drugs of interest for future research in drugs and driving. The data could not be used to determine the relative traffic crash risk of drugs.

5.6 Summary

The panel discussed issues related to drug analysis that affect not only the quality of analytical results but also the outcome of applied research

studies in drugs and driving. Participants outlined the proper collection, handling, and storage of specimens. They also described programs of proficiency testing and quality control needed for the selection and monitoring of laboratories participating in field surveys involving drug analysis. The interpretation of analytical results was discussed in terms of additional information needed to facilitate interpreting these data. Finally, participants provided input on issues in the design of research in the epidemiology of drugs and driving.

6.0 CONCLUSIONS AND RECOMMENDATIONS

Methods of drug analysis play an important role in research on the relationship between drugs and highway safety. The analysis of drugs in body fluids is essential in surveys of drug use among drivers. Some experimental studies correlate the amount of drugs present and their effect on driving skills; these require methods to quantitate drugs in human subjects. This workshop dealt with issues that arise from requirements for analytical methodology in drug and driving research. Its objectives were summarized in a question posed by one participant:

> What analytical techniques--for which specimens, with what limits of sensitivity--are available and applicable today for applied research projects in the area of drugs and driving?

In the Drug Research Methodology project, this workshop preceded another on epidemiology in drugs and highway safety. In one sense, the order of workshops reversed the normal order of planning. In a project to study the prevalence of drugs in driving populations, outlines of objectives and requirements come before consideration of analytical techniques. Yet, significant questions in the design of such studies pertain to issues in drug analysis.

For example, which specimens in what quantity should be obtained? In collecting specimens, what procedures might enhance the quality of analytical results? What information might be obtained by a survey to help interpret analytical data? These questions show the influence of analytical requirements on the design of surveys. Such questions also formed the rationale for the order of workshops in this project.

The purpose of this workshop was to furnish information on the analysis of drugs of interest to the workshop on epidemiological research. Information needs included an identification of techniques most appropriate for dealing with this set of drugs--and any others believed important. An initial pass at analytical issues would ease the task of

designing a survey. In this approach, prior consideration of these issues facilitates the design of surveys; analytical schemes can be modified by practical constraints present in survey research.

The approach used in this workshop did not provide participants with a definite framework for discussion. On the contrary, participants had to conceive a setting of applied research in which to resolve analytical issues. The panel was asked to accept a list of drugs of interest, to set analytical requirements, and to recommend techniques for near-term epidemiological research. To achieve these objectives, participants adopted a scientific viewpoint. They took into account real-world constraints such as cost and the present state of knowledge. They recognized—but did not consider directly--policy-based constraints. The panel attempted to provide guidelines and standards that, if applied in drug and driving research, would yield analytical results acceptable to the scientific community.

Participants accepted the list of drugs of interest, but not without comment. They questioned the rank order of some drugs and classes of drugs. A few drugs not on the list were suggested for addition. The broad diversity of the drugs of interest, however, attracted most attention. The panel concluded that an analytical scheme for screening these drugs--even restricting the range of drugs to those highly ranked--would detect most drugs in human body fluids. The scheme would approximate toxicologic screens for drugs in routine practice. Further, the screen would make irrelevant the specific rank order of these drugs. Participants therefore rearranged the drugs of interest into analytical classes for discussions of requirements and techniques in drug analysis.

Analytical requirements discussed were the types and amounts of specimens for analysis and the limits of detection for drugs. The panel concluded that, for each of four driving populations and for experimental subjects, blood is the specimen of choice. Further, the panel stated that specimens of blood are a minimum requirement in surveys of drug use among drivers. Other specimens, in particular urine, may facilitate screening for drugs. But no body substance can replace blood and still maintain the quality of findings needed for both problem and risk

identification in highway safety. The present state of knowledge precludes use of any other specimen than blood for estimating the magnitude of the drug and driving problem and for estimating the relative traffic crash risk of drugs.

Participants agreed that "therapeutic levels" of drugs should be detected in epidemiologic research. Because the range of concentrations of drugs in blood is so great, precise limits of sensitivity could not be defined for analytical techniques. Therefore, participants set general guidelines (0.05 micrograms per milliliter of blood or lower for detection, 0.1 micrograms per milliliter of blood or lower for quantitation). The panel stated that methodology representing the state of the art should be applied to give the best possible chance of detecting the drugs of interest.

The panel concluded that the present state of the art in methodology could meet these requirements for the analysis of blood for the drugs of interest. In fact, several analytical schemes are possible for use in field studies.

The panel also reached conclusions about the design of surveys involving drug analysis. A central laboratory should be selected for the analysis of specimens. Programs of proficiency testing and quality control should be employed to ensure the quality of analytical data in such studies. The design of epidemiological research should include the measures and guidelines specified to support and supplement the analysis for drugs. Above all, the interpretation of analytical data must reflect their known limitations.

The panel recommended the following research to advance the state of the art in drug analyses as well as the state of knowledge of drugs and driving:

First, the extent and value of existing data on drug use among drivers should be studied. Sources of data and ongoing studies, both local and state-level, should be identified. The feasibility of gathering and analyzing these data should be explored. This research may lead to alternative ways to gather information on the magnitude of the drug and driving problem.

Second, basic research in analytical methodology should continue and

should be supported. Techniques with potential to advance the state of the art in drug screening, such as polyvalent immunoassays and GC-MS-COM, should be emphasized.

Third, the analytical needs of surveys of drugs in drivers should be identified. For example, reference standards for the drugs of interest and their important metabolites should be specified. Designs of programs to ensure the quality of analytical results should be specified. Procedures to support the analyses of drugs (e.g., collection of specimens, collateral information) should be specified in greater detail.

Fourth, basic research is needed to aid in the interpretation of analytical data. In particular, detailed studies of the pharmacokinetics of the drugs of interest are required. Existing information on the presence and amount of drugs in human body fluids, including saliva, should be gathered in a central data bank. Diagnostic aids that distinguish between acute and chronic drug use should be identified.

Fifth, the National Highway Traffic Safety Administration should support one or more studies in the area of analytical methodology. The project(s) should support the development and demonstration of analytical schemes meeting requirements of applied research studies in drugs and driving. The techniques used should detect and quantitate the drugs of interest in specimens similar to those required in surveys of driving populations.

In conclusion, this workshop found that analytical methodology need not be the limiting factor in surveys of drug use among drivers. Analytical requirements do not preclude valid study of the prevalence of a wide range of drugs in various driving populations. Analytical results of a quality acceptable to the scientific community may be obtained with state-of-the-art techniques. The present state of knowledge of pharmacokinetics and the behavioral effects of drugs limits such data to use in problem and risk identification in highway safety. Further research can advance efforts to make more meaningful quantitative findings of drugs in body fluids.

APPENDIX A

THE DETECTION AND QUANTITATION OF DRUGS IN BODY FLUIDS: BACKGROUND INFORMATION

APPENDIX A
THE DETECTION AND QUANTITATION OF DRUGS IN BODY FLUIDS
BACKGROUND INFORMATION

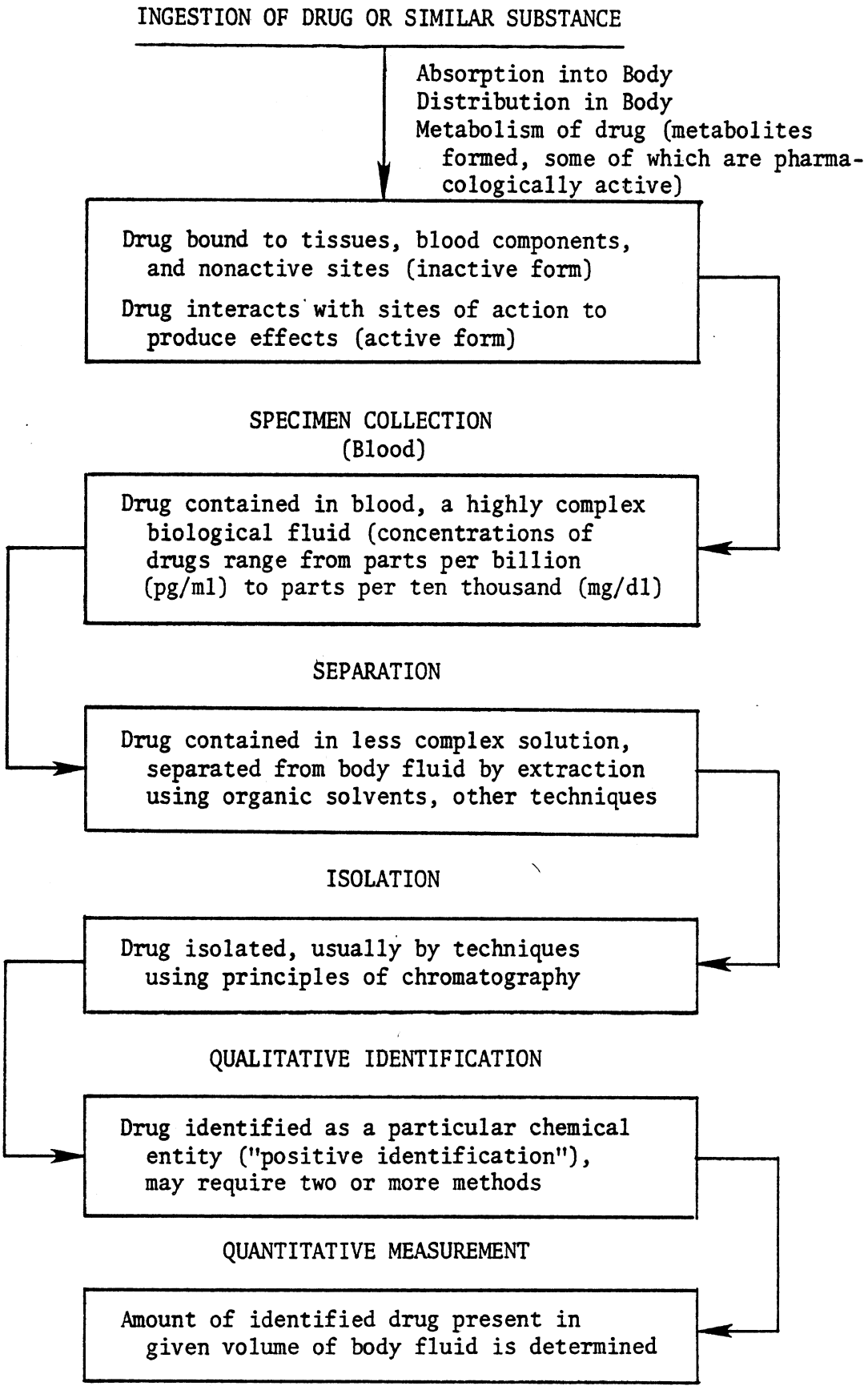
In highway safety research, the analysis for drugs in body fluids supports epidemiologic and experimental studies to define the drug and driving problem. Local efforts to deal with drug-impaired driving have also depended on methods to detect and measure inappropriate drug use by drivers. In the past, the absence of sensitive methods for some drugs (e.g., marijuana, benzodiazepines)--or the unavailability of methods in toxicology laboratories--have hampered both research and enforcement efforts. Discussions of analytical methods and their application are often highly technical. This appendix provides background information on the detection and measurement of drugs in body fluids. Its purpose is to make this workshop report more accessible to the reader unfamiliar with these topics.

The detection and quantitation of drugs in body fluids is a process that starts with collecting a specimen and ends with determining the amount of drug present. Figure A-1 illustrates this process and identifies general steps taken to complete it. Each step is important, though with some modern techniques, a chemist can avoid certain intermediate steps. The process of analyzing for drugs in body fluids has been described in detail elsewhere in the scientific literature (e.g., Sunshine 1975; Joscelyn and Maickel 1977a; Joscelyn et al. 1979), and is briefly summarized here in the context of highway safety. The reader should note that the following discussion contains general statements intended to simplify the description of drug analysis, for which specific exceptions can always be found.

A drug or similar substance can be ingested in several ways; orally, by inhalation, or by injection are the most common routes of administration. As a drug is taken into the body, it is absorbed and distributed by the

FIGURE A-1

THE ANALYSIS FOR DRUGS IN BLOOD



circulation of blood. Both active (free) and inactive (bound) forms of drug are present. Interaction of drugs with enzymes in the body (especially the liver) produced drug-like chemicals known as metabolites. Some of these act like the parent drug and can have effects on behavior. A specimen of blood must be collected from a driver--living, fatally injured, injured, or arrested for impaired driving--in order to detect and to measure the drug or drugs. At present, **blood is the only specimen from which meaningful results can be obtained** (Joscelyn et al. 1979, pp. 292-93). Blood, especially whole blood in which red cells have broken down (hemolyzed blood), is an extremely complex fluid. Most drugs are present in concentrations ranging from parts per billion to parts per ten thousand. The complexity of blood and the presence of drugs in minute amounts require sophisticated chemical tests for its analysis.

Most methods of drug analysis involve four distinct steps:

- **separation** of drug and other substances from blood;
- **isolation** of the drug from other chemicals present in the less complex organic solution;
- **qualitative identification** to establish the presence of a given drug; and
- **quantitative measurement** of the amount of identified drug present in the unit volume of blood.

A separation step is required to extract a drug from blood so that the resulting solution can be more simply analyzed. With the exception of certain techniques, detectors of chemicals are not specific enough to identify the presence of a single drug accompanied by a host of interfering substances. Separation techniques include:

- liquid-liquid extraction,
- molecular sieves (gels, resins),
- ion exchange,
- distillation, and
- chromatography (column, paper, thin-layer, and gas).

Of these, the first two listed are most often used. Chromatography is used more in isolation procedures following initial "clean-up" (Sunshine

1975, p. 392).

Even after separation, an **isolation step** is often necessary to gather together one drug by itself for identification. Chromatographic techniques widely used for this purpose include those mentioned above as well as gel permeation and high-pressure liquid chromatography (HPLC). Because drugs differ in their physical and chemical properties, no one isolation technique will recover all drugs for further analysis. Screening systems comprising several such techniques increase the generality of drug analysis. Use of several isolation procedures for a single specimen is often an advantage, since separate methods are subsequently used to identify different drugs and classes of drugs.

Chemical or electronic detection of the isolated drug follows its isolation from solution. In most analytical procedures, detection and identification of drugs depend wholly on isolation techniques. For example, in gas chromatography, "on-line" detectors measure the presence of drugs separated and moved along a column by a flow of gas. The time a drug takes to move through the column is relatively constant, enabling its identification. Detectors vary in their complexity, analytical characteristics, and cost. Detectors for gas chromatography, for instance, range from simple flame ionization to mass spectrometers, which differ in cost and ability to identify drugs by many orders of magnitude.

Quantitative measurement of the amount of drug originally present in a blood specimen depends on several factors:

- the amount of blood extracted;
- the percentage of drug removed from the blood by extraction (separation);
- the percentage of drug obtained for analysis (isolation); and
- the amount of drug introduced into an instrument for quantitation, once it has been identified.

To simplify calculation of these factors, known quantities of other chemicals are added to blood specimens before the separation step. These chemicals, called internal standards, behave similarly throughout the

analysis and the amounts of internal standards determined at the last step provide an estimate of the concentration of a drug originally in blood.

An important consideration is that, in almost all cases, the analyst **does not know** which, if any, drug(s) are present in a body fluid specimen. Systematic analyses, called drug screens, are required. The analyst can find only those drugs his instruments can detect and identify, at concentrations within the limits of sensitivity of his methods. Because drugs number in the thousands, he will analyze specimens for those drugs of interest whose presence can reasonably be expected. Other drugs will go unnoticed. Costs of extensive drug screening and requirements for special methods to detect certain drugs or groups of drugs limit the range of drugs for which analyses are performed.

Table A-1 lists and defines characteristics of analytical methods. Those terms are often used in comparing different instruments, techniques, and methods for drug analysis. For almost all drugs, more than one kind of method can be applied to its analysis in body fluids. Which method is "best" depends on what information is required of an analysis. As Joscelyn et al. (1979) pointed out, requirements for drug analyses in highway safety research are very stringent, demanding that drugs **not present** be identified along with drugs present in a specimen. For example, epidemiologic research determines the percentage of drivers in a population who use certain drugs; this information can only be obtained if **both** the number of drivers using drugs **and** the number of drivers **not** using drugs are determined. Drug countermeasures based on analyses of body fluids have equally strict requirements, since methods used to provide evidence must meet forensic standards.

General techniques used in analyzing body fluids for drugs include the following:

- thin-layer chromatography (TLC),
- gas chromatography (GC),
- gas chromatography-mass spectrometry (GC-MS),
- immunoassay, and
- high-performance liquid chromatography (HPLC).

Certain techniques may be more appropriate for some drugs than others;

TABLE A-1

CHARACTERISTICS OF A METHOD TO DETECT AND MEASURE DRUGS IN BODY FLUIDS

CHARACTERISTICS	DEFINITION
Specificity	The capability of a method or technique to distinguish between individual drugs or classes of drugs.
Sensitivity	The ability of a method to detect the presence of drugs or classes of drugs.
Speed	The time from start to end of the analytical process using a method.
Simplicity	Usually related to the speed of a method, the requirement for little training for technicians and often associated with highly automated procedures.
Reliability	The dependability of a method. Its ability to reproduce accurate and precise results day to day.
Accuracy	The degree to which a method produces results consistent with actual values.
Precision	The consistency with which a method reproduces results when measuring the same sample.
Economy/Cost	Economic considerations include time of analysis, number of samples processed in a single run, degree of training required of personnel, price of obtaining (and maintaining) instrumentation, price of chemicals and other reagents used in analytical procedure, and overhead of analytical laboratory or other facility.
Safety	The degree to which personnel using a procedure are exposed to risk of injury or long-term toxicity associated with chemicals required by a method.

methods based on the same technique differ, even for the same drug, depending on purposes for which each method was developed. For some drugs there may be a "method of choice," but usually the selection of a particular method depends on the availability of required instrumentation, funding, and the preference of analysts themselves (Sunshine 1975; Maickel 1977; Marks and Fry 1977).

Thin-layer chromatography (TLC), one of the oldest techniques in common use, is rapid, inexpensive, highly specific, sensitive enough for most drugs, and easily adapted to many analytic needs. Most TLC procedures are simple, requiring a minimum of expertise. Its characteristics are applied to best advantage in the preliminary identification of drugs; it is less suitable for measuring the amount of drug in a specimen. Additional techniques are required to confirm and to quantitate results of TLC analysis (Maickel 1977; Marks and Fry 1977; Joscelyn et al. 1979).

Gas chromatography (GC) combines isolation, qualitative identification, and (in some procedures) quantitative measurement. In many laboraties that can afford the initial costs of purchasing the necessary instruments, this technique has largely displaced TLC. The advantages of GC include the variety of available detectors, both "universal" and selective, most of which are highly sensitive. Like TLC, GC methods can detect a wide range of drugs. Unlike TLC, however, only one sample can be analyzed at a time, but quantitative results can be obtained directly. Confirmation of findings for positive identification and accurate quantitation is still required (Maickel 1977; Joscelyn et al. 1979).

The marriage of GC with mass spectrometry (MS), a technique that records a drug's "fingerprint," combines efficient separation of drugs with positive identification of each drug present. GC-MS techniques with computer-operated systems have been increasingly applied to drug analysis in research and forensic laboratory settings (Klein, Kruegel, and Sobol 1979). The power and versatility of this technique are great, but its availability is not. The cost of purchasing, maintaining, and operating GC-MS equipment is beyond the reach of most toxicology laboratories (Maickel 1977).

Immunoassay techniques are relatively new to the area of drug analysis (Butler 1977). Immunoasays are extremely sensitive, highly selective, and rapid procedures; large numbers of samples can be processed simultaneously. There are specific drawbacks to some immunoassay techniques, for example, reagent costs, the need for skilled technicians, facilities for handling radioactive materials (radioimmunoassay [RIA]). On the other hand, separation and isolation steps in the analytical process are avoided, and these techniques serve well when a low percentage of positive findings is expected (Sunshine 1979).

High-performance liquid chromatography (HPLC) is another technique recently and rapidly developed for drug analyses and other applications (Wheals and Williams 1979). Operating at or near room temperature, HPLC instruments can isolate and detect thermally unstable and nonvolatile compounds; these characteristics are complementary to gas chromatography (Parris 1976). Limited primarily by detector systems, HPLC techniques have found special applications but will probably remain in a secondary role in drug analysis, both screening and quantitative measurement, for some time to come (Jane 1975; Bye and Brown 1977).

Once the presence of one or more drugs has been determined and their concentrations measured, the analytic findings must be interpreted. This final and crucial step follows the analysis of body fluids for drugs and depends on the accuracy and precision of the methods used. But interpretation of blood drug concentrations (BDCs) also depends on prior knowledge of what the analytic results mean in terms of driver impairment. This issue--interpretation of analytical results—is basic to any discussion of drug analysis in highway safety research and action programs.

Significant precedents were set when blood alcohol concentration (BAC) as determined by chemical tests was made legally admissable as evidence of driver impairment. Some states even have "per se" laws, making it illegal to drive with a BAC exceeding a statutory limit, for example, 0.10% w/v. Extensive research correlating the behavioral effects of alcohol and BAC supported this approach.

Similar research for other drugs is rarely done. Considerable work in

the separate areas of pharmacokinetics and behavioral effects has been reported, but very few efforts to define the relationship between impairment of driving-related skills and BDCs for any drug other than alcohol have been made (Joscelyn et al. 1979). As a consequence, the ability to detect and measure drugs in body fluids far exceeds the ability to interpret analytic findings in traffic-related cases. Cases in which multiple drugs are detected and measured, an increasingly frequent occurrence, often present even greater problems for interpretation.

The interpretation of analytical results, although extremely important, was not the primary focus of Workshop II and was therefore not discussed in great depth.

APPENDIX B

LIST OF WORKSHOP PARTICIPANTS

APPENDIX B
DRUG RESEARCH METHODOLOGY

THE DETECTION AND QUANTITATION OF DRUGS OF INTEREST IN BODY FLUIDS OF DRIVERS

LIST OF WORKSHOP PARTICIPANTS

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