A STUDY OF SOLVENT INHALATION ABUSE AND THE DEVELOPMENT OF ANALYTICAL TECHNIQUES TO MONITOR THE PROBLEM

by

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"Dinner at Dr. Simpson´s, where Mrs Crowe and another poet drank ether. I had the feeling of being among two insane who laughed with open eyes; there was something frightening in this; I found it outstanding for operation, but not to be used to tempt God."

Hans Christian Andersen, 1847

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CHAPTER ONE

INTRODUCTION

1.0 Introduction.

The object and aim of this thesis was to evaluate and develop analytical techniques to aid in the study of the problem of volatile substance abuse.

To assist with this objective, a detailed study of the problem was required from the available literature.

This Chapter presents the outcome of this study and a review of the characteristics of solvent inhalation abuse. Also it outlines the requirement to monitor the problem.

1.1 A Definition of Volatile Substance Abuse.

Reference to the literature reveals a failure to agree on the best descriptive name for the problem. Throughout this thesis it will be referred to as solvent inhalation abuse, though the terms; "glue sniffing", "aerosol sniffing", "solvent abuse", "solvent sniffing", have all been used to describe the problem.

An acceptable definition of solvent inhalation abuse could be: the voluntary inhalation of the fumes or vapours from volatile organic compounds for the purpose of producing a state of intoxication.

This definition covers many different compounds, ranging from the gases through the volatile solvents to the less volatile alcohols, acetates and oils. Most of these are commonly available to the potential abuser and are obtainable in one form or another often without restriction.

These compounds are abused for their psychedelic properties, or simply as a route to intoxication. The usual mode of use for these volatile drugs is by inhalation. This route can result in quite remarkable psychic and somatic disturbances. The inexperienced may be drunk and sober again before having any idea of being drunk (Nagle 1968). This rather suprising pastime involving the common deliberate use of volatile solvents for purpose of intoxication appears to be a

phenomenon of the past quarter century. The practise illustrates the willingness of some individuals to accept the dangers of exposure to chemicals of unknown or incompletely known toxicity in order to achieve periodic inebriation.

Many of the organic solvents may be considered as being capable of producing some degree of intoxication. The immediate effect appearing to be of pleasant exhilaration, euphoria and excitement, similar to the effect of alcohol. In general the greater the fat solubility the more potent are the narcotic properties of the compound. Alcohols are therefore relatively weak intoxicants and the hydrocarbons, particularly the chlorinated hydrocarbons, are relatively strong. This fact forms the basis of the Meyer-Overton theory of narcosis:- narcotics act on the lipids in the membrane of the nerve cell, and alter its permeability and electrical polarisation (Elkins 1959).

Most industrial solvents are rarely pure chemicals and, at times, the impurities that they contain may be more dangerous to health than the primary component. The possibility therefore exists of ill effects from these impurities as a result of the higher concentrations encountered during solvent inhalation abuse.

Accurate figures on the prevalence and incidence of solvent inhalation abuse are not available. This may be due to the fact that most of those who have this habit are not brought to the attention of the authorities. Often even parents may be

unaware when the practise concerns an adolescent. Most of the recorded reports on incidence come from the police, juvenile authorities or from studies by interested researchers. Such reports are generally in association with some type of disorderly conduct, illness, or atypical behavioural pattern. They are therefore inherently subject to bias from the methods of survey and the data presented may be affected to a considerable extent by the fact that the majority of abusers come to the attention of the reporter only as a result of anti-social acts, performed while under the influence of the solvent.

The sense of smell is appreciated by everyone and many become ecstatic over certain scents, all of which are a combination of volatile compounds. However, solvent inhalation abuse is far removed from the gentle sniffing of air perfumed by relatively low concentrations of these compounds. Inhalation is the more correct term as it implies the deep and sustained breathing of high concentrations of solvent. At adolescence, or often younger, the first experiences of the effects of volatile substances may be discovered by accident, or out of curiosity, or daring. Many children may try sniffing as an experiment, or because it is the current fashion among members of a peer group, but quickly discard the practice (Glaser 1962). It is also assummed that solvent inhalation abuse appears to be principally an activity of children while adults generally resort to other means of achieving the same result. Notable exceptions have been reported in those adults unable to exercise a free choice, such as in prison (Samples 1968).

However, again the sampling bias may distort the real truth. Little if any notice is taken of an adult acting drunk, even in the street, but a child, particularly a young child, acting in a like manner soon becomes the centre of attention.

In most of the previously reported studies there has been a preponderance of boys over girls (Ellison 1965). Indeed it has been suggested that indulgence by girls appears to be restricted to social sniffing, such as with a group of their boyfriends, and that real habituation among girls is rare (Done & Press 1967). Once again the method of survey and referral must be considered as having a possible influence on the results. Of greatest concern is the fact that the solvent inhalation abuser, like the alcoholic or the drug addict, but often at an earlier age, has adopted a method of avoiding the realities of life rather than adapting to them.

The habit presumably starts with the abuse of a relatively small number of products such as household adhesives and plastic glue preparations. As the habit continues experiments might be made with almost any volatile substance to which the abuser has contact, perhaps in realisation that virtually any fat-soluble, volatile organic solvent is capable of producing the desired effect. The popularity of solvent inhalation abuse must therefore continue, owing to the ease of concealment and the ability of the abuser to obtain the material for what would otherwise be presumed as legitimate purposes.

The choice of solvent inhalation abuse over, or in combination with, the ingestion of alcoholic beverages is at first difficult to understand. This behaviour appears even more incomprehensible when, by the standards of many people, the ingestion of alcohol in wines, beers and spirits has become an acceptable form of social conduct. The following reasons may be suggested for this preference:

Availability:- Volatile substances are the most readily available source of intoxication. Supplies of volatile organic compounds in the form of aerosols, paint, lubricants, thinners, adhesives, etc., can be found in most homes, and in the majority of shops and supermarkets. Even in poor households where a stockpile of alcohol, in the form of spirits or wine, may not exist, several solvents in one form or another may be found.

Efficacy:- In general the more volatile the substance being abused the shorter the duration of intoxication. This is due to the fact that most of the solvents abused are excreted unchanged in the breath.

Economics:- The products in which the volatiles may be purchased are usually inexpensive by comparison to alcoholic beverages. Commonly sold over the counter in their many different guises, the volatile substances are easier to obtain and often their sale is not restricted to adults. Alternatively, the same products may be easy to steal and petty theft, particularly shop-lifting, will provide an easy

temptation to the abuser in need of fresh supplies. The incidence of this method of obtaining supplies may only increase with legislative measures to control their sale.

Portability:- The packaging is often compact. A tube of glue or jar of adhesive, lighter fluid refills, spot remover or aerosol can all fit into a pocket and yet they are probably each as capable of intoxicating as a bottle of whisky.

The Crime:- Laws against the abuse and sale of the many products which contain potentially abuseable solvents are by their own implication difficult and to some extent impracticable to enforce. They may include the provision to take action against the sale of "glue sniffing kits", the definition of which must remain difficult in legal terms. Often the only crime committed is the social unacceptability of the abuse, which in the case of an adult maybe of no greater consequence than the abuse of alcohol. The real concern is for the social disordering afforded to the adolescent and young child by participating in this habit.

Consequence:- Headaches seem to be the most common post-intoxication complaint. Indeed the long term physical effects of abusing some solvents in this way may be said to be less harmful than the effects produced by a similar abuse of alcohol. In fact, among the myriad of organic solvents which are capable of producing the intoxication of the type desired by the volatile substance abuser, toluene has been suggested as the safest (Done 1967). This fact should be an especially

important one to take into account when any efforts, particularly at producing legislation, are being made to control the problem.

It is clear that the inhalation of solvents represents a further addition to the adverse factors on the social fabric and rules of society. The possibility also exists of endangering traffic safety, particularly where solvent inhalation abuse is being used in an attempt to circumvent the drink-driving laws. Equally it is clear that when evidence of the described drugs is required in a Court of Law, reliable and rapid analytical methods should be available. These methods could also be used preventively, in the complex assessment of the cause of traffic accidents, or in the assistance in diagnosis of cause of death where the abuse of solvents may be a contributory factor, or simply as an aid in the clinical evaluation of the problem.

1.2 The History of Solvent Inhalation Abuse.

The inhalation of volatile substances and natural vapours as a means of self intoxication is a practice which is as old as history itself. There are reports of the inhalation of a variety of vapours among such groups as the Hebrews, South American Indians and ancient Greeks (Smith 1976). In fact, although their record in history is not obviously evident, the effects of inhalation of toxic fumes and gases, such as carbon dioxide, found in caves and wells, and carbon monoxide from smoke and fire, were undoubtedly already known in prehistoric times. Perhaps the best documented and most frequently reported examples are the priestesses of Apollo, the Pythia in Delphi and the Sybilla in Cumae who reportedly inhaled mephitic and intoxicating vapours to achieve their state of enlightenment (Dietze & Voegele 1963).

No history of solvent inhalation abuse would be complete without consideration of anaesthesia. Discounting the abuse of alcohol which dates back into prehistory man has taken every opportunity to produce an altered state of mind. As the abuse of drugs such as cannabis, opium and heroin followed their discovery and availability to man so it may be said that the abuse of the volatile solvents follows their history.

Early in the eighteenth century Friederich Hoffmann (1660 -1742) introduced his famous Anodyne, produced by the distillation of alcohol and sulphuric acid. Anodyne, and the

similar Ether Drops, which were made by the mixture of one third diethyl ether and two-thirds alcohol, were prescribed for the treatment of many ills including gall stones, intestinal cramps, toothache, earache and dysmenorrhea. The recommended dose was three to twelve drops on a lump of sugar, swallowed with water (Lewis 1761).

The first recorded addict of diethyl ether was a famous London quack by the name of James Graham (1745-1794), proprietor of The Temple of Hymen and The Celestial Bed. He was observed to inhale an ounce or two of ether several times a day with manifest placidity and self-enjoyment (Lee 1847). The abuse of diethyl ether in this way was a popular diversion in the first half of the nineteenth century and, despite an occasional fatality, this abuse provided an opportunity for clinical testing without which its beneficial use in surgery may not have been established.

The practice was by no means limited to the United Kingdom. In 1832, Thomas Mitchell wrote from America that ether inhalation was a popular pastime among the youth of Philadelphia. His paper details an ingenious device for controlling the dose size. A small quantity of ether was placed in a bladder where it was vapourised by the application of hot water then these vapours were inhaled by means of a tube and stopcock. On the effects he reported that in some instances the experiment excited mere playfulness and sprightly movements, but in several cases delirium was induced which sometimes terminated fatally (Mitchell 1832).

The drinking of ether, on some occasions, has almost totally replaced the drinking of alcohol. Perhaps the earliest known, longest continued, and best documented episode happened in Northern Ireland during the nineteenth century (Connell 1965). The habit was first observed in Draperstown during the first half of the nineteenth century. After the introduction of cheap methylated ether in 1856 and the subsequent greater activity displayed in Ireland in supressing the illicit distillation of poteen the practice spread beyond Draperstown (Kerr 1890).

About 1840, a great temperance crusade was led by one Father Matthew throughout England, Scotland and Ireland. It was one of the most successful that had ever occurred, and thousands took the pledge. In Draperstown there lived an alcoholic medical practitioner named Kelly who was carried away by the priest's canting. Aghast at the pleasure that he had given up, but not wishing to break his pledge, he searched about for a substitute. He had prescribed diethyl ether by mouth on occasion and knew of its effects. After a few personal experiments he imparted the knowledge to his friends and patients who had also taken the pledge (Kerr 1890). Meanwhile the crusade had died down and matters might have ended with this small circle, except for the matter of taxes.

The British Government in 1855 placed an onerous tax upon ethanol and the beverages in which it was contained. Following this the efficient constabulary put an end to the home

distillation of poteen. However, a combination of British ingeniousness and the Irish thirst found a loop-hole in the law. The tax did not include methylated alcohol. By careful distillation of methylated alcohol with sulphuric acid, diethyl ether could be produced. The London distillers did not exactly attempt to produce a pure product and as much as twenty per cent alcohol could be found in some samples. This methylated ether was then shipped by the ton to Northern Ireland where it made a welcome substitute and even was in some ways preferred, and especially among the poor, to the now prohibitively expensive whiskey.

The quantities of ether consumed were by no means small and on one occasion a single trader was known to have bought as much as fifty gallons. The rector of Cookstown testified that in addition to the large quantity transported secretly by rail or by private conveyance, more than two tons of ether were openly carried every year on the railway into the surrounding district (Kerr 1890). As ether, an explosive, was dangerous to transport it had to be shipped on deck. This resulted in an extra rate being levied, and gave opportunity for the establishment of a thriving smuggling industry around ether distribution and transport. The supply of ether remained regular and was available at most public houses and groceries.

In 1875, a young philosophy student engrossed in theological mysticism tried the inhalation of ether in the hope of freeing himself from his material surroundings. He claimed to have

seen magnificent visions during these intoxications but, not satisfied, he found himself increasing the strength and frequency of the dose, and he soon became addicted. He was observed to inhale over four ounces of ether in ninety minutes without loss of conciousness (Ewald 1875).

In 1878, a charming essay was written on travels through Ireland in which it was noted that the main street of Draperstown smelt like a surgery. It was in this essay that the correct method of drinking was first described. "First rinse the mouth out with cold water, then down about a tablespoonful of ether, followed quickly with a glass of cold water" (Richardson 1878). Though Richarson strongly disapproved of the habit, he agreed with the majority of medical opinion, and admitted that there appeared to be less chronic damage than with alcohol. The principal hazards of the habit seemed to be chronic gastritis, death from overdosage and burns from smoking while drinking.

By 1890, owing to the frequency of frenzied outbursts under the influence of ether, pressure from the temperance societies, an article by the editor of the British Medical Journal (Hart 1890), and loss of tax revenue, a Parliamentary Committee was established to investigate ways of controlling the problem (British Parliamentary Papers 1890-1891). Suggestions were made on measures to control the problem. One such suggestion was the addition, after the conversion of the methylated spirit into ether, of the nauseant wood naptha which would act as a deterrent to even moderate drinking.

Another plan was for the abolition of the retail trade, the ether for drinking being usually sold in small quantities. Also it was suggested that the sale of ether might be confined to druggists, who would be legally compelled to take out a licence authorising the sale in a definite substantial quantity. Subsequently, regulations limiting the sale of ether were imposed. Nevertheless, by 1910 it was reported that ether abuse was still prevalent in the area (Caldwell 1910), but by 1920 the practise seems to have died out and was replaced by alcoholic beverages that were again cheaper and more easily available.

Subsequent reports are rare and usually limited to the medical literature although during the Second World War the Germans were known to have substituted ether when alcohol was scarce (Blatherwick 1972). In 1962, it was reported that of two patients who used ether to get drunk the one who inhaled ether appeared to be addicted yet the ether drinker did not (Bartholomew 1962). Also it has been reported that a couple who were addicted to ether inhalation abuse at the rate of a pint per day, each consummed a pint of methylated ether while already intoxicated from alcohol. The next morning the man found his wife dead (British Medical Journal 1963).

The invention of chloroform brought with it a new faster acting and more potent solvent for the abuser and possibly for this reason it acquired its first addicts more easily than ether had done before it. The first and probably most significant was Horace Wells who, while experimenting with

chloroform, began to use it to get drunk. One night, disorientated from its effects, he ran into the street and squirted acid on passing women. This violent behaviour led to his arrest and subsequent suicide (Nagle 1968).

At the same time, February 1848, the death of a druggist's apprentice was reported. Apparently he was in the habit of inhaling chloroform almost daily for the sake of the excitement and the pleasurable feelings he experienced from its use. His death followed after he poured some chloroform onto a towel, inhaled it and fell face forward into the towel. Resuscitative measures were not successful and he became arguably the first person to die from chloroform inhalation abuse. The press of the time, with much of the same venom as the news media of today, commented: "It is not denied that an overdose of chloroform will cause death ..... The present fatal result should act as a warning against the unassisted and unprofessional use of it. It is a gross abuse of one of the greatest curative agents ever invented, to employ it as a medium of temporary excitement or intoxication. There is good reason to fear, however, that such a use of chloroform does prevail to some extent, and it is to be hoped that the present case will have the good effect of checking such a pernicious and dangerous practice" (Medical News 1848). However, this warning went unheeded, in the same way as have similar warnings in our own time. The use of chloroform, both medicinally and for pleasure, became widespread.

The physician of the mid-nineteenth century had few potent drugs and chloroform became highly valued for its sedative and analgesic properties. It was frequently used as a remedy for sleeplessness and as a therapeutic agent for the prompt and effectual suppression of pain, being recommended for convulsions, tetanus, and angina pectoris. In fact Simpson, considered a founder of modern gynaecology and the obstetrician who introduced the use of ether and chloroform in childbirth, treated his own angina with it (Simpson 1870).

This widespread use of the drug meant that many more potential addicts came into contact with it and, although chloroform never achieved the spectacular success of ether in replacing alcohol in whole areas, individual case reports have been published in most countries. Indeed the total number of such reports is greater than with ether since a dead person is more likely to be written about than a chronic drunk. Although the use of chloroform for inhalation abuse is now considered rare, it cannot be assumed to be totally extinct.

It appears that if ether or chloroform had lacked their ability to produce drunkeness there would have been no extensive clinical trials and perhaps no discovery of their anaesthetic powers and relative safety. Unfortunately, even after the discovery of their usefulness in anaesthesia, they continued to be used as an intoxicant (Nagle 1968).

As progress was made in science and in the discovery of new volatile compounds so the number of solvents available for

potential inhalation abuse increased and as these substances became more obtainable there developed an explosion of the occurrences of their abuse.

The synthesis of trichloroethylene made available yet another chemical agent which found ready application in both industry and medicine. Trichloroethylene has excellent solvent properties and rapid penetration which enables it to be used as a degreasing fluid, commercial solvent and dry-cleaning agent. It was first used in medicine nearly 70 years ago (Baader 1927). Due mainly to its analgesic and narcotic properties, it was widely prescribed for angina pectoris, as an analgesic and a general anaesthetic for operations of short duration (Kleinfeld & Tabershaw 1973). However, it appears that it was mostly industrial workers who stumbled on its narcotic action. Very few reports of trichloroethylene abuse by members of the medical profession occur in comparison to those of ether and chloroform (Alapin 1973).

The first extensive study on trichloroethylene abuse was that carried out by Steuber (1931), who collated two hundred and eighty four cases of trichloroethylene poisoning, including twenty-six fatalities. She reported that the toxic effects involved primarily the central nervous system, although gastrointestinal and circulatory effects were also observed.

It has been suggested that most of the deaths attributed to trichloroethylene in the European literature were due to the

formation of phosgene, which with hydrochloric acid, occur as decomposition products when trichloroethylene comes into contact with heat (Flinn 1946). The production of phosgene was also proposed by Von Oettingen (1937) who described a further seven fatal and twenty-two non-fatal cases. He also implied that some of the earlier deaths attributed to trichloroethylene were due to impurities formed during its manufacture as pure trichloroethylene did not become available until 1936.

The abuse of volatile substances spread to the abuse of petrol vapour during the 1950's and was first reported in 1951 (Clinger & Johnson 1951). This report described two adolescent boys who were habituated to the effects produced from the abuse of petrol. The first boy, a sixteen year old, had been inhaling petrol for periods of four to eight hours for as long as ten years. He was reported as having experienced visual and auditory hallucinations as well as erotic sexual fantasies during this period. In time sexual gratification became the object of his inhalation. The other boy, a thirteen year old, found that petrol inhalation abuse gave him a sense of power and physical strength. Both subjects were reported to have given up the habit after prolonged psychiatric treatment.

On isolated Australian Aboriginal settlements alcohol and other drugs are not readily available and petrol is comparitively plentiful. Its use as an intoxicant therefore has paralleled the increased number of cars, engines and

tractors. Though unrecorded in the Australian literature except for single case reports, petrol inhalation abuse is thought to be a common practice (Bianchi, Cawte, Money & Nurcombe 1970).

Another report recorded that a nineteen year old Marine, who had a seven year history of petrol inhalation abuse, used carbon tetrachloride as a substitute when he was unable to find a source of petrol. He inhaled for one or two hours from a can containing 60% carbon tetrachloride while he was intoxicated by alcohol. An acute illness ensued, commencing with pancreatitis, proteinuria, and hematuria (Chipman & Durden 1967).

Amyl nitrite, used by ophthalmists as a muscle relaxant and vasodilator was reported to be used by teenagers and young adults for inhalation abuse specifically as one of its effects was claimed to be an enhancement and extension of duration of the sexual experience (Louria 1970).

The beginning of the modern epidemic of solvent inhalation abuse was first recognised in the 1940's, and one of the earliest reports in the medical literature was published soon afterwards (Clinger & Johnson 1951). It is often suggested that the habit started in the United States of America and spread as publicity became more widespread. This is a fair comment but in reality it is not the case. Although the practice of abusing glues for their volatile compounds was probably first recognised in the United States, solvent

inhalation abuse is more likely an inevitable consequence of solvent availability, though the advertising effect of publicity can never be ignored.

The practice did not come to the attention of the public until 1959 and engendered sufficient concern to be mentioned at the White House Conference on Narcotic and Drug Abuse as a "hazardous teenage fad necessitating a community education approach" (Freer 1963). The authorities and police soon became concerned with the problems of law-breaking, particularly by children, who were participating in solvent inhalation abuse. Arrests in the city of Denver of children intoxicated by solvents increased from 30 in 1960 to 134 in 1961, and to 184 in 1964, a six fold increase in four years (Corliss 1969). Explanation for this dramatic increase could be the increased awareness and vigilance of the police to the problem. In New York in 1963 a total of over two thousand cases were reported (Allen 1966).

It must be remembered that the number of cases which receive attention are in no way a reliable indicator of the extent of the practice since only those caught are counted. Neither is solvent inhalation abuse confined to youngsters, or even just those who get into trouble with the law, since many other children who worry parents, and patients who confuse doctors by suddenly complaining of weakness, headaches, and dizziness could perhaps be suffering from volatile substance abuse hangovers.

In 1964, solvent inhalation abuse was first reported in Canada when a pharmacist from Winnipeg noted an increase in the number of sales of nail polish remover (Gellman 1968).

In the same year, it was reported that the inmates of the state prison on McNeil Island did not appear to be habitual solvent inhalation abusers but just used the practice in order to defy authority and to create status for themselves among the prison population. It was also noted that inmates would try the practice on several occasions and then lose interest but as they quit others would pick it up, the younger inmates adopting the habit more readily. The detectable offence rate was about five to eight per month (Samples 1968).

In Finland solvent inhalation abuse had been known since the early fifties, and between 1968 and 1971 twelve deaths were attributable to the practice. During the first half of 1971 three hundred cases were recorded by officials, and it was also noted that ten drink-driving offences in that year involved solvent inhalation abuse (Alha, Korte & Tenhu 1973).

Attention in Lanarkshire, Scotland, was rekindled by the appearance of several cases during the early seventies. Most of these were children brought to the attention of the police after theft of industrial solvents or their appearance in a state of semi-conciousness at school breaks. At the same time a marked increase in sales of cleaning materials and adhesives to young people was noticed by shop keepers in the area (Watson 1976).

Legislation prohibiting the sale of certain solvent based products to persons under a proscribed age or the open display of the same products for self-service may result in some temporary success in controlling the problem, but as with all prohibitions concerned with forms of drug abuse, a criminal sub culture is encouraged to develop with consequences possibly more dangerous than the habit itself (Silberberg & Silberberg 1974).

History has shown us that solvent inhalation abuse is not a recent development in man´s quest for intoxication, however its popularity as a means of escape from reality has increased with the availability and with the number of solvents discovered. Although the practice has received widest publicity and investigation in the United States it is by no means confined to that area of the world. In fact it may be perfectly reasonable to assume that solvent inhalation abuse is as prevalent as alcohol abuse though, due mainly to the social acceptability of alcohol, it has not nearly so many devotees. Therefore the number of observed cases cannot reflect the magnitude of the problem and often the method of sampling is so unavoidably biased, so that an unrealistic view of the severity of the problem is reported.

1.3 Volatile Substances - The Choice Available.

There is a great choice of volatile solvents available to the abuser. They consist of a group of organic chemicals which are used in a wide variety of industrial and household products. These compounds may be available in a pure form but, more commonly, are present in their semi-pure forms, and are often mixed with other chemicals to obtain the required properties for their intended application. A list of the more common solvents and some of their uses are listed in Table 1.3.1.

Very little is known about the pharmacology of these compounds but it must be assumed that their lipid solubility is a direct cause of their central nervous system depressive effect as a direct result of impairing neural transmission due to changes in membrane permeability. Their actions will therefore tend to be potentiated by other central nervous system depressants, particularly alcohol, which may be consumed with them for this very purpose. Occurences of death observed are usually due to respiratory arrest or ventricular arrhythmia though it is probable that chronic use would demonstrate liver necrosis in much the same way as chronic alcohol poisoning.

Solvent	Commercial Use
Methanol	denatured alcohol,dyes,anti-freeze,paint removers,glues.
Ethanol	laquers,paints,antifreeze.
Propanols	cosmetics,antiseptics,lotions.
Butanols	dewaxers,polishes,perfumes, adhesives,dyes,paint removers.
Pentanols	adhesives,laquers,dyes.
Hexanols	inks,brake fluids,lubricants.
Heptanols	dry cleaning soaps,degreasers.
Cyclohexanol	polishes,laquers,inks.
Benzyl alcohol	cosmetics,inks,laquers.
2-Propen-1-ol	resin glues,denaturant.
2-Methyl-2-pentanol-4-one	metal cleaners.
2-Chloroethyl alcohol	resin and cellulose glues, dyes,cleaning agents.
Acetone	rubber and plastic adhesives, varnishes,dyes,paints.
Methylethylketone	cosmetics,paints,adhesives.
Pentanone	laquers.
Hexanone	laquers.
Heptanones	laquers,adhesives.
2,6-Dimethylheptan-4-one	rubber adhesives.
Methylisobutenylketone	synthetic rubber,glues,inks, plastic glues,dyes.
Cyclohexanone	paints,laquers,degreasers, paint removers.
Phenylmethylketone	perfumes,cellulose solvent.
Trimethylcyclohexene	resin and cellulose adhesive.
Toluene	adhesives,resins,varnishes, paint remover,rubber solvent.
Xylene	epoxy resins,paints,adhesives, inks,degreasers.
Isopropylbenzene	cellulose thinner,paints.
Styrene	glues,resins.
Isopropyltoluene	paints,thinners.
Methyltertbutylbenzene	resins,glues.
Trimethylbenzene	paint thinners.
Tetralin	polishes,turps substitute, asphalt,thinners,degreasers, paint removers.
Cyclohexane	rubber solvent,adhesives, perfumes.
Methylcyclohexane	adhesives.
Decalin	thinners,resins,glues.
Butane	lighter fluid.
Propane	camping gas.

Table 1.3.1a - List of common commercial solvents and their uses.

Solvent	Commercial Use
Carbon Tetrachloride	drycleaner,degreaser.
Trichloroethylene	polishes,adhesives,paints, glues,degreaser,dry cleaner.
Tetrachloroethylene	dry cleaner,inks,resin glues.
Tetrachloroethane	spotting agent,glues.
Methyl Chloride	refrigerant.
Dichloromethane	paint removers,refrigerant, cleaning fluid,degreaser.
Dichloroethanes	plastic adhesive,cleaning agent,rubber cement,degreaser.
Trichlorethanes	resins,degreaser,spot remover.
Pentachloroethane	dry cleaning.
Chloroform	antiseptic,dry cleaner.
Nitroethane	cellulose thinners,adhesives.
Nitropropane	paints,varnishes,laquers, resins,adhesives.
Chloronitropropane	rubber cement.
Pyridine	denaturant,paints.
Paraldehyde	varnishes,dyes,lubricants.
Furfural	laquers,resins.
Acetal	paints,varnishes.
Diethyl ether	general solvent,polishes.
Isopropylether	paints,varnishes.
Butyl ether	general solvent.
Dichloroethyl ether	spotting agent,paints,resins, laquers.
Dichloroisopropyl ether	cleaning agent,paint remover.
Ethyl acetate	laquers,adhesives,cements, paint removers.
Butyl acetate	stain removers,polishes.
Amyl acetates	laquers,varnishes,polishes, perfumes.
Benzyl acetate	laquers,perfumes.
Vinyl actetate	adhesives,resins.
Methyl formate	refrigerant.
Ethyl lactate	laquers.
Butyl lactate	paints,varnishes.
Ethylene glycol	dyes,antifreeze,skin lotions.
Ethylene glycol alkylether acetates	adhesives,resins,laquers,inks, glues,cements.
Diethylene glycol	antifreeze,plastic adhesives, resins,face creams,laquers.
Tetrahydrofuran	resins,laquers.
Dichloroethylene	dry cleaner.
Freons	aerosol propellants,cleaners, refrigerants.

Table 1.3.1b - List of common commercial solvents and their uses.

1.4 Volatile Substance Abuse - The Method.

1.4.1 Methods of Volatile Substance Abuse.

The problem of solvent inhalation abuse is often referred to as "sniffing". This is an over simplification of what has been developed into an art form by those who are addicted to the practice.

The most popular products are adhesives, glues and cleaning fluids, although the choice of product may to some extent depend on the substance being used by the abuser's associates.

The most common method for abusing these products appears to involve the placing of the product in a plastic bag, such as an empty crisp packet, and then bringing the bag into close proximity to the nose and mouth. Other methods which have been employed involving soaking a piece of cloth with the product and then covering the face with the cloth or placing it in the mouth and sucking it. Inhalation direct from the product container is also known. Aerosols may be inhaled directly from the spray or placed into a balloon or bag first. Cases have also been noted of swallowing balls of cotton soaked in glue (Vega 1967). The solvents may even be drunk, although most are somewhat irritating to the upper gastrointestinal tract and may produce vomiting and so this method is rarely used.

In order to increase the concentration and effect, plastic bags are sometimes placed over the head or even entire body, with the obvious consequence of asphyxiation. This additional risk in using a plastic bag has caused the more cautious abuser to use paper bags, crumpled before use to minimise noise and chance of detection (Laury & Preble 1967). Other methods of increasing the effect have been developed; heating the material in a can or by adding hot water to increase the vapour concentration (Glaser & Massengale 1962), dispersing the solvent over a wider area (Quintanilla 1961), the use of atomisers (Christiansson & Karlsson 1957), filling a bathtub with thinner (Lindstrom 1970), and even by attempting intravenous injections (Ferguson 1975).

The dose is measured by a subjective assessment of their effects and the quantity required to produce this effect may be increased over a period of time as an apparent tolerance is acquired. Inhalation usually takes place through the mouth and this technique may be referred to as "huffing", a description of the short but deep breathing which is exhibited (Adams & Barker 1963).

The practice may be as infrequent as a single experimental exposure, or as often as multiple use daily for periods of several years. The young volatile substance abuser may first experience the effects of solvent vapours by accident or out of curiosity or daring. It is probable that most children try these intoxicants by a trial inhalation of the currently popular solvent once or a few times then desist. However, a

small number may become heavy abusers, inhaling daily for several years, not abandoning the habit until their late teens. This maturing out of the habit of solvent inhalation abuse has been reported with the observation that many chronic abusers often progress to chronic alcoholism or barbiturate addiction (Cohen 1975).

The abuse of solvents is usually a group practice but may be carried out in private where the risks of over-dosing and ill effects are greater though the risk of detection is less. As the action of the drug develops, even group "glue parties" are marked by withdrawl and low levels of communication between the members. Like alcohol, solvent inhalation abuse disinhibits and permits behaviour to occur which would be suppressed under more sober conditions (Cohen 1975). In fact, one of the main reasons for their popularity could be this behavioural disinhibition, particularly when used alongside or in combination with alcohol.

Solvent inhalation abuse is a low status avocation and no one, except abusers themselves, has a good word for it. Perhaps this experimental use of volatile substances by adolescents can be understood as the natural exploratory behaviour of being willing to try anything once. However, justification for chronic abuse is difficult in anyone who can possibly have any respect for the state of health of their bodies and minds.

1.4.2 Physiological Effects of Volatile Substance Abuse.

Man enjoys getting drunk. It can be described as an extremely pleasant condition, and to achieve it countless substances have been tried. Indeed, the desire to get drunk can even be said to have led to the discovery of anaesthesia.

Drunk is the correct word for the condition achieved by the volatile substance abuser. Various degrees of drunkenness may be observed from the slight befuddlement enjoyed by the subject perhaps experimenting with the substance for the first time, to the possibility of producing a coma accidentally or by overenthusiastic abuse. This coma can be in all respects similar to that deliberately produced by the anaesthetist for surgery (Nagle 1968).

The immediate physiological effect of the inhalation abuse of solvents can usually be described as that of a pleasant exhilaration, excitement, and even euphoria. In this way the abuse of the volatiles closely simulates the early effects produced by the ingestion of alcohol. The symptoms of this early stage of abuse consist of dizziness, slurred speech, a state of imbalance, and sometimes even drowsiness. The abuser then begins to act drunk, exhibiting ataxia and slurred speech. Sometimes irrational impulsiveness, excessive irritability, and over-excitement develop, and it is during these periods of overactivity that injuries can take place. Diplopia and tinnitus may be experienced by the abuser during

this phase of the intoxication, lasting for up to thirty minutes after the initial inhalation. As the condition deepens hallucinations and delusions may develop. An euphoric, dreamy high is then usually described, with drowsiness, stupor, and eventually sleep as the frequent endpoint. Unconsciousness may then ensue, the subject perhaps remaining unresponsive for periods of an hour or more. Respiration maybe depressed, pupils dilated, and heart rate accelerated. The abuser may even be found to be amnesic regarding actions taken during the acute stages of intoxication (Chapel & Taylor 1970).

Treatment of acute intoxication is best performed by the administration of fresh air or oxygen and of course removal from the source of the volatile substance. Habitual abusers may develop a persistant cough, and a rash around the nose and mouth has also been described (Cohen 1975).

The chronic volatile substance abuser often has an unpleasant odour to his breath, and excessive oral secretions resulting from irritation to the mucous membranes of the nose and mouth by the vapours. Other chronic effects have been described as: fatigue and reduced physical activity, mental depression, loss of appetite, weight loss, irritability, inattentiveness, irritation of skin and respiratory tract, forgetfulness, bone marrow inhibition, low blood pressure, various anaemias and white blood cell abnormalities, congestion and haemorrhage of the lungs, cerebellar degeneration, elevated spinal fluid pressure, and liver and kidney damages (Corliss 1969). The

symptoms are potentiated by other central nervous system depressants such as alcohol. Indeed volatile substance abusers often come to the attention of the authorities because of their resemblance to the chronic alcoholic (Glaser & Massengale 1962).

There is an apparent wide variation in the symptoms demonstrated by different abusers, some do not develop any at all, while others can exhibit quite severe abnormalities. In one case acute hepatic and renal damage developed in a nineteen year old abuser after the inhalation of the vapours from a cleaner, the main constituent of which was toluene (Hobby, O'Brien, & Yeoman 1971). This compound is present in most glues and adhesives and is probably responsible for the pleasurable effects obtained from abusing these products. A second case described the inhalation of petrol fumes as the cause of severe hepatic damage and lead encephalopathy from the lead alkyl additives (Louria 1969). A third case was illustrated by the inhalation abuse of a spot remover containing trichloroethylene resulting in acute renal tubular and hepatic necroses (Baerg & Kimberg 1970). Additionally one review of the subject of inhalation abuse reports a total of one hundred and ten cases of sudden death many of which resulted from the inhalation of fluorinated hydrocarbons (Bass 1970).

Many of the hazards associated with volatile substance abuse are due to the impaired judgement and irrational, reckless behaviour expressed by the abusers. These people are accident

prone and have been known to injure other people in their vacinity (Cohen 1975). Visual hallucinations and visions are common and frequently terrifying. The most common of these are the feelings of being able to fly and of posessing superhuman powers resulting in the abusers throwing themselves off of buildings or standing in the path of oncomming vehicles (Chapel & Taylor 1970). Some abusers have felt an insensitivity to pain and self administer cuts and lacerations. This self-mutilation is uncommon but some cases have been reported. Perhaps the best documented of these involved a recluse in his early twenties who was found floating in the water of a pit near where he lived. He was nude, and his left testis was missing. Thin parallel cuts were noted on the skin of the scrotum proximal to the previous location of the organ. He was known to have been inhaling fumes of paint thinner the night before. His bizarre behaviour resulted from mental derangement related to the inhalation of the paint thinner and it is thought that his death was a direct complication of the self-mutilating act (Eckert 1977).

The abused volatiles appear to be producers of aggression and a simple explanation for this may be that these drugs extinguish control over behaviour before they extinguish control over motor activity. Thus the intoxicated person has lost his inhibitions about acting out while he still remains capable of acting.

A partial tolerance to these volatile substances may develop

with their frequent abuse which may eventually lead to addiction. Addiction is technically not the correct word for the condition as no solvent has been proven to cause physical symptoms upon withdrawl in the habitual user, the usual requirement to satisfy the definition of addiction (Nagle 1968). The term, however, is too deeply imbedded in the literature on the subject to allow the easy substitution of any other word.

The detection of the solvent inhalation abuser is an important stage in the effective application of treatment and can usually be obtained from contributive evidence such as breath odour, dilation of the pupils, slurred speech and actions. The early detection must in itself provide a deterant to the casual abuser. Otherwise treatment should consist of withdrawl from the source of solvent and education on the potential consequences arising from continuation of the habit. Despite the knowledge of the possible physical dangers or the threat of punishment a relapse from treatment has been observed to be frequent (Cohen 1975).

1.4.3 Psycological Effects of Volatile Substance Abuse.

A common feature of the psycological make up of the abusers of volatile substances is the presence of excessive feelings of shyness, insecurity, or inferiority. Their abuse of compounds in this way may be observed as an effort at self treatment. However for any lasting effectual treatment the abuser's self

image must be changed. For this reason care should be taken during any treatment to reduce the abuser's demoralisation (Cohen 1975). Although the abuser may inhale solvents once or twice purely as a result of curiosity, repeated abuse may suggest an emotional imbalance.

This state of emotional imbalance is naturally more particular to the adolescent age group, and is one of the main factors to consider in explaining the greater occurrence of abusers in this age group than in any other. The frustrations induced by the inability to meet the goals set by society, schools, or parents; rejection by parents and friends who may not meet their emotional needs; or anything which may bring about a sense of insecurity, such as quarrelsome parents, an apparent lack of understanding by adults or authority, can all be considered as causes of a person turning to the escapism of solvent inhalation abuse (Cohen 1975).

The habit has a disproportionally greater popularity in urban areas than in rural areas. The lack of access to the materials is one suggestion for this phenomenon which on examination is found to be invalid as the products are universal. Another suggestion is the possibility of stronger social controls as it is often found that in rural areas the family is more closely bound, and there is an interactive community of neighbours, friends and relations (Adams & Barker 1963). The simple lack of knowledge of the behaviour can now only be claimed to contribute to this imbalance in extremely remote areas as the media has taken the position of

advertising the practice thoroughly, though not intentionally, by their reporting of incidents and current awareness profiles. There may even be reason to believe that the wide publicity given to the practice of solvent inhalation abuse has increased rather than decreased its incidence. The fact remains that the pressures of living and of meeting the targets set by society leads to the feeling by some people that the only solution to their difficulties can be found through intoxication by whatever means available.

The psycological effects of volatile solvent abuse can be classified into five categories of mental disturbance.

1 - Emotional disturbances: These are characterised by the euphoric moods which are usually exhibited by the abuser in the early stages of the intoxication. This is then followed by psychomotor disinhibition up to the state of excitation, as well as feelings of bliss, relaxation and general indifference.

2 - Perceptional disturbances: Solvent inhalation abusers often demonstrate an illusory perception of reality, with visions and visual hallucinations and acoustical experiences of elementary sounds, like murmur, creaks, or drones. Sometimes even kinesteric sensations of floating and flying are manifest (Malcolm 1968). It is probably for these disturbances that the abuser is searching.

3 - Attention disturbances: During the very early phase of intoxication a subjective feeling of an improved ability of concentrated attention may occur revealing itself as the simultaneous inability to apply any sustained concentration.

4 - Consciousness disturbances: Subsequent phases of the intoxication may lead to a kind of dream like experience of fantastic and imaginary happenings, and the apparent existence of infinite space. This may be accompanied by a confused perception of the flow of time and this inexact estimate of time eventually leads the abuser into a state of confusion.

5 - Action disturbances: Under deep intoxication the abuser may experience a disinhibition or inhibition of reflexive actions and a heightened heterosuggestibility which may lead to impulsive and aggressive actions and in even to criminal activity.

The behaviour problems associated with volatile substance abuse are a major concern to teachers, doctors, and police as well as parents. Most young abusers of volatiles are truant and experience some difficulty with reading, writing, and arithmetic (Silberberg & Silberberg 1974). Steps in applying some form of discipline or even of taking legal action where available, is of unknown effect and indeed may be retarditive in long term treatment, for which the underlying causes must be identified and redressed (Samples 1968). An educational program should be made to inform both adults and children of

the potential risks of solvent inhalation abuse. Perhaps the regulation of the availability of certain solvents by restricting their sales could be considered or even to tighten regulations concerning the formulations of products containing the abused solvents. Though it must be realised that legal restrictions on the supply of solvents may well serve to compound the problem by forcing abusers to resort to more dangerous substitutes for their intoxication or even lead to the support of a criminal subculture (Chapel & Taylor 1970).

1.4.4 Sociological Influence on Volatile Substance Abuse.

When people are faced with the situation where they are unable to alter or effect their environment to produce changes, the direct attempt to improve their psycological state by some pharmacological method often remains as their only apparent solution.

The literature on chronic solvent inhalation abuse is almost unanimous in its suggestion that the cause of the problem lies in the disorganised existence of the abuser, although there is some difference of opinion as to the main cause of the disorganisation. Some of the critical variables have been arranged into a proposed causal model for volatile substance abuse. This model is a generalisation of the many sociological factors which have been suggested through the literature.

Of the four environmental conditions proposed the first, the presence of low social assets, is perhaps the most vocally supported by the media. Under this general heading are grouped many socioeconomic and background variables, the measurement of which, as a social assets scale, is frequently used in sociological influence determination (Katch, Luborsky & Todd 1973). Examination of the literature indicates that all of these variables may not be present or indeed be predictive of volatile substance abuse at any given time, however, it seems probable that the sum of the social assets of an individual would be in some way indicative of their psychological vulnerability. The occurrence of familial disorganisation has also been suggested as a variable of high importance. This has been evidenced by the high level of actual broken homes, the presence of unloving parents in those families still intact, and of alcoholic or drug addictive mothers and fathers among abusers. The fathers, in particular are often seen by the abuser as cruel, unjust, unapproachable and uncaring, so that even an abandonment of the family may be considered an improvement in its predicaments. Social disorganisation is also blamed by other observers for the high incidence of abusers in minority groups. The filth, misery and hopelessness of the inner city which provides no alternatives for its children are seen as the etiologic agents for volatile substance abuse (Cohen 1973).

The second environmental condition which has been postulated as important in producing psycological vulnerability and

therefore perhaps leading to the abuse of volatiles is acculturative stress. This condition is the type of psychological distress or discomfort that would be present in any group undergoing social and cultural changes. Adolescents in particular experience a type of acculturative stress as they progress from childhood into adulthood (Latorest, Smart & Whitehead 1971).

The third environmental condition is the relationship between parental drug use and alienation in their children (Latorest, Smart & Whitehead 1971). Parental drug use could also influence the drug use of children directly by providing an example.

The fourth environmental condition of the causal model is peer and sibling influence. Peer influence is acknowledged as being an important predictor of adolescent behaviour and especially drug use. Studies have shown that sibling drug use is also important in predicting the abuse of solvents (Jackson & Smart 1972).

The presence of psychological vulnerability is shown in the causal model as providing the mediating link between the environmental conditions and solvent inhalation abuse. There are two proposed symptoms of this vulnerability : a) the learned helplessness syndrom - including hopelessness, anxiety, passivity and depression; and b) alienation.

It has been postulated that whenever an organism has been

exposed for a length of time to uncontrolability in its environment, a condition of learned helplessness will result. The first symptom being anxiety which later gives way to depression, passivity and eventually hoplessness. The motivation to respond is lost and adaptive responses are not made even when there are adaptive responses available (Seligman 1975). Evidence is available to suggest that this learned helplessness is present in solvent inhalation abusers (Fejer & Smart 1973). The poor academic performance of abusers also tends to support this lack of motivation. There are also suggestions that poverty and overcrowding could be conditions which would lead to this state of learned helplessness (Busse, Hall & Weise 1973).

It is estimated that about five times as many adolescents experiment with solvent inhalation as become regular abusers and it is emotional disruption that stands out as the common cause which pushes the individual into persistent abuse (Barnes 1979). The solvent vapours are therefore used in a desperate effort by the abusers to treat themselves and in doing so reduce the interpersonal barriers and the daily frustrations of their lives.

Obviously the final choice of solvent to be abused depends upon other variables. The solvent being used by the microculture to which the abuser contributes, the availability of the product and the secondary gains of using that particular intoxicant. When solvent inhalation abuse is the "in" thing among the peer group, this may be the overwhelming

variable, outweighing all others in importance.

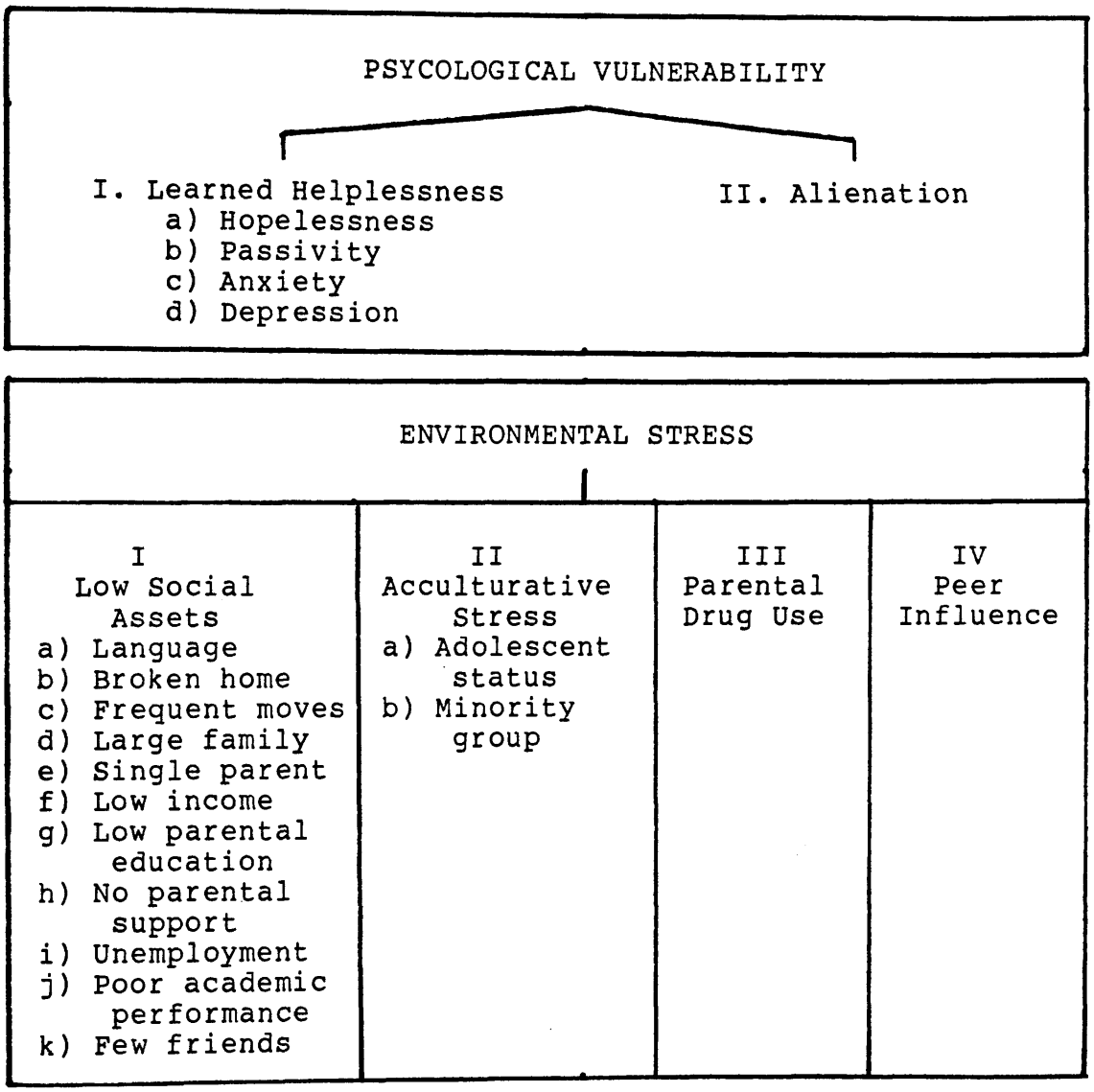


Table 1.4.4.1 - The Causal Model for the Sociological Influence on Volatile Substance Abuse.

1.5 Conclusion - The Provision for Clinical / Forensic Monitoring of Volatile Substance Abuse.

Solvent inhalation abuse is a subject of concern to many people who deal with youth, but it must be recognised that the fears surrounding it and the measures used to prevent it may do more harm than is actually caused by the practice. In attempts to tackle the problem drug education programs have been devised. Some of these, however, only qualify as "How To Do It" courses. Indeed in consideration of such programs, the greater readiness for the general public to accept the myth rather than the reality of the presence of the abuse, and the prohibition atmosphere surrounding the habit, it may be fortunate that more individuals are not practicing than actually do.

Solvent inhalation appears to carry with it a potential for significant detriment to the abuser's physical and emotional health. More serious and lasting forms of misbehaviour such as alcoholism, drug addiction, or criminal activity may be implicit in the nature of the abuse (Glaser & Massengale 1962)

The practice itself has been indicated to be a form of passive retreat and the abusers possess many of the basic personality characteristics of alcoholics and drug addicts: low intelligence rating, low socioeconomic background, membership of a minority group, and weak personality structure (Ellison 1965).

In recent years the media has been suggesting that the problem of solvent inhalation abuse is no longer as great as it used to be. It has even been suggested that the "harmless" activity of solvent inhalation is merely a passing fashion which if ignored will disappear. It is arguable that the publicity given to the problem by the media may even have done more to increase the problem than to decrease or even control it. Certainly adolescents constitutes the section of the public most likely to be encouraged to experiment with the practice by such advertising and perhaps the age group most likely to rebel against parental and authoritative controls.

It is undoubtable that the majority of parents are concerned over the problem and in particular on the possible effects and consequences on the health of their children. However, there are exceptions, and it has been reported that some actively encourage children to take up or continue the practice of solvent inhalation. Shop owners have been reported to complete the sale of a can of adhesive or tube of glue with a plastic bag, the obvious intention being that the product is to be abused (Chapel & Taylor 1970).

Similar concern is shown by the police and in particular in relation to the problems of property damage, theft and arson, which all too frequently follow the sessions of abuse by adolescents. However, legislation to prohibit the sale or restrict the distribution of solvents, or even the products which contain them, may work very much in the same manner as

the Prohibition, in that the prohibition of alcohol actually made drinkers out of many who previously were non-drinkers (Kupperstein & Susman 1968).

It must be remembered that the numbers referred by police and hospitals are only a small proportion of the overall problem, they reflect the trends in the extremes of abuse and only present information on overdosing or in connection with interrelated psycological conditions. The statistics so obtained do not reflect the social offence of solvent inhalation, which must comprise of the majority of abusers; adolescents who indulge in a short, though perhaps very regular, abuse during school lunch breaks, or after school hours, and it does not include those who are solitary in their abuse in the privacy of their homes. It can be said that these abusers have learned respect and some degree of control of their intoxication to a similar extent to the accepted social control of alcohol intake. Such control has been demonstrated to be possible, sufficient to produce inebriety without producing drunkenness and the resultant drawing of attention by parents, teachers and police. Another very important factor to be remembered when dealing with statistics on the problem is the fact that the younger the abuser the more likely the intoxication is to become apparent to the public gaze and the greater the outcry of concern.

The focussing of public attention on the problem has done little to halt the spread of the habit. This may be in part due to the fact that denials by the abuser are often difficult

to disprove, and that there is a feeling that nothing is being done, or indeed can be done, by the health authorities in combating the problem. Besides the additional hinderance that parents are often not willing to admit that their child abuses volatiles, like drug addiction in any form it is a social black mark, they are also afraid that the blame for the child's addiction will be levelled at them, or suggesting their failure as a competant parent. Teachers probably have similar feelings about their classes also hoping that if ignored the habit will go away.

From the above discussion certain questions become apparent:

1. Is the abuse of volatile substances more dangerous to the individual than the abuse of alcohol and if so, should some form of legal measures be taken to control it ?

2. Should more information on the problem be available to teachers, parents and police; is any service being offered to them to control or monitor the abuse ?

3. What priority does this control or monitoring have over the similar problem of alcohol abuse, or the abuse of more dangerous drugs ?

The answers to these questions are interdependant and no attempt will be made here to provide specific answers. However, without the provision for monitoring the abuse no solution can be offered, little information on harmful effects

be given, and no case can be proposed for legislation. Yet without this control, consent or legislation any attempt at monitoring must be ineffective and open to statistical misinterpretation. Therefore until some equitable solution to this dilemma can be reached the statistical results produced from a programme of monitoring the abuse, such as the one included in this thesis, must be regarded with great suspicion.

CHAPTER TWO

QUALITATIVE MEASUREMENT OF VOLATILE SUBSTANCES IN BLOOD AND TISSUES

2.0 Introduction.

This Chapter describes analytical techniques for the qualitative measurement of solvents of abuse. In addition to a detailed description of the theory behind these techniques, and a review of the available literature, there are details of a case involving the practical use of the developed methods.

Any acceptable analytical procedure to be employed for qualitative assessment should be capable of encompassing a wide range of the available solvents, in particular those which are to be found in household products and are easily obtainable for abuse. In the following sections the development of a gas chromatographic method to accomplish this task is described. To improve on the selectivity of this method, mass spectroscopy was employed in conjunction with a computerised mass spectral data system. In this way a library of known solvent spectra was created and this was then available for the subsequent indentification of unknowns.

2.1 Review of the Equipment Available.

2.1.1 Theoretical Aspects of Gas Chromatography.

Gas chromatography is a process by which a mixture is separated by a gas phase moving over a stationary solid or liquid phase. Gas solid chromatography is employed for the separation of gases and relatively non-polar solutes of high volatility, whereas gas liquid chromatography is more broadly applied to the separation of a wider range of solutes.

The experimental procedure for obtaining a gas chromatogram is relatively simple. A small quantity of the sample mixture is introduced into the column by way of a self-sealing inlet after which an unadsorbed carrier gas moves the solutes through the column at a rate determined by the solute/sorbent interactions. Each solute in the mixture will then leave the end of the column after a characteristic volume of carrier gas has passed through. The entire process is known as elution analysis and the solutes emerge as peaks the shape of which is determined by the isotherm for that particular adsorbent-solute pair. Symmetrical peaks allow for the calculation of the characteristic retention volume of the solute.

One of the characteristics of gas chromatography is the simplicity of the apparatus. Even the most refined equipment still consists of four basic components: a carrier gas supply and flow control; a column and column temperature control; a

detector system; and, a sample port and means of sample introduction.

Mobile Phase

The main characteristics of gas chromatography, speed, high efficiency and sensitivity are due, in the most part, to the fact that the mobile phase is a gas, and therefore these characteristics are also determined by the particular gas used. The most commonly used gases are helium, nitrogen, argon and hydrogen. The choice of gas is largely determined by the column efficiency and detector sensitivity. The inert gases present no reactivity problem and are thus preferred to hydrogen which may under certain conditions present an explosion hazard.

The purity of the mobile phase is also of great importance. The impurities result in detector instability and variation in response, and these effects increase in direct proportion to the sensitivity of the detector.

After the selection of a suitable carrier gas the flow rate must be selected and regulated. This is particularly important with the increasing resolution of gas chromatographic columns, since the time of analysis is inversely proportional to the linear gas velocity and therefore a compromise usually has to be made. This variation of column efficiency, represented by height equivalent to theoretical plate (H), with linear gas velocity is represented

by the Van Deemter equation:

$$H = \frac{A + B + C * u}{u}$$

The inevitable requirement is that flow rates must be controlled accurately, usually with precision needle valves or diaphram valves, and are usually in the range of ten to one hundred millilitres per minute for packed columns.

Column and Column Temperature

The column is a tube which contains the stationary phase fixed in a finely dispersed form, thus providing a large contact surface area with the mobile phase in which the vapourised samples move down the column. In elution gas chromatography the sample is applied as a plug into the mobile phase at one end of the column, distribution then occurs between stationary and mobile phases and the components of the sample then emerge from the other end of the column as separate bands.

To exploit the potential of gas chromatography many different columns have been developed, and therefore the column technology, the practical problem of selecting column construction and stationary phase, has arisen as a major task of all work with gas chromatography.

Standard columns are constructed of either metal or glass tubing having an internal diameter of two to nine millimeters. In general the wider the diameter of the column, the higher the amount of stationary phase per unit length of column and

therefore the larger the maximum tolerable sample size. The granular packing material usually has a particle size of between eighty and six hundred micrometers, and in the case of gas liquid chromatography this support is usually impregnated with one to thirty percent by weight of a liquid stationary phase. The length of a packed column is usually between one and three metres.

The ideal support has not yet been found and although those that are available produce satisfactory results, their individual influence on the efficiency of the column may be considerable.

The most common supports are the porous granules such as diatomatious earths, fluorine containing polymers such as Teflon, and other synthetic polymers. Non-porous material such as glass microbeads have also been used.

The preparation of a packed column is relatively simple and this contributes significantly to their popularity over open tubular columns. Firstly the column tube is thoroughly washed with detergent and solvents and then dried. The support material is agitated in a solution of the liquid phase while the solvent is evaporated leaving a uniform predetermined coating of stationary phase on the support. The method of filling the tube with the packing material is usually by slowly pouring the coated support into one end of the tube while applying a reduced pressure to the other end. Vibration of the column to produce a more densely packed column must be

applied with care, especially if the support is of poor mechanical strength.

The requirements to be considered when choosing the liquid stationary phase for a particular application are low vapour pressure at the operating temperature, chemical stability and low viscosity, adequate wetting of the suppport and, selectivity and resolving power for the solutes to be separated. It must also be soluble in some solvent in order to facilitate coating of the support.

The liquid phase in the column always has a vapour pressure and as it is exposed to the mobile phase there is always some column bleeding. This bleeding will increase with increasing temperature, ultimately contaminating the eluting solutes, reducing detector efficiency and changing the column's separation characteristics. The thermal stability of the phase must also be considered. A small amount of oxygen in the mobile phase can cause the oxidation of some stationary phases.

The basic measure of the efficiency of a column is the separation of a sample represented by the chromatogram and is a result of the entire chomatographic process. Column efficiency may be defined by considering either a pair of solutes, which is only convenient for a specific case, or a single solute, which gives a more general appraisal of column effectivenes, since the selectivity of the stationary phase is not involved.

This efficiency is related to the relative broadness of the peaks which in ideal cases results in the peak appearing on the recorder in the form of Gaussian curve. The peak width (w) therefore represents the most convenient method for chromatogram evaluation.

Assuming that the column is uniform the variance of the solute distribution at the end of the column is proportional to the column length (L). This proportionality, the height equivalent to a theoretical plate (H) is then defined by the following equation:

$$H = \frac{L * w^2}{16 * t^2}$$

Two peak efficiency is expressed in terms of resolution (R) which is equal to the ratio of spacing between the peak maxima to the mean base width of the peaks.

$$R = \frac{2 * (t^2 - t^1)}{(w^1 + w^2)}$$

For more closely spaced peaks the resolution can be determined from the following equation:

$$R = \frac{(a - 1) * k * n^{0.5}}{(4 * a * (1 + k))}$$

Where (k) and (n) refer to the slower solute peak.

The resolution is a measure of the degree of separation of adjacent peaks, therefore when R = 1.5 the separation is 99.7 %, though for most practical purposes the value R = 1.0, 98 % separation, is sufficient.

The Detector

The purpose of the detector is to sense changes in the composition of the effluent gases and to translate those changes into an electrical signal which may be amplified and recorded.

By far the most popular detector is the flame ionisation detector (FID) and the majority of gas liquid chromatographs are today fitted with this type of detector as standard. In this detector hydrogen is mixed with the effluent gases and then burnt in an atmosphere of oxygen. The ions which are produced in the flame then conduct a current from the flame jet, which acts as one electrode, to a collector plate or tube above or around the flame, which acts as the other electrode. This ion current is then amplified and fed to a recorder. The size of this current therefore depends on the number of ions which are in the flame, and thus on the quantity of eluted solute entering the flame. The sensitivity of the detector is relatively independent of the flow rate of the carrier gas, especially where the collector electrode is in the form of a tube around the flame. However for best performance the size of the flame, and therefore the hydrogen and oxygen flow rates, is critical.

Another slightly different group of detectors are the radioactive source ionisation detectors. In these an inert gas is ionised by radiation from a suitable radioactive

source. The collisions between electrons and the inert gas molecules are elastic and there is little loss of energy. A potential between two electrodes in the ionised gas produces a standing current. On the introduction of a solute into the carrier gas there are inelastic collisions between the electrons and the molecules of the solute. The molecules may capture the electrons producing ions, or if the electrons are of high enough energy dissociation may take place.

$$A.B + e^- \longrightarrow A.B^- + \text{energy}$$

or $$A.B + e^- \longrightarrow A + B^- \pm \text{energy}$$

Under the influence of the applied potential the heavier of these ions ($A.B^-$ and B^-) have a lower velocity than the electrons and therefore reduce the value of the standing current. This reduction in ion current is proportional not only to the concentration of molecules but also to the electron affinity of the molecules. Thus the hydrocarbons give a poor response compared to halogenated compounds. The electron capture detector (ECD) is mainly used in the detection of halogen containing or organo-metalic compounds. The size of the applied potential required to maintain the standing current also depends on the design and the temperature of the detector, on the distance of separation of the electrodes and the pressure of the carrier gas. To control the electron energy to as constant a level as possible the potential can be applied in the form of short pulses of about one microsecond in duration at a frequency of every twenty to one hundred microseconds. Under these conditions the electrons interact with the molecules under the influence of a zero field strength and it is only during the

pulse that uncaptured electrons are collected at the electrode.

The majority of modern electron capture detectors use a Nickel-63 radioactive source (Beta emitter). The low energy radiation it produces has a short range and it has a half-life of about eighty five years. The usual carrier gas for a Nickel-63 detector is nitrogen. The use of argon or helium is not recommended as their excitation energy is greater than the ionisation energy of the majority of organic compounds. However, by the addition of a suitable purge gas, such as methane, an adequate decrease in standing current can be obtained.

There are many other types of detector in use, such as the katharometer or thermal conductivity detector (TCD), the flame photometric detector (FPD), the thermionic detector (TID) and infra-red detectors (IRD). As these will not be used in this thesis they are only listed by name to complete the overall description on detectors.

Today one of the most specific and sensitive detectors is a mass selective detector. The use of a mass spectrometer and its coupling to a gas chromatograph as a detection system will be described in a subsequent section.

Sample Introduction

Samples are introduced into the column through an injection port, the design of which varies from one manufacturer's system to another. The injection port is sealed with a rubber septum through which the sample is introduced via the needle of a syringe. The temperature of the port is usually controllable and is maintained at about fifty degrees above the operating temperature of the column. This temperature is necessary in order that the sample is swept on to the column as a plug and not evaporated slowly as a band dependant on the solute boiling points. The efficiency of separation is also dependant on the sample size, which for standard packed columns is of the order of one to ten microlitres.

The sample to be injected into the chromatograph should be gaseous or easily vapourised, and the methods of introduction of the sample into the column vary according to the physical state of the sample and the requirements for the analysis. With the majority of samples the physical state is a liquid. In these cases and also in the case where the sample is a solid the sample is introduced as a solution. This is very convenient as the sample or the solution of the sample can then be injected directly into the injection port using a precision syringe.

This method of injection is not, however, as precise as expected, and even with extreme care it is difficult to inject reproducable amounts. This is due to the fact that the sample

is injected into a system which has a pressure greater than atmospheric and a higher than ambient temperature. The needle of a syringe will contain a volume of the sample and depending on the length of time that the operator holds the syringe in the injection port this sample may vapourise and enter the column. The absolute quantity of sample injected is therefore reasonably operator dependant. To improve on this area of possible error a "hammer" injection is usually made where the needle of the syringe is rapidly inserted and the plunger forced home with one quick movement. This problem is best overcome by the use of internal standards in the sample thus making the analysis relatively independent of the volume injected.

Gas samples and volatile liquids are injected into the chromatograph using one of two methods. The first method involves direct injection using a gas tight syringe, this is usually used for headspace samples and for gaseous samples which cannot be handled by other methods. The errors in this method are in the difference in pressures between syringe and injection block and also the difference in temperatures. The alternative method involves the use of a gas sampling valve whereby a known volume of sample is swept on to the column with the carrier gas. The sample loop may involve a cold trap and a heating element to flash the sample on to the column.

Headspace Analysis

In headspace analysis, as with other chromatographic methods, the measurement made which is proportional to the concentration of the sample is the area under the chromatographic peak. At low pressures in the vapour phase above the sample this peak area is proportional to the partial vapour pressure of the component. This partial pressure is usually expressed in terms of the vapour pressure of the pure component, the mole fraction in the sample and its activity coefficient. In a few cases, ideal mixtures, the activity coefficient has a value of unity, however in the majority of cases the mixture is non-ideal and the value of the activity coefficient may be greater or less than unity. Its value is dependant on the nature of the solute and the other solutes in the mixture, on the mole fraction of all the other solutes, on the temperature and to some extent on the pressure. Thus the quantitative evaluation of gas chromatographic headspace analysis depends on an equilibrium.

At a given temperature the vapour pressure is characteristic of a compound and therefore calibration must be performed under the same experimental conditions as those used for the analysis. This necessitates a precise control of the temperature owing to the temperature dependance of vapour pressure. It is also important to know the time required to establish the vapour pressure equillibrium, as this can be dependant on the viscosity of the sample. However, owing to the fact that the activity coefficient is not purely

characteristic of a solute, as it is dependent on the mixture composition, the calibration must be performed with a mixture which corresponds in composition to that of the sample. Introduction of another factor to correct for differences between samples and the mixtures used for calibration is also required.

Retention Index Theory.

The usual method for the standardisation of chromatographic data is by the use of Kovats indices (Kovats 1958). These values are accepted to represent the "equivalent carbon number" of each solute compared to a set of references and may be calculated from the following equation:

$$I = 100 * \frac{(\log t_s - \log t_z)}{(\log t_{(z+1)} - \log t_z)} + 100 * z$$

Where the retention time of the solute is (t_s) and (t_z) and ($t_{(z+1)}$) are the bracketing retention times of n-alkanes with (z) and (z+1) carbon atoms respectively.

For any one column, especially in the absence of stationary phase, there is a linear relationship between temperature and Kovats index for a given solute at the same carrier gas flow rate. Deviations from linearity are due to: decomposition; temperatures below the freezing point of the solute; changes in the carrier gas viscosity due to increasing temperature. The first two have a marked effect on the index value but only impose limitations on the extent of use of this relationship. The third cause of possible deviation, change in carrier gas flow and viscosity, has only a small overall effect and can

usually be discounted in practice.

A plot of temperature against retention index should therefore produce a straight line as represented by the equation:

$$I = K * T + C$$

Where (K) and (C) are equation parameters and (T) is the temperature in degrees absolute.

If these parameters are known for a series of solutes the retention indices and therefore the retention times may be calculated at any given operating temperature above the freezing point of the solute and below the temperature at which decomposition occurs. Conversely it would be possible to use retention time data to indicate the possible identity of an unknown solute peak.

2.1.2 Mass Specroscopy - Theory.

A mass spectrometer is an instrument for sorting ions according to their masses. There are several types of mass spectrometer, but all have components to perform the following basic functions: ionisation of the sample; acceleration of the ions by an electric field; dispersion of the ions according to their mass to charge ratio; and the conversion of detected ions into an electrical signal.

There are several methods used to produce ions in the source but the most widely used method is the bombardment of the sample molecules with a beam of electrons. The ionising

electrons are liberated by thermionic emission from a heated tungsten or rhenium filament and are accelerated by an adjustable potential. This potential controls the kinetic energy available for the ionisation of the gaseous molecules. Bombardment of molecules by energetic electrons in this way results in the production of more positively charged ions than negatively charged ions, it is therefore not surprising that the majority of mass spectral applications are with positively charged ions.

The ions are extracted from the source point by an electric field applied between positively charged repeller plates and negatively charged accelerator plates. They are then directed through a series of focussing electrodes and ultimately a narrow slit into the mass analyser unit.

There are several possible ways to separate these ions according to mass number. Perhaps the most widely used method is by use of a magnetic field as the ions passing through the field result in a circular trajectory the radius of which is inversely proportional to the magnetic field strength. The geometric construction requires that the two slits and the apex of the magnetic sector are colinear and thus the most commonly used sectors are of sixty, ninety and one hundred and eighty degrees. This results in the ions of equal momentum being focussed on the collector slit. Providing the possibility to select which ions are focussed on the exit slit by varying the magnetic flux or the applied voltage.

The resolution of this type of mass analyser is not only dependant on the spread of kinetic energies but also on the fact that the magnetic field boundary is not sharply defined. This boundary error can be considerably reduced by the use of a one hundred and eighty degree sector and enclosing both the source and the collector within its boundary.

Another method of analysing the ions is by use of an electrostatic sector. This assumes a circular trajectory in passing through two concentric cylindrical electrodes.

The use of an electrostatic sector and a magnetic sector are combined in a double focus instrument which is capable of resolution greater than ten times the resolution obtainable on a single focus instrument.

The detection of the ions is usually carried out by an electron multiplier which is essentially similar to the photomultiplier used in ultraviolet and visible detection, but it has the primary cathode optimised for the detection of ions rather than photons. As it is in the vacuum of the spectrophotometer it does not require encasing in a glass envelope.

Sample introduction into the mass spectrometer can be by several methods, the choice depending on the physical properties of the sample. Most modern spectrometers accept samples in more than one way. A probe is used for solid samples. This is a stainless steel rod which is designed to

hold a small quartz glass cup and to fit precisely into the source through a vacuum lock. The sample may then be heated or may be volatile under the conditions at the source. About one nanogramme is usually more than adequate for this method of sample introduction but it does not last long in the source and is soon pumped away. Another method of sample introduction is through a molecular leak. This is a small hole in a thin gold membrane, typically ten micrometres in diameter, through which gases and very volatile liquids may slowly diffuse from a glass or metal bulb containing the gaseous sample.

Perhaps the most important method of sample introduction is via a gas chromatograph. The combination of a highly efficient separation technique with the specificity of the mass spectrometer as a detection system provides an extremely versatile analytical technique. The major problem to be overcome with this sort of coupling is the fact that the high pressures of gas chromatography are incompatable with the high vacuum required for mass spectroscopy. The carrier gas is usually helium and the outlet of the gas chromatograph is connected to a molecular separator before entering the mass spectrometer. There are several designs for this separator but perhaps the most common is the jet separator. Here the exit from the gas chromatograph is opposite a small jet whose internal diameter is greater than the outlet jet of the gas chromatograph. The sample molecules then leave the gas chromatograph into the evacuated chamber of the separator. The heavier the molecules the greater their momentum and

therefore the greater the number of them which will traverse the gap between the two jets, the rest will be pumped away.

2.1.3 Literature Survey.

The exposure to high levels of organic solvents either by inhalation or by ingestion has been well documented (Browning 1953). However much of the literature on the subject refers to toxicity and to specific threshold limits, and not to to measurement or identification of these volatiles in biological fluids.

The problem of industrial over exposure to solvents has been recognised as a potential hazard to occupational safety for several years and the exposure levels are now carefully governed by the Health and Safety at Work Acts and are under constant review by the Health and Safety Executive.

Solvent inhalation abuse, though far from being a new problem, has not received the attention of the analyst until recent years when the incidence of cases, often of a fatal nature, has increased. Owing to the fact that in the majority of the cases of abuse the type of solvent is unknown, the requirement is primarily for a good separation technique. For this reason there is an almost exclusive use of gas chromatography.

Several methods have been published in the literature mainly concerned with the separation of a single solvent from body fluids, a few suggest the expansion of the methods to cover

more than one solvent and the majority of levels found are from fatal cases.

The determination of ethanol, the most abused solvent and perhaps the least considered in reference to volatile substance abuse, has had many methods published for its analysis in blood and urine (Curry, Simpson & Walker 1966, Davis 1966). These methods employ direct injection of the blood or extracted blood into the gas chromatograph. Some headspace methods are also reported for ethanol and other alcohols (Dahl & Wallace 1966, Glendening & Harvey 1969). There are many arguments for and against the use of headspace analysis as opposed to direct injection. The arguments against headspace are often purely of a technical nature concerned with the sample handling stage. The advantages are of longer column life, which is important when many analyses are to be performed, and less interferences from co-eluting compounds.

The extension of the methods available for ethanol to other volatiles represents no problem and has been successfully accomplished for quite a wide range of solvents (Jain 1971, Hessel & Modglin 1964).

An investigation into the possible columns available for the separation of solvents has led to the development of novel stationary phases (Schluneggar 1969). However, more recent workers have found several commercial columns to be suitable and recomend the use of Porapak Q, Carbowax 1200 or 400 on

Chromasorb W, and other polystyrene derivatives such as Chromasorb 102 (Jain 1971, Collom & Winek 1970).

The quantity of blood required for headspace analysis has been shown to be very small and in the region of one hundred microlitres (Fujiwara, Nakajima & Sato 1975). This particular method involved the use of a syringe as an equilibration vessel but required the use of a mercury seal to prevent losses, however, a sensitivity as low as ten nanogrammes per millilitre was claimed. Not all methods use such a small sample volume of blood. One publication gives details of a method where one hundred and fifty millilitres of blood is required for analysis (Nomiyama & Nomiyama 1978). This of course is an unreasonably large volume and it would be very difficult to obtain such a volume in post-mortem cases, although they appeared to have no problem in obtaining results for three fatalities.

Detailed surveys of the problem of solvent inhalation abuse from the point of view of the solvent concerned are few. Perhaps two of the more relevant to this study are those carried out by Bass and Oliver (Bass 1970, Oliver & Watson 1977), the latter of which provides the most relevant information. This study involved the area of Lanarkshire and although no blood solvent levels were given the results give some indication as to the scale of the problem of volatile substance abuse in the area of study.

2.2 The Examination of Stationary Phases and Supports for the Packed Column Gas Chromatographic Separation of Solvents.

2.2.1 Introduction.

Gas chromatography is commonly used for the separation of mixtures of compounds of similar chemical properties. Although the choice of stationary phase and packing material for the gas chromatographic column may to some extent be predicted in terms of polarity and molecular weights of the components to be separated, the main criteria for the selection of a particular combination of stationary phase and support is that the resulting column should give acceptable resolution with sharply defined component peaks.

The category of chemicals loosely defined as solvents consists of many chemically dissimilar compounds ranging from the non-polar aliphatic and aromatic paraffins to the polar esters and alcohols. A table giving listing some of these compounds has already been given in the previous chapter (Table 1.3.1). Most of the solvents mentioned have had some history of misuse, although the table cannot be considered as complete as any solvent or volatile compound is open to potential abuse. It does however illustrate the magnitude of the problem.

A number of column packing materials were therefore examined in order to acertain their suitability for the separation of a representative selection of these solvents.

2.2.2 Method.

Gas Chromatographs

Two gas chromatographs were used: Pye 104 and a Varian 2800 with flame ionisation detectors. Both instruments were equipped with heated injection and detector blocks.

Preparation of Columns

Glass columns (1.5m x 6mm id) were packed by the application of a reduced pressure to one end of the column while pouring the packing material into the other end through a funnel. Slight vibration of the tubing was allowed to provide even packing throughout the column.

The packing material support was coated with stationary phase by stirring 10 grammes of the material in a solution of the stationary phase in 100 millilitres of an appropriate solvent. The solvent was subsequently evaporated using a rotary evaporator as this technique was observed to give a more even coating than static evaporation.

Each of the columns were conditioned before use by programmed heating at a rate of two degrees centigrade per minute up to their respective maximum operating temperatures. A flow of oxygen free nitrogen of about thirty millilitres per minute was maintained through the column during this process. Conditioning was carried out in the oven of the gas chromatograph taking care not to connect the detector end of

the column and in this way preventing contamination of the detector with bleed from the column.

A representative sample of solvents were selected from those given in Table 1.3.1 which, it was anticipated, would include the more commonly abused volatiles. Solutions of these were prepared at an approximate concentration of one microgramme per millilitre by serial aqueous dilution. In the cases of solvents which were immiscible with water at this concentration an aqueous solution of ten percent methanol was used. This did not appear to affect retention times but did precipitate the requirement that all of the solutes must separate from methanol in addition to each other.

A one microlitre sample of each solvent was injected on each of the columns under test. Injections were repeated several times and in some cases the carrier gas flow rate was changed in the attempt to optimise separation of the components in the mixture.

2.2.3 Results.

The twelve columns tested and their respective operating conditions are given in Table 2.2.1. Retention times were measured using a stopwatch and were subsequently calculated relative to ethanol. The list of solvents used and these relative retention times are given in Table 2.2.2.

Column No.	Support/ Packing	Stationary Phase	% Loading	Column Temp.	Carrier Flow
1	Chrom.W	-	-	105	40 P
2	Porapak P	-	-	215	40 P
3	Carbopak	SP2100	-	185	40 P
4	Chrom.W	E 301	5	120	40 P
5	Chrom.W	Carbowax400	10	80	40 P
6	Chrom.W	Carbowax20M	3	100	35 V
7	Chrom.W	Carbowax20M	30	100	40 P
8	Chrom.W	Apiezon L	10	80	40 P
9	Chrom.W	Apiezon L	20	80	40 P
10	Tenax G.C	-	-	100	35 V
11	Chrom.G	OV-17	2.5	100	40 P
12	Chrom.W	N.P.G.A.	3	120	40 P

NB. Carrier gas oxygen free nitrogen. (ml/min)
Flame Ionisation Detection on both Pye 104 (P) and Varian 2800 (V) instruments.

Table 2.2.1 - Operating conditions for the twelve columns examined.

Column No.	1	2	3	4	5	6
Methanol	1.00	0.64	0.47	1.00	0.93	0.94
Ethanol	(0.65)	(1.40)	(1.90)	(0.55)	(2.30)	(0.85)
n-Propanol	1.00	1.71	1.42	1.09	1.67	1.05
i-Propanol		1.36	1.10	1.00	1.39	1.00
2-Butanol		2.57	2.16	1.09	1.52	1.11
n-Pentanol		5.00	5.26	1.54	4.22	1.41
Benzene		4.21	3.47	1.45	0.78	1.05
Toluene		7.71	7.05	1.64	1.13	1.17
Xylene				2.18		1.53
Acetone		1.28	1.10	1.00	0.47	0.82
M.I.B.K.				1.45	1.13	
CH2.Cl2			1.26	0.81	0.65	0.88
CH.Cl3			0.73	1.00	1.00	
CCl4				1.27	2.26	
n-Hexane				1.00		
cy-Hexane			1.31			

Column No.	7	8	9	10	11	12
Methanol	0.66	0.90	1.00	0.73	1.00	1.00
Ethanol	(1.80)	(0.55)	(1.00)	(0.55)	(0.65)	(0.80)
n-Propanol	1.50	1.72	1.45	2.00	1.00	1.06
i-Propanol	0.88	1.09	1.10	1.36	1.00	1.06
2-Butanol	1.44	2.00	1.90	3.00		1.25
n-Pentanol	4.05	5.27	4.30	8.81		1.87
Benzene		9.09		5.09		1.00
Toluene		15.64		11.36		1.25
Xylene						1.87
Acetone		1.72	1.60	1.18		0.81
M.I.B.K.		8.36	6.40	11.64		
CH2.Cl2		1.54	1.50	0.64		0.87
CH.Cl3		2.27	1.60	1.27		
CCl4		6.54	5.50	3.81		
n-Hexane		1.00		2.45		
cy-Hexane		1.18	13.60	3.27		

Table 2.2.2b - Retention Times Relative to Ethanol on Columns 1 to 12.

2.2.4 Discussion.

The resolution of even the alcohols was found to be incomplete on most of the columns tested. There are three columns, however, which stand out from the rest as being of some possible use and merit further analysis. These columns are: Tenax G.C. ; Apiezon L; and Carbopak SP2100. The Carbowax 400 column showed quite good peak shapes for most of the solvents used but the resolution of many of the peaks was unsuitable. Tenax G.C. gave good resolution but poor peak shapes on some of the solvents. The other columns tested tended to be quite good for particular chemical series but not for general mixtures.

Consideration was thus given to the other properties of these columns. Tenax G.C. is a porous polymer of 2,6 diphenyl-p-phenylene oxide, it has a high maximum column operating temperature, in excess of 300^{o}C, and is reported by the manufacturers as being inert to all solvents. It has also negligible column bleed even at elevated temperatures which is an important consideration for mass spectroscopic detection gas chromatography and for temperature programming. Carbopak SP2100 is sensitive to both thermal and mechanical shock though perhaps an even more restrictive limitation is its poor capacity requiring the use of low sample volumes, preferably less than 0.1 microlitre. The solvent has the undesirable effect of distilling off the stationary phase resulting in deterioration of the column. Apiezon L arguably produced the best separation of the solvent mixtures but has a considerable

column bleed making it totally unsuitable for mass spectroscopic detection gas chromatography and for temperature programming.

2.2.5 Conclusion.

From the above results it was decided that the most suitable column for separation of mixtures of solvents would be Tenax G.C. This column was therefore selected to establish a library of retention data for use in the qualitative analysis of solvents.

2.3 The Standardisation of Retention Data for the Qualitative Analysis of Solvents on Tenax G.C.

2.3.1 Introduction.

The requirement for the rapid identification of the solvents from abused preparations necessitates the standardisation of column operating conditions. The use of one column temperature as a standard condition has already been shown to be unsatisfactory due to the marked effect on peak shape, retention time and therefore resolution. However, by the application of retention indices, retention times and their theory it should be possible to derive a method by which the temperature may be selected to give the maximum resolution. Consequently by the measurement of the retention times of component peaks in an unknown mixture an identity could be proposed for each individual peak from a previously established library of standard data.

A series of fifty five solvents were selected for examination all of which are of interest as possible solvents of abuse. In addition to these solvents the first twelve n-alkanes were also examined to provide the necessary retention data for the calculation of Kovats indices.

2.3.2 Method.

Gas Chromatographs

A Pye 104 gas chromatograph was used with a flame ionisation detector. This instrument was equipped with heated injection and detector blocks. The ideal hydrogen and air pressures for the ionising flame were established and then maintained throughout the experiment. Oxygen free nitrogen, at a flow rate of forty millilitres per minute measured at the detector under ambient temperature, was used as the carrier gas.

Preparation of Solutions

Aqueous solutions were prepared of each of the solutes by serial dilution to a concentration of approximately ten microgrammes per millilitre. Acetone being used as a co-solvent when the solute was immiscible with water.

Experimental

One microlitre samples were injected and chromatographed on a glass column (1.5m x 6mm) packed with Tenax G.C. and the retention times of each solute were measured with a stopwatch.

The samples were chromatographed at four different isothermal temperatures: 100^{o}C, 150^{o}C, 180^{o}C, and 210^{o}C, and repeated in triplicate, the retention times being calculated as the mean of the three.

2.3.3 Results.

The retention time data obtained for the n-alkanes and used to determine the Kovats indices on Tenax G.C. under the operating conditions described are given in Table 2.3.1. The calculated Kovats indices with their appropriate vales of (K) and (C) parameters are given for the fifty five solvents tested in Table 2.3.2.

n-Alkane	Formula C	H	Kovat Index	100°C Rt	100°C logRt	150°C Rt	150°C logRt	180°C Rt	180°C logRt	210°C Rt	210°C logRt
Methane	1	4	100	87	1.940	24	1.380	12	1.079	7	0.845
Ethane	2	6	200	126	2.100	35	1.544	18	1.255	9	0.954
Propane	3	8	300	193	2.286	51	1.708	28	1.447	17	1.230
Butane	4	10	400	269	2.430	75	1.875	41	1.613	26	1.415
Pentane	5	12	500	398	2.600	109	2.037	59	1.771	34	1.531
Hexane	6	14	600	550	2.740	161	2.207	92	1.964	55	1.740
Heptane	7	16	700	724	2.860	236	2.373	138	2.140	89	1.949
Octane	8	18	800	1000	3.000	346	2.539	206	2.314	132	2.121
Nonane	9	20	900	1479	3.170	507	2.705	308	2.489	195	2.290
Decane	10	22	1000	2089	3.320	743	2.871	461	2.664	295	2.470
Undecane	11	24	1100	3090	3.490	1091	3.038	689	2.838	466	2.660
Dodecane	12	26	1200	4266	3.630	1600	3.204	1030	3.013	692	2.840
logRt = m * KI + c			m	0.1516		0.1660		0.1756		0.1830	
			a	1.8118		1.2112		0.9074		0.6477	
Correlation			r	0.9995		1.0000		1.0000		0.9992	

Table 2.3.1 - Retention Times (seconds) and Log Values for the n-Alkanes on Tenax G.C.

Solvent	100°C Index	150°C Index	180°C Index	210°C Index	K	C
Carbon Tetrachloride	872	741	662	583	2.62	1134
Chloroform	730	665	626	587	1.30	860
Dichloromethane	845	675	573	471	3.40	1185
Methanol	269	406	488	569	2.72	3
Acetaldehyde	322	465	551	637	2.86	35
2-Chloroethanol	843	775	734	692	1.37	980
Ethanol	409	476	516	555	1.32	276
Ethylene Glycol	796	801	804	807	0.10	786
Trichloroethylene	830	769	732	695	1.22	952
111-Trichloroethane	816	869	901	933	1.06	709
Acetone	549	553	555	557	0.07	541
Allyl Alcohol	623	607	597	587	0.32	655
3-Chloropropane diol	1505	1249	1095	603	7.68	2343
Dichloropropanol	1435	1182	1031	941	4.58	1880
2-Methoxy Ethanol	684	722	745	768	0.76	607
n-Propanol	567	584	594	603	0.32	534
iso-Propanol	513	518	539	560	0.42	464
Propylene Glycol	855	850	847	844	0.10	865
n-Butanol	402	724	917	1109	6.42	240
2-Butanol	352	649	827	1004	5.92	240
n-Butyric Acid	489	868	1095	1322	7.57	268
22´Dichloroethylether	702	1357	1750	2143	13.10	608
Diethyl Ether	301	526	661	796	4.50	149
Diethylene Glycol	648	1323	1728	2133	13.50	702
1,4 Dioxan	643	1001	1216	1431	7.16	73
2-Ethoxy Ethanol	271	824	1156	1488	11.06	835
Ethyl Acetate	351	646	823	1000	5.90	239
Methyl Ethyl Ketone	419	666	814	961	4.92	73
Tetrahydrofuran	412	702	876	1050	5.80	168
Cyclopentanol	1235	939	761	583	5.92	1827

Table 2.3.2a - Kovats Indices, K and C values for Fifty Five Standard Solvents on Tenax G.C.

Solvent	100°C Index	150°C Index	180°C Index	210°C Index	K	C
Cyclopentanone	1316	985	786	587	6.62	1978
Furfural	1436	1070	850	630	7.32	2168
Furfuryl Alcohol	1943	1108	854	353	14.08	3317
n-Pentanol	1011	820	705	590	3.82	1393
iso-Propyl Acetate	413	723	909	1095	7.39	343
Pyridine	1105	919	807	695	3.72	1477
Aniline	1141	1173	1192	1210	0.62	1078
Benzene	921	786	705	624	2.70	1191
2-Butoxy Ethanol	1259	1113	1025	937	2.92	1551
Cyclohexane	864	768	710	652	1.92	1056
Cyclohexanol	672	849	955	1061	3.53	318
Cyclohexanone	1444	1174	1012	850	5.40	1984
Methyl i-Butyl Ketone	1027	884	754	624	3.66	1408
Paraldehyde	1132	986	898	810	2.92	1424
Amyl Acetate	1693	1070	696	321	12.47	2940
Benzaldehyde	561	923	1140	1357	7.23	162
Benzyl Alcohol	618	998	1226	1454	7.60	142
Methyl Cyclohexane	781	774	770	766	0.13	794
Methyl Phenyl Ether	1498	1223	1058	893	5.50	2048
Toluene	1122	937	826	715	3.70	1492
Di-n-Butyl Ether	1383	1067	877	687	6.32	2015
Ethyl Benzene	1148	1058	1004	950	1.80	1328
Styrene	1254	1121	1042	961	2.66	1520
Xylene	1386	1106	938	770	5.60	1946
Tetrahydronaphthalene	686	966	1134	1302	0.10	961

Table 2.3.2b - Kovats Indices, K and C values for Fifty Five Standard Solvents on Tenax G.C.

2.3.4 Discussion.

The method does appear to work in ideal situations and test unknowns, randomly selected from the fifty five solvents examined and run through the procedure were identified correctly. This method of identification is of course dependant on the fact that the values of (K) and (C) have been determined for the solvent in question.

Another problem which requires highlighting is the case where two solvents have the same retention time at the temperature being used and it is important to take this into consideration when confirming the solvent identity by the judicious selection of a second temperature.

2.3.5 Conclusion.

The above method provides a quick source of qualitative information on unknown solvent mixtures. The data lends itself well to computerisation and a database could be established to provide quick access to the information and to suggest possible secondary temperatures for confirmation of the suggested identities. This form of data is considerably more useful than retention time lists as it is quick and dependant only on the carrier gas flow rate. Retention time data alone is of course also dependant on temperature which, in the majority of cases, cannot be fixed until component separation has been achieved.

Although this method of solvent indentification is reliable in the majority of cases, there are occasions when, as the database grows, two or more possible alternative identities would be proposed or the solvent does not already exist in the database. In these cases and where absolute identification is required a more selective detection system is required, ultimately the use of mass spectroscopy.

2.4 The Use of Mass Spectroscopy in the Identification of Solvents.

2.4.1 Introduction.

Information on the type of product, and therefore the identity of the solvents involved, is not always made available when samples are submitted for analysis from cases of suspected volatile substance abuse. The method of retention index measurement can be useful in selecting possible identities, however, alone it is insufficient due to the imposed limitations of previously calculated data and on the near coincidence of data for some of the solvents. The variations in column temperature required to provide separation and identification can become tedious and can be exasperated by the additional fact that many different solvents may have been abused.

The use of mass spectroscopy is an important tool in the indentification of abused solvents and provides a means of positively confirming the solvent suggested by retention index measurements.

The manual procedure for identifying compound structures from their mass spectra can be a time consuming process which may be aided to some extent by referral to a previously constructed library of pure solvents. This library was assembled from the mass spectra obtained from each of the

available solvents thought of as the most likely candidates for abuse.

2.4.2 Procedure.

Instrumentation

A Pye 104 gas chromatograph was used equipped with a glass column (1.5m x 6mm) packed with Tenax G.C. and operated at a temperature of one hundred and fifty degrees. A V.G. Micromass 16F mass spectrometer was used as a detection system, the operating parameters being those given in Table 2.4.1. The carrier gas used was CG grade Helium at a flow rate of thirty millilitres per minute. The mass spectrometer was controlled by the VG2035 data system with data aquisition parameters as given in Table 2.4.2.

Parameter	Value	
Mass Range	20 - 250	m/e
Electron Energy	70	eV
Source Temperature	240	oC
Interface Temperature	275	oC
Multiplier	2.0	KV
Magnet Scan	4.0	KV
Gain	1 E-6	A
Response Time	0.001	seconds
Scan Law	Exponential Down	
Scan Time	3.0	seconds
Inter-scan Delay	3.0	seconds
Source Pressure	2 E-6	Torr

Table 2.4.1 - Operating Parameters for the V.G. Micromass 16F when used for the Collection of Reference Spectra Data.

Parameter	Value
Lock Mass	40 m/e
Lock Mass Tolerance	3 m/e
Run Duration	30 minutes
Start Delay	5 seconds
Scan Control	Data System
Scan Law	Exponential Down
Store Times	Yes
Enter All Scans	Yes
Multiplier Threshold	500
Low Mass Limit	20 m/e
Inter-scan delay: Initial	3.0 seconds
Final	3.0 seconds
Peaks	Yes
T.I.C.	Yes
Retention Time	Yes

Table 2.4.2 - Data Aquisition Parameters for the VG2035 Data System when used for the Collection of Reference Spectra Data.

Experimental

A 0.1 microlitre sample of pure solvent was injected on to the column. Data in the form of total ion current and individual ion intensities was collected by the data system from the mass analyser. For each solvent this represented a collection of about two hundred scans. The scan which coincided with the retention time of the solvent peak in the total ion current trace was then examined before selection for inclusion in the reference library. Figure 2.4.1 shows a typical total ion current trace, obtained in this example for toluene, and the mass spectrum of the selected scan for this run is shown in Figure 2.4.2.

After manually selecting the best scan from the total ion current trace the data is then transfered to the library file using the library creation parameters given in Table 2.4.3.

Figure 2.4.1 - Typical total ion current trace obtained for toluene.

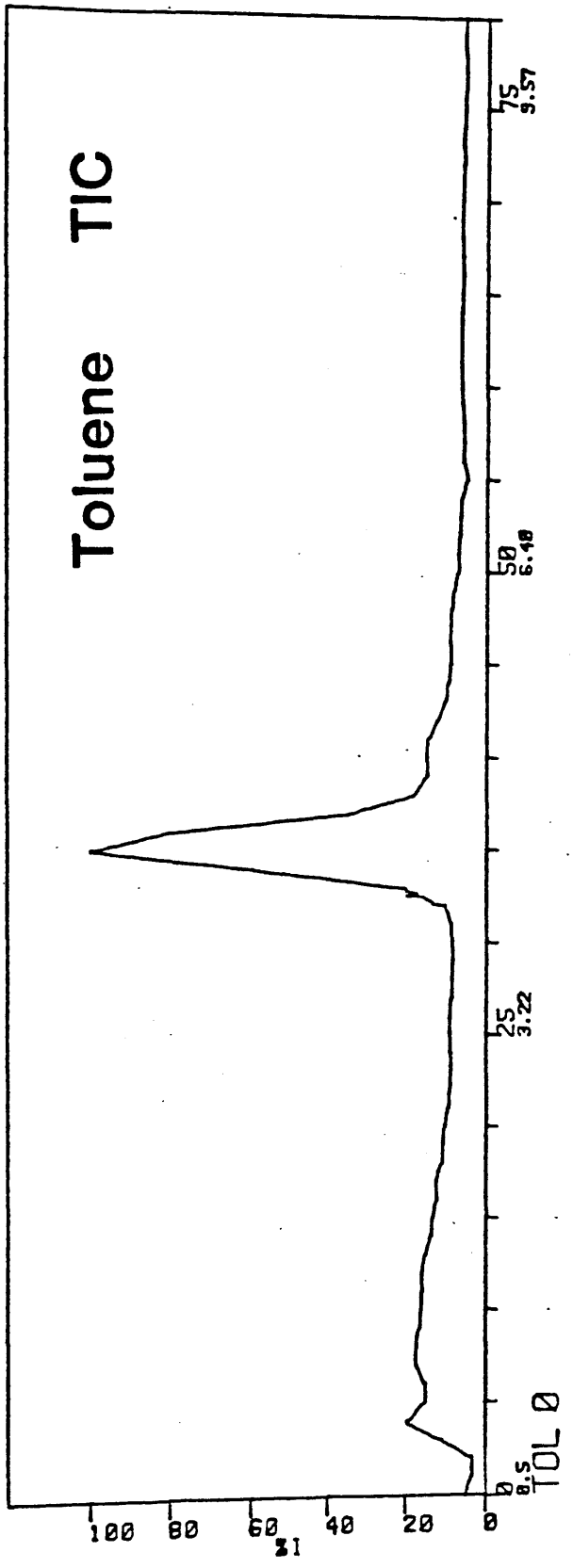
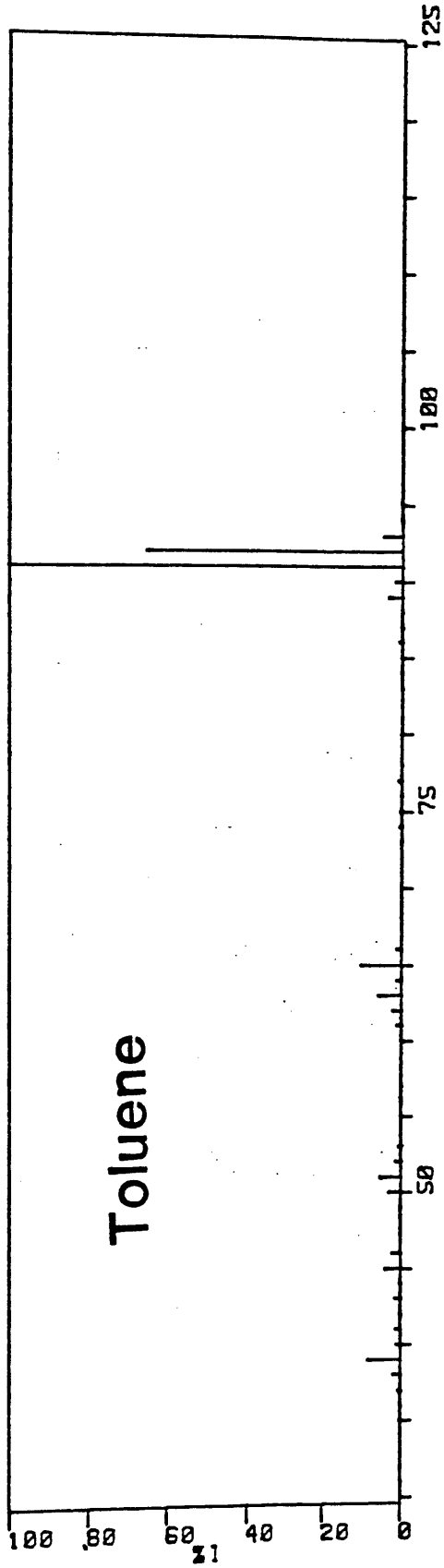


Figure 2.4.2 - Mass spectrum of the selected scan for toluene.



Parameter	Value
MN1 EC	Elemental Composition
MN2 CN	Compound Name
MN3 MW	Molecular Weight
Excluded Masses	None
Low Mass Limit	20
High Mass Limit	300
Extended Filters	No
Library File Type	F
Search Peaks	12
Minimum Fit	850
Matches	5
Minimum Base Intensity	750
Minimum Peak Match	2
Filters	No
Elements	No
Q to Report	Yes

Table 2.4.3 - Data Library Creation Parameters for the Solvent Reference Library on the VG2035 Data System.

2.4.3 Results.

The resulting library was stored on disc for future reference. The data was examined and the six peak index given in Table 2.4.4. was assembled to facilitate manual searches of the data in the event of failure of the data system.

Solvent	Six Peak Index m/e						Mol.
	1	2	3	4	5	6	Wt.
2-Chloroethanol	31	27	43	29	49	51	80
Ethylene Glycol	31	33	29	45	43	42	62
2-Ethoxy Ethanol	31	59	29	45	43	72	90
Tetrahydrofuran	42	41	72	71	27	43	72
Acetone	43	58	27	42	26	39	58
Methyl i-Butyl Ketone	43	58	57	100	41	85	100
3-Chloropropane diol	43	61	44	79	31	29	110
iso-Propyl Acetate	43	61	87	59	41	42	102
Methyl Ethyl Ketone	43	72	29	57	27	42	72
2-Methoxy Ethanol	45	29	31	43	47	76	76
2-Butanol	45	41	59	31	56	55	74

Table 2.4.4a Six Peak Index Stored in the Solvent Library Reference File.

Solvent	Six Peak Index m/e						Mol. Wt.
	1	2	3	4	5	6	
Paraldehyde	45	43	89	87	117	131	132
Diethylene Glycol	45	75	43	31	76	58	106
Dichloromethane	49	84	86	51	47	88	84
Cyclopentanone	55	41	84	56	27	42	84
Cyclohexanone	55	42	41	98	39	69	98
Cyclohexane	56	84	41	55	42	69	84
Allyl Alcohol	57	29	31	39	58	27	58
Di-n-Butyl Ether	57	29	41	56	87	27	130
Cyclopentanol	57	29	41	67	39	27	86
2-Butoxy Ethanol	57	45	41	29	87	39	118
Cyclohexanol	57	82	67	41	39	29	100
Benzaldehyde	77	105	106	51	50	78	106
Benzene	78	77	52	51	50	39	78
n-Pentanol	79	43	81	45	39	49	88
Pyridine	79	52	51	50	78	39	79
Dichloropropanol	79	81	43	49	29	27	128
Benzyl Alcohol	79	108	77	107	51	39	108
Methyl Cyclohexane	83	55	41	98	42	39	98
Chloroform	83	85	47	87	48	49	118
1,4 Dioxan	88	29	58	31	27	43	88
Toluene	91	92	65	39	51	63	92
Ethyl Benzene	91	106	51	39	65	77	106
Xylene	91	106	105	51	77	39	106
22´Dichloroethylether	93	63	27	65	95	31	142
Aniline	93	66	65	39	92	94	93
Trichloroethylene	95	130	132	60	97	35	130
Furfural	96	95	39	29	38	37	96
111-Trichloroethane	97	99	61	26	27	63	132
Furfuryl Alcohol	98	41	81	97	53	42	98
Tetrahydronaphthalene	104	91	132	117	115	51	132
Styrene	104	103	78	51	77	50	104
Carbon Tetrachloride	108	65	78	39	77	51	108
Methyl Phenyl Ether	108	65	78	39	77	51	108

Table 2.4.4b - Six Peak Index Stored in the Solvent Library Reference File.

2.4.4 Discussion.

The efficiency of a mass spectral library is largely dependant on the number of individual reference spectra that it contains. Although several volumes exist containing peak indices their practical usefulness is often restricted by the use of specific operating conditions and it is well known that instrumental variation alone can cause quite large discrepancies between results on different spectrometers. This is particularly true for low molecular weight, simple structured compounds where the fragmentation pattern is not very extensive. The single ion trace of toluene is a good demonstration of this fact, Figure 2.4.2, where the peaks of only two ions have intensities greater than ten per cent and only five ions above five per cent. It must therefore be preferred to establish, whenever possible, reference file data obtained on the instrument to be used in assessing unknowns, and ideally under the same standard operating conditions.

It should also be recognised that the six peak index alone does not confirm the identity of an unknown. Attention is drawn to the similarity between the data for carbon tetrachloride and methyl phenyl ether, despite the fact that these two compounds are chemically quite different. It is therefore important to compare the complete mass spectra and even retention data before assigning any specific identity to an unknown.

2.4.5 Conclusion.

The mass spectra of the forty four solvents listed in Table 2.4.4 were successfully obtained and were stored for future reference as a library file.

This file was then available for use in the unequivocable identification of unknown solvents in cases of volatile substance abuse or where the identity of a solvent in a product was of some forensic interest.

2.5 Conclusion.

The use of gas chromatography with a Tenax G.C. column has been demonstrated as a valuable instrument in the qualitative analysis and identification of solvents. In addition the use of mass spectroscopy has been illustrated to provide further qualatative information. The limitations of the methods involved depends to a great extent on the availability of data on the solvents concerned and information about the formulation of the product.

During the development work and the selection of suitable standard chromatographic conditions a considerable database of retention indices and mass spectra have been established. This database should provide a starting point for the identification of volatiles of abuse both in the suspect products and in blood or tissue samples.

To provide a more clinical appraisal of the state of solvent inhalation abuse it is important that wherever possible not only the identity of an abused solvent is established but also that quantitative information is obtained. The methods described in this chapter will therefore form the basis for the development of quantitative methods and their subsequent use in providing statistics concerning the status of volatile substance abuse.

CHAPTER THREE

QUANTITATIVE MEASUREMENT OF VOLATILE SUBSTANCES IN BIOLOGICAL FLUIDS

3.0 Introduction.

This chapter details the development of a quantitative method for the measurement of abused solvents in blood and tissue samples. This method, initially intended for the measurement of toluene was later modified to determine levels of trichloroethylene, trichloroethane, methanol, ethanol and chloroform. Results from cases involving these solvents are also described.

In addition to the development of the analytical technique and its validation, details are given of the results obtained from a survey of blood samples taken from suspected volatile substance abusers in the Glasgow area.

The investigation of a number of fatalities resulting from the abuse of volatiles is also detailed. Some discussion is made on correlation between the effects produced on the individual and the measured levels of the abused solvent.

The possible use of metabolite measurements of various volatile substances is examined in particular the two which have appeared as most popular in the period of this study,

toluene and trichloroethylene. A detailed review of the possibility of using metabolite measurements to monitor the problem of halothane hepatitis is also undertaken.

Discussion is also made of the possible use of breath measurements and their possible virtue as a non invasive method for monitoring volatile substance abuse.

Analytical methods are established for the measurement of hippuric acid as a metabolite of toluene, trifluoroacetic acid as a metabolite of halothane and trichloroacetic acid and trichloroethanol as metabolites of trichloroethylene.

The potential problem in the use of dichloromethane is illustrated by the observation of carbon monoxide as its metabolite.

3.1 Evaluation of Accuracy in Preparing Standard Solutions of Toluene.

3.1.1 Introduction.

The initial use of Tenax G.C. as a column packing material for the qualitative analysis of solvents has demonstrated it to be both reliable and applicable to a wide range of solutes. Its high thermal stability and very low bleed characteristics make it ideal for both programmed temperature gas chromatography and for use with a mass spectometric detection system.

The choice of toluene as the first volatile of abuse to be studied arises from the apparent popularity of this solvent among abusers (Watson 1976) and its use along with acetone as a solvent base for the majority of contact adhesives, a popularly abused product. It was decided to defer the measurement of acetone due to its low toxicity and its natural occurrence in blood.

Owing to the fact that low levels of toluene, less than one hundred microgrammes per millilitre, are expected, one of the foremost requirements in the development of any quantitative method for measuring these levels is the preparation of accurate standards. Aqueous standards are easier to prepare than blood standards and can usually be prepared with more precision. However, the same problem exists with both, in that the preparation of standards at such low levels requires

many dilution steps and the transfer of small volumes of volatile solvent. These dilution errors are smaller for solutes which are completely miscible with water, acetone, methanol, ethanol, etc., but additional errors are involved when toluene, benzene and other immiscible solutes are used. These can to some extent be overcome by the use of a co-solvent to increase the water solubility of the solute. However, this is really undesirable as it represents another component to be separated which would be present in a comparitively high concentration. This may result in an effective screening of the solute peaks or cause other interferences.

The accuracy of the preparation of such a standard was investigated by preparing and analysing a set of toluene standards by gas liquid chromatography.

Benzene was chosen as an internal standard as this compound has very similar properties to, and was known to be resolved clearly from, toluene. As both solutes are only very slightly soluble in water acetone was used as a co-solvent.

3.1.2 Method.

Preparation of Standard Solutions

A series of standard stock solutions were prepared using the following procedure: One millilitre of benzene (AnalaR grade) was transfered into each of six volumetric flasks of twenty-five millilitre capacity using a bulb pipette (Grade

A). To these were then added 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 millilitre aliquots of toluene (AnalaR grade) respectively. The flasks were then diluted to volume with acetone (AnalaR grade).

A fifty microlitre aliquot was then removed from the stock solution using a Hamilton syringe and diluted to one hundred millilitres in a volumetric flask with distilled water. This dilution step was repeated for each standard. The entire procedure was replicated ten times.

In turn a one microlitre sample was removed from each of the prepared solutions and injected into the gas chromatograph.

Gas Chromatographic Conditions

A Pye 104 gas chromatograph was used equipped with a flame ionisation detector. The results were recorded and integrated using a Hewlett Packard 3370B integrator. The chromatographic column used was 1.5m x 6mm glass packed with Tenax G.C. and was operated at an isothermal temperature of 180^{o}C with a nitrogen carrier gas flow rate of thirty five millilitres per minute.

3.1.3 Results.

The resulting area integrations were used to give a peak area ratio for toluene : benzene. These ratios, for each set of standards, were plotted against concentration of toluene in microgrammes per millilitre and the data obtained subjected to

linear regression analysis. The value was thus obtained for the concentration gradient. The results are shown in Table 3.1.1.

ug/ml	0	10	20	30	40	50	Gradient	r
1	0.000	0.438	0.853	1.406	1.727	2.151	0.0434	0.9984
2	0.000	0.667	0.865	1.457	1.728	2.150	0.0415	0.9916
3	0.000	0.488	0.790	1.309	1.845	2.457	0.0482	0.9953
4	0.000	0.649	0.833	1.298	1.833	2.348	0.0450	0.9930
5	0.000	0.790	0.983	1.552	1.659	2.086	0.0419	0.9833
6	0.000	0.626	0.933	1.401	1.860	2.216	0.0436	0.9967
7	0.000	0.572	0.914	1.312	1.774	2.346	0.0450	0.9969
8	0.000	0.458	0.845	1.292	1.987	2.350	0.0480	0.9962
9	0.000	0.839	1.059	1.327	1.813	2.292	0.0419	0.9832
10	0.000	0.534	0.710	1.312	1.668	2.223	0.0432	0.9937
Mean	0.000	0.606	0.879	1.367	1.789	2.226	0.0442	0.9928
SDev	0.000	0.135	0.099	0.085	0.100	0.116	0.0024	0.0054

Table 3.1.1 - Toluene to Benzene Peak Area Ratios for Ten Sets of Standards.

3.1.4 Discussion.

The accuracy, represented as the mean relative standard deviation over the concentration range, was determined to be 5.4% This level is considered acceptable cosidering the steps involved in preparing the standard solutions. The peak shape as observed on the chromatogram was also acceptable and no interference resulted from the use of acetone as a co-solvent. It was noted, however, that peak tailing was causing problems at the ten microgramme per millilitre level due to the low peak height to width ratio.

It is reasonable to assume that this degree of accuracy would be similar to that obtained for other immiscible volatile solute standards where a co-solvent would be required.

3.1.5 Conclusion.

Any unknown sample level obtained from calibration curves using the above method should be quoted as $\pm$ 5 microgrammes per millilitre.

Owing to the fact that water separates on a Tenax G.C. column and the importance of maintaining extended column life, the possibility of using headspace samples rather than direct injection has to be investigated.

3.2 The Determination of Optimum Headspace Equilibrium Temperature for Samples Containing Toluene, Benzene and Acetone.

3.2.1 Introduction.

Tenax G.C. separates water from other solvents and as this peak is eluted first with a high degree of tailing the use of aqueous samples restricts the use of this column for solvents. To reduce the quantity of water that is injected on to the column without reducing the quantity of solvent is difficult. However, due to the fact that the majority of solvents dealt with are particularly volatile the use of headspace (vapour phase) samples should provide a method of reducing the volume of water with only a small reduction, by comparison, of the quantity of solute.

Headspace samples have other advantages. They are clean, in that they do not contain cell debris or high boiling, and therefore slow eluting, liquids. This has the effect of prolonging column life, reducing column bleed and improving peak shape. Another advantage is that the volume of headspace injected can be as great as one millilitre without drastic changes in the column efficiency. An equivalent volume of aqueous solution would, under normal operating conditions, severely damage the column by the distillation of the stationary phase or fracturing of the column packing.

The quantity of solute present in the headspace is dependent on both the temperature and the concentration in the liquid phase. It is therefore important to establish an optimum equilibrium temperature for analyses using a headspace method. Toluene, benzene and acetone solutions were incubated at a number of temperatures between 40°C and 100°C to establish the optimum equilibration temperature for analysis. Headspace samples were analysed by gas liquid chromatography.

3.2.2 Method.

Preparation of Standards

A solution of toluene, benzene and acetone was prepared by dissolving two grammes each of AnalaR grade toluene and benzene in twenty grammes of acetone and diluting to a total volume of one litre with distilled water. A stock solution was then prepared by the dilution of one millilitre of this solution to one hundred millilitres in a volumetric flask with distilled water. The resulting solution therefore contained twenty microgrammes of each of toluene and benzene and two hundred microgrammes of acetone per millilitre.

One millilitre aliquots of this stock solution were then transferred by bulb pipette (Grade A) to each of twenty-one Hypo-vials which were then sealed with a butyl-rubber septum and aluminium crimp seal.

3.2.3 Results.

The peak area measurements for each of the solutes is given in Table 3.2.1. The results were plotted on a graph and the resulting curves are given in Figures 3.2.1 to 3.2.3.

Temperature		40°C	50°C	60°C	70°C	80°C	90°C	100°C
Toluene	1	789	1219	1234	1263	1350	1552	1983
	2	640	1117	1280	1389	1354	1573	1959
	3	1018	1197	1358	1519	1662	1805	1930
Mean:		815	1118	1291	1390	1455	1643	1957
Benzene	1	1382	2270	2561	2713	2813	3659	4572
	2	1528	2021	2465	2909	3303	3698	4043
	3	1702	2476	2468	2465	2803	3608	4627
Mean:		1537	2255	2498	2695	2973	3655	4414
Acetone	1	1079	1603	1671	2274	2796	3760	4511
	2	775	1427	2014	2602	3124	3646	4103
	3	1494	1866	1923	2195	3150	4151	6031
Mean:		1116	1632	1869	2357	3023	3852	4881

Table 3.2.1 - Peak Areas for Toluene, Benzene and Acetone Taken at Seven Different Equilibration Temperatures.

Figure 3.2.1 - Plot of Peak Area vs. Equilibration Temperature for Toluene.

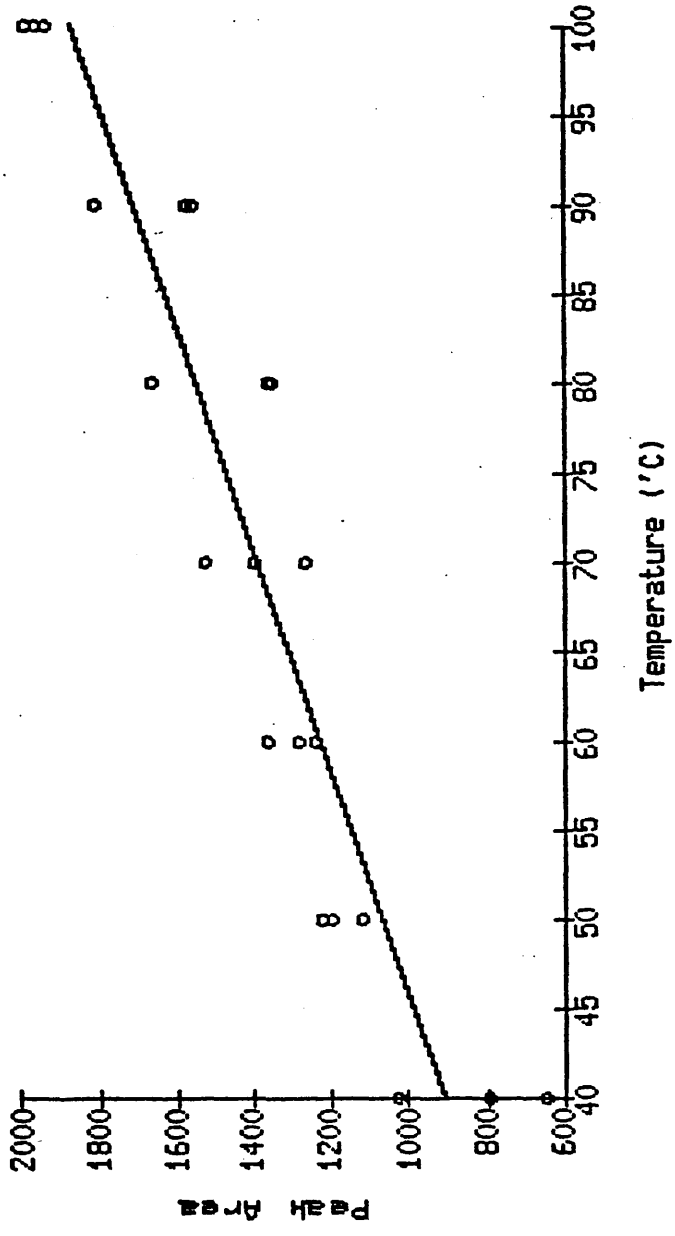


Figure 3.2.2 - Plot of Peak Area vs. Equilibration Temperature for Benzene.

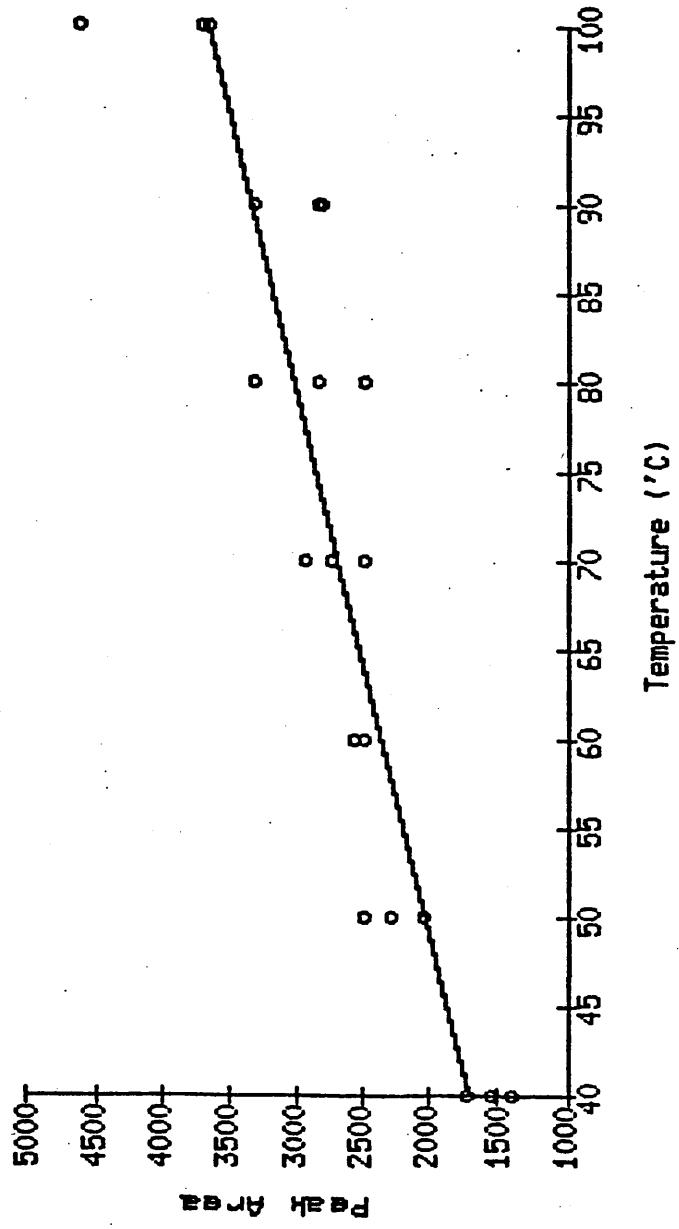
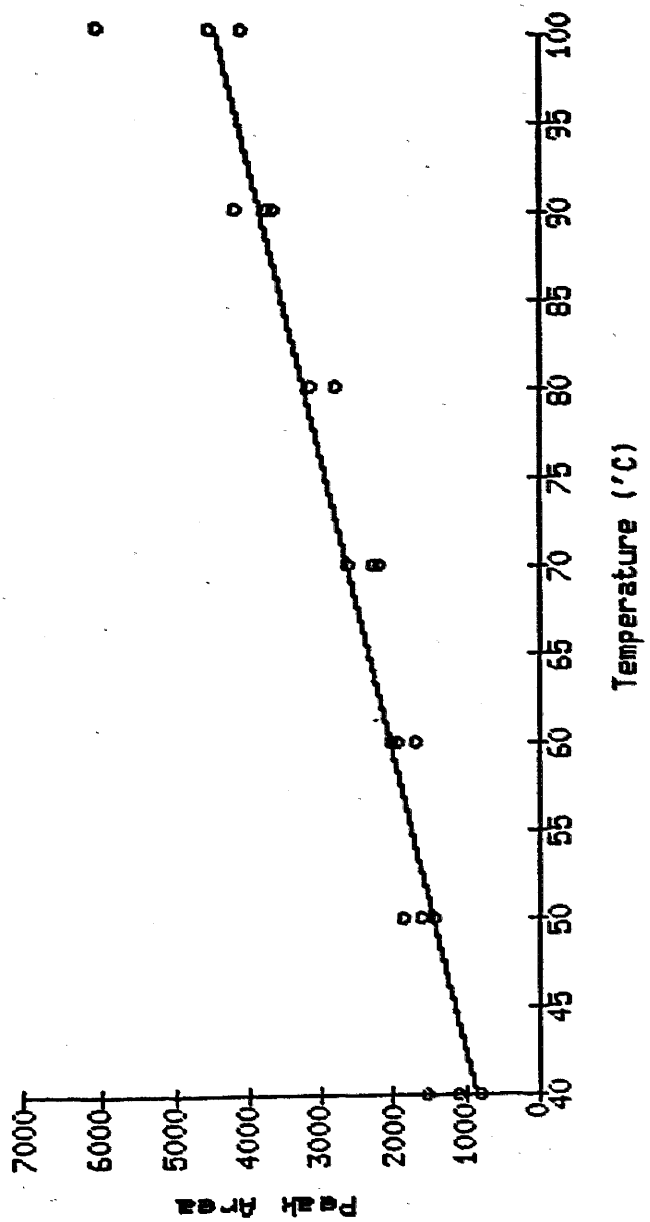


Figure 3.2.3 - Plot of Peak Area vs. Equilibration Temperature for Acetone.



3.2.4 Discussion.

Elevated temperatures are used to increase the quantity of the solute in the vapour phase. This concentration will continue to increase until saturation occurs. As the solutions that are being analysed are aqueous and the solutes are in very low concentrations this saturation will be unlikely. Indeed at these low concentrations the isotherm may be assumed to be linear. The selection of optimum temperature therefore mainly depends on convenience and on the sensitivity of the detection system to the concentrations being measured.

The choice of 60^{o}C as equilibrium temperature was therefore reached for the following reasons:

1) The quantity of water in the vapour phase will not be too great. This has particular importance when using a column such as Tenax G.C. which chromatographs water.

2) The temperature is not so high as to present condensation problems. A gas tight syringe may be heated only to about 50^{o}C before it becomes too hot to handle with comfort.

3) The temperature is elevated sufficiently to increase the vapour concentration of the majority of volatile substances abused and therefore increases the method sensitivity.

3.2.5 Conclusion.

The results of this experiment demonstrate the relationship between toluene, benzene and acetone concentration with increasing temperature of equilibrium for a constant equilibration period.

An optimum equilibration temperature of 60^{o}C was selected as a reasonable compromise under the experimental conditions. At this temperature the rate of change of concentration of solute in the vapour phase was observed to be at a minimum.

Equilibrium of a vapour phase over liquid phase is dependent primarily on temperature but the time allowed for the equilibration process may also be important. To determine the importance of this factor it is necessary to perform an experiment to examine equilibration time.

3.3 The Dependence of Vapour Phase Samples of Toluene on the Equilibration Time at 60°C.

3.3.1 Introduction.

The quantity of any volatile component in the vapour phase is dependent on an equilibrium state between the vapour in the vial and the liquid. This equilibrium is dependent on the concentration in the liquid phase, the temperature and the volume of vapour.

Equilibration can be considered as a process of evaporation from the liquid phase. This evaporation will continue until a particular vapour pressure is reached at which point the concentration of the solute in the vapour will become constant and dependent only on the concentration of the solute in the liquid.

It is therefore important to allow sufficient time to elapse for this process to occur in order that any head-space sample is a true representation of the concentration of the solute in the liquid phase.

In order to determine the optimum equilibration time a series of identical solutions were prepared and examined after different equilibration periods of between 0 and 240 minutes at 60°C.

3.3.2 Method.

Preparation of Standards

An aquous solution of toluene was prepared by dissolving one gramme of AnalaR grade toluene and two and one-half grammes of redistilled ethyl benzene, a proposed internal standard, in ten millilitres of acetone and diluting to a total volume of one litre in a volumetric flask with distilled water. A one millilitre aliquot of this solution was then further diluted to one litre in a volumetric flask with distilled water. The resulting standard solution contained toluene at a concentration of one microgramme per millilitre and ethyl benzene at a concentraion of two and one-half microgrammes per millilitre.

Twelve, six millilitre Hypo-vials were then prepared by transferring by bulb pipette (Grade A) a one millilitre aliquot of this standard solution to each vial. The vials were then sealed with a butyl-rubber septum and an aluminium crimp seal.

Sample Incubation

The vials were then placed in an aluminium heating block (Tecam Driblock DB3) previously equilibrated at 60^{o}C. Head-space samples of five hundred microlitres were then removed using a Hamilton gas-tight syringe, pre-heated to 60^{o}C to reduce condensation of the vapour phase in the syringe. Samples were removed at intervals after placing in the heating block and immediately injected into the gas chromatograph.

Gas Chromatographic Conditions

As detailed in Section 3.1.2., and operated at an isothermal temperature of 150°C. The areas of the peaks due to toluene and ethyl benzene were measured and their ratio determined. The procedure was repeated in triplicate.

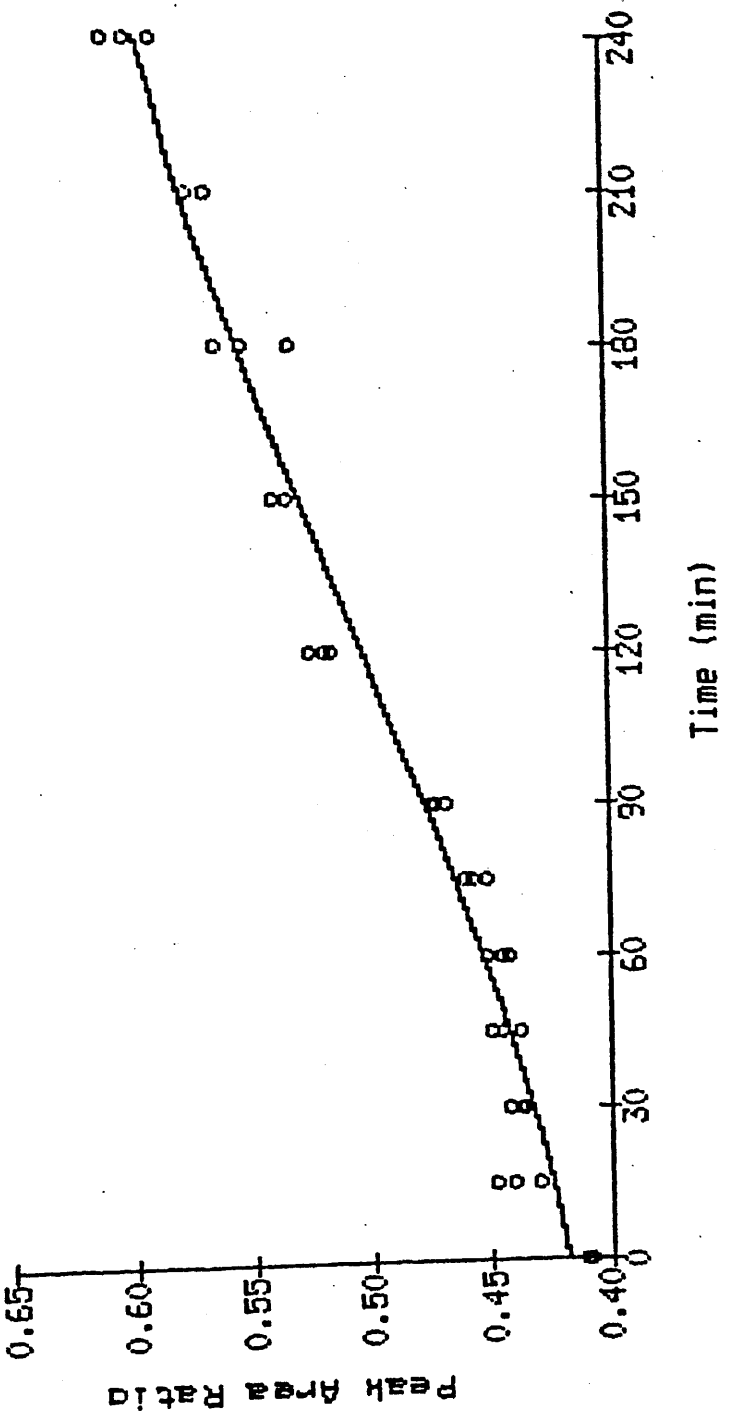
3.3.3 Results.

The peak area ratios of toluene to ethyl benzene are given in Table 3.3.1. These values were plotted as a function of time and the resulting graph is shown in Figure 3.3.1.

Time.(minute)	Run 1	Run 2	Run 3	Mean Ratio
0	0.407	0.409	0.408	0.4082
15	0.429	0.448	0.440	0.4396
30	0.435	0.442	0.437	0.4384
45	0.437	0.449	0.445	0.4439
60	0.442	0.451	0.445	0.4464
75	0.451	0.459	0.457	0.4560
90	0.468	0.473	0.472	0.4717
120	0.515	0.524	0.518	0.5195
150	0.534	0.540	0.534	0.5365
180	0.552	0.563	0.562	0.5597
210	0.566	0.574	0.574	0.5719
240	0.588	0.607	0.599	0.5984

Table 3.3.1 - Peak Area Ratios of Toluene to Ethyl Benzene as a Function of Equlibration Time.

Figure 3.3.1 - Plot of Peak Area Ratios of Toluene to Ethyl Benzene as a Function of Equlibration Time.



3.3.4 Discussion.

As can be seen from the graph, Figure 3.3.1, there is no point where true linearity exists. However, the period between fifteen and seventy five minutes the variation in the peak area ratio is less than two per cent and therefore may be approximated to linearity. Therefore a period of about forty five minutes for equilibration to occur can safely be used for the analysis.

3.3.5 Conclusion.

A period of forty five minutes should be allowed, in sealed vials of six millilitres total volume at a temperature of $60^{o}C$, for the vapour phase equilibrium of two millilitres of an aqueous solution containing one microgramme of toluene per millilitre.

3.4 Determination of the Blood : Water Vapour Concentration Ratio for Toluene.

3.4.1 Introduction.

The activity coefficient of the solute plays an important role in head-space gas chromatographic analysis. If the product ($m\ c_i * p_o * y_i$) is known then the concentration of component (i) in the samples can be directly calculated from the peak area. In almost all practical situations this product must be evaluated by measurement.

c_i = concentration, p_o = vapour pressure, y_i = activity coefficient

The practical application of gas chromatographic head-space analysis is almost exclusively applied to trace analysis where the solutions can be considered as ideal. Under these conditions the activity coefficient is constant and therefore the above described product is also constant provided that the detector used also demonstrates a proportional response. The flame ionisation detector does exhibit a proportional response for low concentrations ($m\ c_i$ = constant).

In addition to the above calibration problem we must consider the fact that water is used to prepare standards and not blood. This difference in sample matrix requires the determination of a correction factor to compensate for the different effects on vapour pressure produced by blood and water.

In order to determine this correction factor, the blood to water vapour concentration ratio, a series of identical standards of toluene were prepared in both water and whole blood and examined after equilibration under the conditions established in the previous experiments.

3.4.2 Method.

Preparation of Standards

A stock standard solution of toluene was prepared by diluting one gramme of toluene (AnalaR Grade) in one litre of a mixture of acetone and distilled water (1:9). Ten standard solutions were then prepared by further diluting one millilitre aliquots of this stock solution in one litre volumetric flasks with either distilled water or whole blood. Ten, six millilitre Hypo-vials for each standard were then prepared by transferring a one millilitre aliquot of the corresponding standard solution to each vial. The vials were then sealed with a butyl-rubber septum and an aluminium crimp seal.

Sample Incubation

The vials were then placed in an aluminium heating block previously equilibrated at 60^{o}C. After an equilibration time of forty five minutes a five hundred microlitre aliquot of the head-space above the liquid in the vial was removed with a gas-tight syringe, preheated to 60^{o}C, and injected into the gas chromatograph.

Gas Chromatographic Conditions

As detailed in Section 3.1.2., and operated at an isothermal temperature of 150°C. The area of the peak due to toluene was measured and the correction factor for blood : water vapour concentration ratio calculated from the results obtained.

3.4.3 Results.

The peak areas obtained for toluene and the blood to water vapour concentration ratio for each analysis are given in Table 3.4.1.

Blood	Water	Blood:Water Ratio
72433	44680	1.621
62957	40430	1.557
75637	42400	1.784
75727	42537	1.780
72173	44137	1.635
77427	38837	1.994
67867	45543	1.490
73610	41187	1.787
60143	39937	1.506
67933	39917	1.702
Mean Ratio		1.686
cov %		9.2 %

Table 3.4.1 - Peak Areas for 1 ug/ml of Toluene in Blood and Water.

3.4.4 Discussion.

The problem with the determination of a blood to water vapour concentration ratio in this way is the fact that direct measurement peak areas has to be used. It is not valid to use

an internal standard as the ratio for this would also be unknown. The use of peak areas also creates another problem in that the errors in the sampling are greatly increased. This is illustrated in the results by the wide variation in the areas between samples.

The average blood : water vapour concentration ratio, or correction factor for toluene was found to be 1.69 with a standard deviation of 0.15.

3.4.5 Conclusion.

The above experiment shows that the concentration of toluene in the vapour phase over blood was greater than that above water by a factor of 1.7.

The inconsistency of peak area measurement of toluene alone was also demonstrated. The most likely resource for improvement of this sampling inaccuracy might be offered by the use of an internal standard.

3.5 The Use of Ethyl Benzene as Internal Standard.

3.5.1 Introduction.

In section 3.1 the use of benzene as an internal standard for toluene analysis was examined. In general, it is expected that for the majority of cases to be encountered in volatile substance abuse investigation, toluene will be the most commonly abused solvent. However the fact that many other solvents are open to abuse indicates the importance of selecting a suitable internal standard. Although benzene would appear to be a good choice, as its availability for abuse is limited, there are several other solvents which elute at about the same time under the proposed chromatographic conditions. The most significant of these is ethanol. For this reason another internal standard had to be chosen.

Ethyl benzene is not used commercially to any great extent and separates well from toluene under the conditions proposed. Owing to its similar chemical properties to toluene it makes a more suitable internal standard.

The toluene levels obtained from the few cases quoted in the literature on volatile substance abuse indicate that blood levels of up to twenty micrograms per millilitre are to be expected (Schluneggar 1969, Collom & Winek 1970, Anthony, Bost, Thomson & Sunshine 1978, Nomiyama & Nomiyama 1978).

To examine the suitability of using ethyl benzene as an internal standard in the proposed method for measuring toluene, and to determine an estimate of the precision of preparing aqueous standards by this method, a series of standard curves were prepared and examined under the conditions established by the preceeding experiments.

3.5.2 Method.

Preparation of Standards

As both solutes are only very slightly soluble in water acetone was used as a co-solvent.

A stock solution of internal standard was prepared by diluting three grammes of redistilled ethyl benzene in one litre of a mixture of acetone and distilled water (1:9).

A stock solution of toluene was similarly prepared by diluting one gramme of toluene (AnalaR Grade) in one litre of a mixture of acetone and distilled water (1:9).

A series of standard solutions were then prepared by tranferring one millilitre of stock internal standard solution into each of six, one litre volumetric flasks. To these flasks an aliquot of either 0, 10, 20, 30, 40, or 50 millilitres of the toluene stock solution was added, and the resulting mixture then diluted to volume with water.

A one millilitre aliquot was then removed from each of the

standard solutions and placed in a six-millilitre hypo-vial. The vials were then sealed with a butyl-rubber septum and an aluminium crimp seal.

The procedure was repeated ten times to obtain information on the precision available in preparing standard curves in this way.

Sample Incubation

As detailed in Section 3.4.2.

Gas Chromatographic Conditions

As detailed in Section 3.4.2. The area of the peaks due to toluene and ethyl benzene were measured and the ratios calculated from the results obtained.

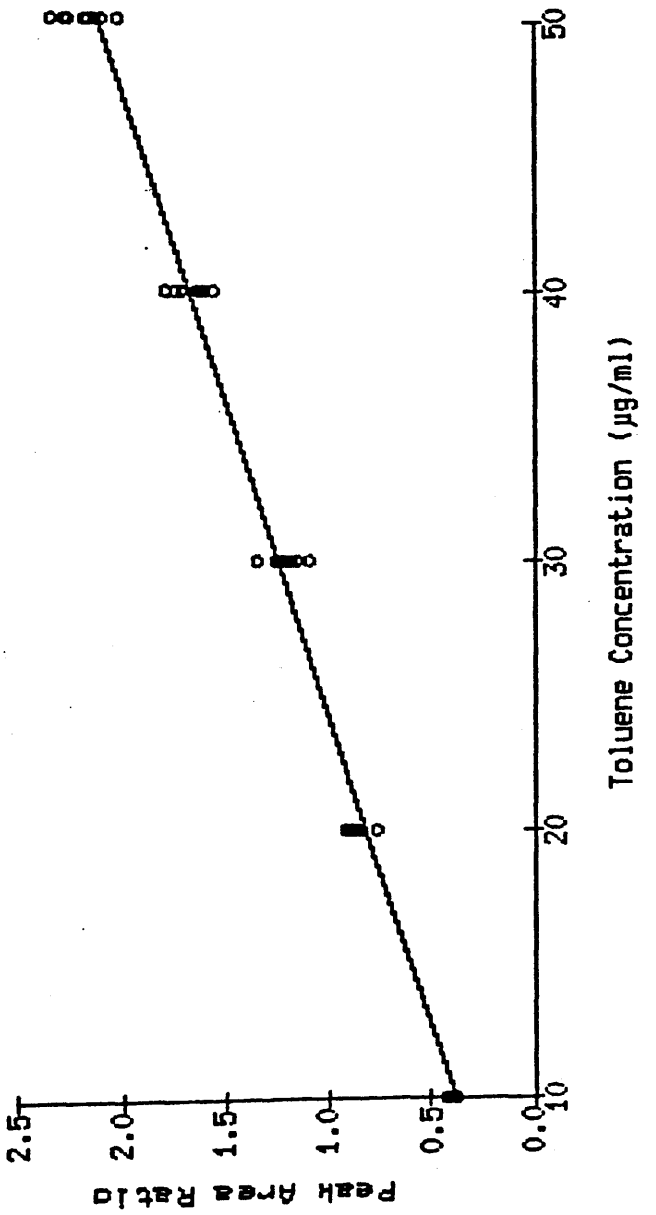
3.5.3 Results.

The peak area ratios for toluene to ethyl benzene for each set of standards are given in Table 3.5.1. These results were then plotted in the form of a calibration graph which is shown in Figure 3.5.1. The slope of this curve was found to be 0.045 with a correlation coefficient of 0.993 by linear regression analysis.

Conc.ug/ml	10	20	30	40	50
Std.Set 1	0.369	0.748	1.174	1.566	2.165
2	0.370	0.827	1.147	1.621	2.034
3	0.391	0.851	1.213	1.644	2.178
4	0.393	0.900	1.245	1.572	2.142
5	0.387	0.884	1.261	1.753	2.271
6	0.368	0.827	1.194	1.723	2.101
7	0.369	0.862	1.099	1.634	2.182
8	0.377	0.869	1.144	1.799	2.177
9	0.389	0.912	1.187	1.723	2.336
10	0.412	0.906	1.353	1.609	2.257
Mean	0.383	0.859	1.202	1.664	2.184
Std.Dev.	0.014	0.049	0.072	0.080	0.075

Table 3.5.1 - Toluene to Ethyl Benzene Peak Area Ratios for a Series of Standards.

Figure 3.5.1 - Calibration graph of Toluene to Ethyl Benzene Peak Area Ratios for a Series of Standards.



3.5.4 Discussion.

The error level observed in the standard curves with ethyl benzene was approximately 3.5 % This level is lower than that obtained with benzene as internal standard, Section 3.1, under the same chromatographic conditions, though this may be in part due to the weighing of standards rather than the use of small volume pipettes.

The straight line calibration graph and the possibility for increasing the sensitivity indicate that the method may be applicable to lower concentrations. The peak shapes are also greatly improved in the use of head-space samples by the removal of the large volume of water from the column.

3.5.5 Conclusion.

Ethyl benzene is a suitable internal standard for the quantitative measurement of toluene and possibly many other volatiles of abuse using the head-space method of analysis. The developed method for toluene could now be used to measure levels in blood from subjects exposed to toluene vapour.

3.6 Determination of the Blood : Water Vapour Concentration Ratio for Ethyl Benzene.

3.6.1 Introduction.

In section 3.4 the problems of using aqueous standards for the determination of volatiles in blood were discussed and the correction factor for toluene was obtained. In the same way a similar correction factor was determined for the internal standard ethyl benzene. This is necessary as it is assumed in using an internal standard that the vapour phase concentration produced in the head-space above blood will be the same as that produced in the vapour phase above water.

In order to determine this correction factor, the blood to water vapour concentration ratio, a series of identical standards of ethyl benzene were prepared in both water and whole blood and examined after equilibration under the conditions established in the previous experiments.

3.6.2 Method.

Preparation of Standards

An aqueous solution of ethyl benzene was prepared by dissolving two and one half grammes of redistilled ethyl benzene in three millilitres of acetone and diluting to a total volume of one litre in a volumetric flask with distilled water. One millilitre of this solution was then further

diluted to one litre in a volumetric flask with either distilled water or whole blood. The whole blood was obtained from outdated blood transfusion stock. The resulting standard solutions contained ethyl benzene at a concentraion of two and one half micrograms per millilitre.

Sample Incubation

As detailed in Section 3.4.2.

Gas Chromatographic Conditions

As detailed in Section 3.4.2. The area of the peak due to ethyl benzene was measured and the correction factor for blood : water vapour concentration ratio calculated from the results obtained.

3.6.3 Results.

The peak areas obtained for ethyl benzene and the blood to water vapour concentration ratio for each analysis are given in Table 3.6.1.

3.6.4 Discussion.

As was found with toluene in section 3.4 the errors are reflected by the high standard deviation obtained and are once again primarily caused by volume changes in the syringe. This adds further support to the requirement for the use of an internal standard.

Blood	Water	Blood:Water Ratio
15637	18577	0.842
15363	17217	0.892
14827	27277	0.544
14273	25227	0.566
13863	30937	0.448
14500	28210	0.514
13340	28777	0.464
14857	29590	0.502
14827	21243	0.698
14267	22367	0.638
Mean Ratio		0.611
cov %		25.4 %

Table 3.6.1 - Peak Areas for 2.5 ug/ml of Ethyl Benzene in Blood and Water.

The average blood : water vapour concentration ratio, or correction factor for ethyl benzene was found to be 0.61 with a standard deviation of 0.15.

3.6.5 Conclusion.

The above experiment shows that the concentration of ethyl benzene in the vapour phase over blood was less than that above water by a factor of 0.6. This is the inverse of the result observed for toluene and the ratio of the calibration factors of toluene to ethyl benzene is about 2.5. The values may therefore cancel each other out in practice.

3.7 Determination of the Calibration Factor for Aqueous Standards.

3.7.1 Introduction.

In sections 3.4 and 3.6 the blood : water vapour ratios were separately measured for toluene and ethyl benzene and observed to be 1.69 and 0.61 respectively. The method used to obtain these values was subject to large errors due to the use of a gas-tight syringe. These errors should be overcome by the use of an internal standard. The fact that the quantity of solute in the vapour phase is dependant on the composition of the liquid phase of the sample was discussed in section 2.1.1 and the explanation of dependance of activity coefficients on sample composition was given.

It therefore becomes necessary to examine if blood standards give the same toluene : ethyl benzene ratio as water standards, and to calculate any calibration factor required for water standards.

In order to determine this calibration factor a toluene and ethyl benzene standard was prepared in both water and whole blood then examined after equilibration under the conditions established in the previous experiments.

3.7.2 Method.

Preparation of Standards

A stock solution of the ethyl benzene internal standard was prepared by diluting two and one half grammes of redistilled ethyl benzene in one litre of a mixture of acetone and distilled water (1:9).

A stock solution of toluene was similarly prepared by diluting one gramme of toluene (AnalaR Grade) in one litre of a mixture of acetone and distilled water (1:9).

One millilitre of the toluene stock standard solution and one millilitre of the ethyl benzene stock standard solution were then further diluted to one litre in a volumetric flask with either distilled water or whole blood. The whole blood having been obtained from outdated blood transfusion stock. The resulting standard solutions contained toluene at a concentration of one microgram per millilitre and ethyl benzene at a concentraion of two and one half micrograms per millilitre. One millilitre aliquots were then removed from each standard and placed in individual Hypo-vials. The vials were then sealed with a butyl rubber septum and aluminium crimp seal.

Sample Incubation

As detailed in Section 3.4.2.

Gas Chromatographic Conditions

As detailed in Section 3.1.2, and operated at an isothermal temperature of 150°C. The analysis was repeated ten times and peak area ratios for toluene to ethyl benzene obtained. These ratios were then used to obtain a blood : water ratio.

3.7.3 Results.

The toluene to ethyl benzene ratios were calculated for both the blood and water standards and the ratios of blood to water standards determined. The results are given in Table 3.7.1.

Blood	Water	Water:Blood Ratio
0.569	0.545	0.958
0.593	0.583	0.983
0.596	0.561	0.941
0.573	0.575	1.003
0.586	0.574	0.980
0.585	0.574	0.981
0.596	0.508	0.852
0.582	0.567	0.974
0.594	0.551	0.928
0.576	0.533	0.925
Mean Ratio		0.953
cov %		4.03 %

Table 3.7.1 - Peak Area Ratios for 1.0 ug/ml of Toluene with Ethyl Benzene as Internal Standard in Blood and Water.

3.7.4 Discussion.

The observed water to blood ratio of 0.95 is of some suprise when considered with the fact that the individual ratios observed for toluene and ethyl benzene were 1.69 and 0.61

respectively. A Wilcoxson's summed ranks test on the water and blood ratios indicated that there is no significant difference between the two sets of data and it could therefore be acceptable to presume that the water : blood ratio is unity. This assumption would considerably simplify calculations and would if anything result in a underestimation of actual concentrations.

3.7.5 Conclusion.

The water to blood correction factor for standards of toluene with ethyl benzene internal standard can be assumed to be unity.

It is now possible to use the method for the measurement of toluene levels in actual blood samples from individuals exposed to toluene vapours. However it is important to first establish baseline levels and limits of detection.

3.8 Examination of Blood for Toluene to Establish "Normal" Levels.

3.8.1 Introduction.

The possibility that toluene has a "normal" level in blood of non-abusers and abusers alike must be considered when any investigation is carried out to establish abnormal levels. The origin of this toluene must be primarily environmental owing to the considerable use of toluene in commercial products. The other possible explanation for these levels could be founded in the possible formation through some metabolic process.

To establish these background levels ten samples of blood were obtained from volunteers selected from a population of non-abusers and persons not exposed to toluene during the course of their work. These samples were then examined for levels of toluene.

3.8.2 Method.

Preparation of Samples

i) One millilitre aliquots of the "normal" blood samples were then placed in individual Hypo-vials.

ii) A stock solution containing one milligram per millilitre of toluene was prepared as outlined in Section 3.5.2. A ten

millilitre aliquot of this solution was diluted to one litre with triple distilled water. Then a one millilitre aliquot of this ten microgram per millilitre solution was further diluted to one litre with triple distilled water. All dilutions were performed in volumetric flasks. One millilitre of this solution containing ten nanogrammes of toluene per millilitre of water was added to another Hypo-vial.

iii) To two other Hypo-vials was added a one millilitre sample of the triple distilled water.

iv) A stock solution of internal standard was prepared by diluting two and one half grammes of redistilled ethyl benzene in one litre of a mixture of acetone and distilled water (1:9). A one millilitre aliquot of this solution was diluted to one litre with triple distilled water. Then a one millilitre aliquot of this twenty-five microgram per millilitre solution was further diluted to one litre with triple distilled water. All dilutions were performed in volumetric flasks.

One millilitre of this internal standard solution containing twenty-five nanogrammes of ethyl benzene per millilitre of water was added to all the vials except for one containing only triple distilled water (Blank). All of the vials were then sealed with a butyl rubber septum and aluminium crimp seal.

The vial prepared containing just water and internal standard

was used to test the purity of the ethyl benzene and thus obtain a limit of detection.

Sample Incubation

As detailed in Section 3.4.2.

Gas Chromatographic Conditions

As detailed in Section 3.7.2.

The analyses were repeated in duplicate and the concentration of toluene in each sample calculated by the comparison of peak area ratios for toluene to ethyl benzene obtained with that from the standard.

3.8.3 Results.

The peak area ratios for toluene to ethyl benzene in the standard, blank, ethyl benzene and samples are given in Table 3.8.1. The concentrations in each sample have been calculated by simple standard ratio.

3.8.4 Discussion.

The measurement of levels as low as ten nanogrammes per millilitre requires several dilution steps in the preparation of standards and care must be taken in the use of toluene free water. Although the water used in this case was triple distilled a trace of toluene was still observed.

Ratios		Sample No.	Toluene (ng/ml)
0.005	0.005	Blank	0.12
0.055	0.047	Ethyl Benzene	1.21
0.417	0.428	Standard	10.00
0.191	0.196	"Normal" 1	4.58
0.193	0.193	"Normal" 2	4.57
0.206	0.200	"Normal" 3	4.80
0.203	0.210	"Normal" 4	4.89
0.176	0.213	"Normal" 5	4.60
0.200	0.212	aNormal" 6	4.88
0.204	0.191	"Normal" 7	4.67
0.208	0.207	"Normal" 8	4.91
0.191	0.196	"Normal" 9	4.58
0.189	0.215	"Normal" 10	4.78
		Mean Baseline	4.73

Table 3.8.1 - Normal Baseline Blood Toluene Levels.

The toluene level observed in the ethyl benzene internal standard represents a purity of slightly more than ninety per cent. Ethyl benzene is not available in high purity commercially. The above results demonstrate that it is difficult to purify by double distillation even using a one metre fractioning column filled with glass helices.

The "normal" blood level results show a remarkably uniform distribution of toluene in each of the samples. The measured levels evidently being a result of the sum of the impurity level in the ethyl benzene and to a normal baseline level present in blood. Subtraction of the level resulting from the ethyl benzene impurity from each of these results gives a mean baseline "normal" blood toluene level of 3.5 nanogrammes per millilitre.

3.8.5 Conclusion.

A mean "normal" blood toluene level of 3.5 nanogrammes per millilitre was observed in the samples analysed. The level was consistent throughout the samples and was shown to be not due to either water or internal standard contamination.

3.9 Examination of Possible Loss of Toluene into the Septum during Analysis.

3.9.1 Introduction.

As observed in section 3.3 the quantity of toluene in the head-space is dependant on the time allowed for equilibration to be reached. This period was determined to be approximately forty five minutes. There then appears to be no change in concentration of toluene in the head-space until after a further forty five minutes have elapsed. During this period there is the possibility that the butyl rubber septum absorbs some of the toluene with the consequence that the results may be slightly lowered.

To establish if the septum was absorbing the toluene an experiment was performed using three different types of septum.

3.9.2 Method.

Preparation of Samples

A stock standard solution containing one microgramme of toluene and two and a half microgrammes of ethyl benzene per millilitre of water was prepared by serial dilution as in previous experiments. The vials used were sealed with three different types of septum: a) a butyl rubber septum; b) a teflon lined septum; c) a small piece of aluminium foil backed

with the teflon septum. All three types of septum were held in place with the usual aluminium crimp seal.

Vials were filled with one millilitre of the stock solution and one millilitre of whole blood obtained from outdated blood transfusion stock. The vials were then sealed with one of the three types of septum. Four vials of each type were prepared.

Sample Incubation

As detailed in Section 3.4.2.

A vial of each septum type was sampled at one of the four sampling times (45, 60, 75, and 90 minutes).

Gas Chromatographic Conditions

As detailed in Section 3.4.2..

3.9.3 Results.

The area ratios of the toluene to ethyl benzene peaks were calculated and the results are given in Table 3.9.1. These results were examined statistically using analysis of variance and were found to have no significant level of variance.

Septum \ Time (m)	45	60	75	90
a) Butyl Rubber	0.469	0.449	0.462	0.473
b) Teflon lined	0.385	0.494	0.500	0.510
c) Foil lined	0.486	0.523	0.516	0.485

Table 3.9.1 - Peak Area Ratios for Septum Seal Comparison.

3.9.4 Discussion.

There does not appear from the above results to be any significant absorption of toluene by the butyl rubber septum or the teflon septum on the assumption that the third type of septum also does not absorb toluene. The conditions used are those established for the method and it would seem that there is no reason for not using butyl rubber septa in this application.

The manufacturers of the septa (Pierce & Wariner, U.K.) recommend that they are not used with chlorinated solvents and in these instances the teflon septa should be used. Although it may be considered that at the concentrations expected to be encountered, of the order of one microgramme per millilitre, any changes in concentration due to septum absorption would be negligible.

3.9.5 Conclusion.

No absorption of toluene was observed. Therefore the butyl rubber septa are suitable for use in the developed analytical method.

3.10 Determination of Blood Levels in Industrial Workers Exposed to Toluene Vapour.

3.10.1 Introduction.

Toluene is widely used throughout industry as a solvent base for a large number of products though perhaps the most common is its use in contact adhesives. These adhesives are used in considerable quantities by the building, packaging, vehicle manufacturing and furnishing industries. In these occupations the employees are often operating in confined spaces and for long periods in direct exposure to the fumes of solvents from the adhesives used. They therefore make an ideal control group for the study of blood levels from abusers of similar products. Their blood levels can be expected to be above baseline but not as high as levels expected from volatile substance abuse.

To establish this control level a series of blood samples were obtained from volunteers who had undergone occupational exposure to toluene.

3.10.2 Method.

Sampling Procedure

Twenty-three blood samples were obtained from volunteers who had been exposed to toluene through their use of contact adhesives in fixing fabric upholstery linings to the interiors

of cars. The quantities of adhesives used, and duration of exposure to the fumes were ill-defined. The space worked in was confined and without air extraction equipment. Protective gloves were not used when handling the adhesive.

At the end of the day's shift the operators were in the habit of washing excess adhesive from their hands with hexane - an apparently acceptable practice. Exposure to the hexane vapour was minimal. None of the volunteers complained of any subjective or physical ill-effects from either the hexane or the adhesive.

Preparation of Samples

i) A one millilitre aliquot of sample blood was pipetted into a Hypo-vial.

ii) A stock solution containing one microgramme of toluene per millilitre was prepared as outlined in Section 3.8.2. A series of five standards and a blank were prepared in the range of zero to five hundred nanogrammes of toluene per millilitre of water by dilution of this stock standard with triple distilled water in volumetric flasks. One millilitre of each of these solutions was added to individual vials.

iii) To all the vials was added a one millilitre aliquot containing twenty-five nanogrammes of ethyl benzene as internal standard. This solution was prepared as outlined in Section 3.8.2. The vials were then sealed with a butyl rubber septum and aluminium crimp seal.

Sample Incubation

As detailed in Section 3.8.2.

Gas Chromatographic Conditions

As detailed in Section 3.8.2.

The analyses were repeated in duplicate and the concentration of toluene in each sample calculated by the comparison of peak height ratios for toluene to ethyl benzene with a calibration curve obtained from the standards.

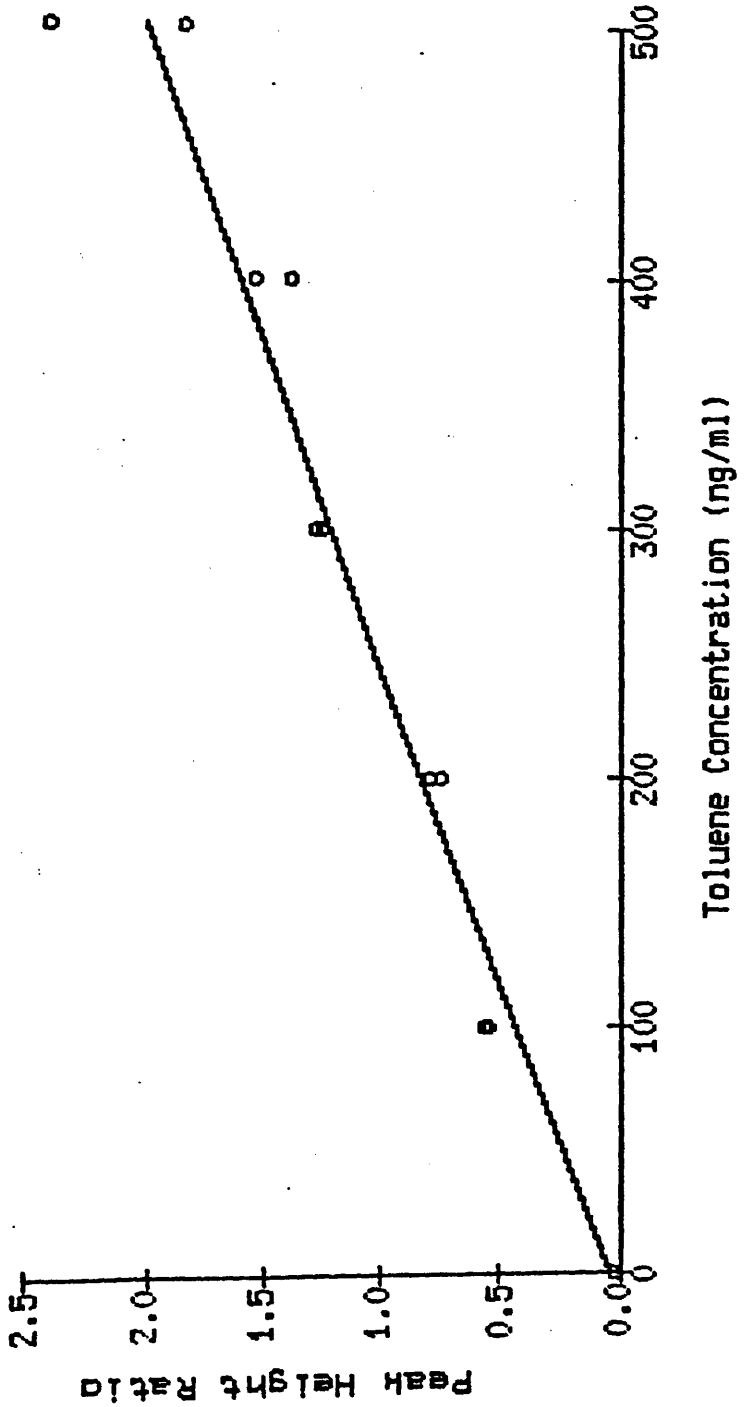
3.10.3 Results.

The peak height ratios obtained for the standards and samples are given in Table 3.10.1. The calibration graph and the statistically determined best fit straight line to the data are given in Figure 3.10.1. The sample concentrations were then obtained from the graph.

Sample	Peak Height Ratios		Toluene (ng/ml)
Blank	0.010	0.013	0
Std. 1	0.533	0.553	100
2	0.731	0.790	200
3	1.249	1.266	300
4	1.377	1.532	400
5	1.832	2.382	500
Subj.1	1.292	1.084	292
2	0.371	0.315	77
3	0.694	0.606	155
4	0.306	0.262	61
5	0.432	0.399	95
6	0.876	0.988	227
7	0.354	0.419	88
8	0.308	0.371	76
9	0.401	0.412	93
10	1.249	1.196	301
11	0.231	0.213	46
12	0.371	0.320	77
13	1.486	1.522	373
14	0.205	0.236	45
15	0.222	0.297	55
16	0.837	0.825	201
17	0.331	0.339	75
18	0.242	0.274	55
19	0.246	0.271	55
20	0.122	0.154	24
21	0.245	0.261	54
22	0.364	0.303	74
23	0.285	0.238	56

Table 3.10.1 - Peak Height Ratios for Standards and Samples from Industrial Exposure to Toluene Vapour.

Figure 3.10.1 - Standard Calibration Graph.



3.10.4 Discussion.

No hexane was observed in any of the samples. This was not unreasonable considering that the period of contact with hexane was short and the volatility, and therefore the elimination rate, of hexane is high.

The samples were found to contain toluene in the range of 20 to 400 nanogrammes per millilitre. This is higher than baseline levels and sufficiently high to indicate exposure to toluene.

3.10.5 Conclusion.

Blood levels of toluene to be expected from exposure to toluene based adhesives over a prolonged period in an industrial environment are in the region of 20 to 400 nanogrammes per millilitre.

This level will be considered as the lowest indicator of possible volatile abuse and should provide a base from which analysis of blood from abusers may be compared.

3.11 Determination of Toluene Levels in Blood Taken from Volatile Substance Abusers Using Toluene Based Products.

3.11.1 Introduction.

In the three years between 1977 and 1980 blood samples were collected from suspected volatile substance abusers. With the collection of each sample some data was also requested from both the abuser and from the medical practitioner removing the blood sample. The majority of the samples were obtained by the cooperation of the Police Community Involvement Team based at Tobago Street in the East End of Glasgow. Other samples were obtained from the casualty wards of several Glasgow hospitals, and from health centres and institutes.

3.11.2 Method.

For each of these samples the following standard procedure was applied:

Sample Preparation

An aliquot of approximately one millilitre of sample blood was pipetted into an empty, clean, pre-weighed six millilitre hypo-vial. The vial was then reweighed. A standard vial was also prepared containing one microgramme of toluene in one millilitre of water. A one millilitre aliquot of internal standard solution containing approximately two and a half microgrammes of ethyl benzene per millilitre was then added to

each vial.

Sample Incubation

The vials were then sealed with a butyl rubber septum and aluminium crimp seal before being placed in a heating block previously equilibrated at sixty degrees. After an equilibration time of forty five minutes a five hundred microlitre aliquot of the head-space above the liquid in the vial was removed with a preheated gas-tight syringe and injected into the gas chromatograph.

Gas Chromatographic Conditions

A Pye 104 gas chromatograph equipped with a flame ionisation detector was used. The Tenax G.C. column was operated at an isothermal temperature of one hundred and fifty degrees with a zero grade nitrogen carrier gas flow of thirty five millilitres per minute.

Calculation of Results

The concentration of toluene in the sample was calculated from the standard using peak area ratios. Analyses were always repeated in duplicate and the average of the two results taken.

3.11.3 Results.

There have been a total of one hundred and fourteen samples submitted during the three year period. Of these, sixty six were found to contain a significant level of toluene. Table

3.11.1 gives details of the levels observed in these cases.

Sample No.	Toluene ug/ml	Sample No.	Toluene ug/ml	Sample No.	Toluene ug/ml
63	0.67	96	3.31	139	0.14
64	0.25	98	0.05	140	2.40
65	1.09	101	0.16	143	2.04
66	0.44	102	0.20	144	0.62
67	0.95	103	0.19	145	5.40
68	0.59	104	0.10	147	1.08
69	2.72	106	1.08	148	1.68
70	0.58	107	0.24	149	0.83
71	0.42	111	0.54	150	0.62
72	0.22	113	0.89	153	2.39
73	0.15	114	0.94	154	4.09
74	0.44	115	1.81	155	5.77
75	0.37	116	3.16	156	2.33
76	0.66	119	1.76	159	3.06
77	0.47	120	1.49	163	14.82
78	9.63	122	0.33	164	8.06
87	0.48	125	4.89	165	8.02
88	1.11	126	1.50	168	1.37
89	0.41	133	1.24	169	1.80
93	1.35	134	0.41	171	0.53
94	1.20	135	1.82	179	0.15
95	0.29	138	0.76	180	0.42

Table 3.11.1 - List of Blood Levels of Toluene Found in Samples from Suspected Volatile Substance Abusers.

3.11.4 Discussion.

From the above table it can be seen that the levels observed range from as low as 50 nanogrammes per millilitre, a level possible from severe industrial over-exposure to toluene, to as high as 15 microgrammes per millilitre, a three hundred fold difference.

These levels on their own are almost totally meaningless in so far as prediction of toxic or even fatal levels. However from the mean of the results it may be suggested that a level of

about 800 nanogrammes per millilitre is almost certainly indicative of the abuse of toluene. Without the consideration of other data little else can be deduced from these results.

3.11.5 Conclusion.

A blood level of toluene in the range of 0.05 to 14.82 microgrammes per millilitre is indicative of exposure to abnormally high levels of toluene vapour. A blood level above 0.8 microgrammes per millilitre is evidence of toluene abuse.

The examination of these results with other available data on the abuser should provide further information on the extent and severity of the problem of toluene abuse.

3.12 Examination of the Etiological Data Obtained with Volatile Abuser Blood Samples.

3.12.1 Introduction.

From the start of this investigation into the extent of the problem of solvent inhalation abuse a considerable effort was made to attempt to gain as much useful information as possible. In addition to the collection of blood samples and the recommendation for the collection of breath samples the answers to a short questionnaire were also requested of the doctor carrying out the examination of the suspected volatile substance abuser.

These simple questions were designed to provide basic etiological information regarding the nature, extent and effects of the practice.

3.12.2 Method.

The questions asked of the abusers are given in Table 3.12.1.

Name
Age
Sex
Substance Abused
Period of Abuse
Time of Last Abuse
Appearance of Abuser

Table 3.12.1 - Questionnaire Asked of Each Abuser.

3.12.3 Results.

The response obtained from the questionnaire was to some extent disappointing and in fact the last question was hardly ever answered.

Of the sixty referrals which were found to have positive blood solvent levels one abuser was referred on three separate occasions and three were referred twice.

The male to female ratio of the referred abusers was 5.7 to 1.

Of the fifty six different questioned abusers the average age was found to be 14.5 years. The age distribution is shown in Figure 3.12.1.

The distribution of time of abuse is shown in Figure 3.12.2.

3.12.4 Discussion.

It is interesting to note that only two of the referrals were over eighteen years old and in fact both of these were fatalities. It is thought that this may be more of a reflection of the referral procedure than the true state.

As may be expected from this age group the most frequent time of abuse is the early evening, after school hours or during school meal breaks. Though here again the results are heavily

Figure 3.12.1 - The age distribution of the abusers.

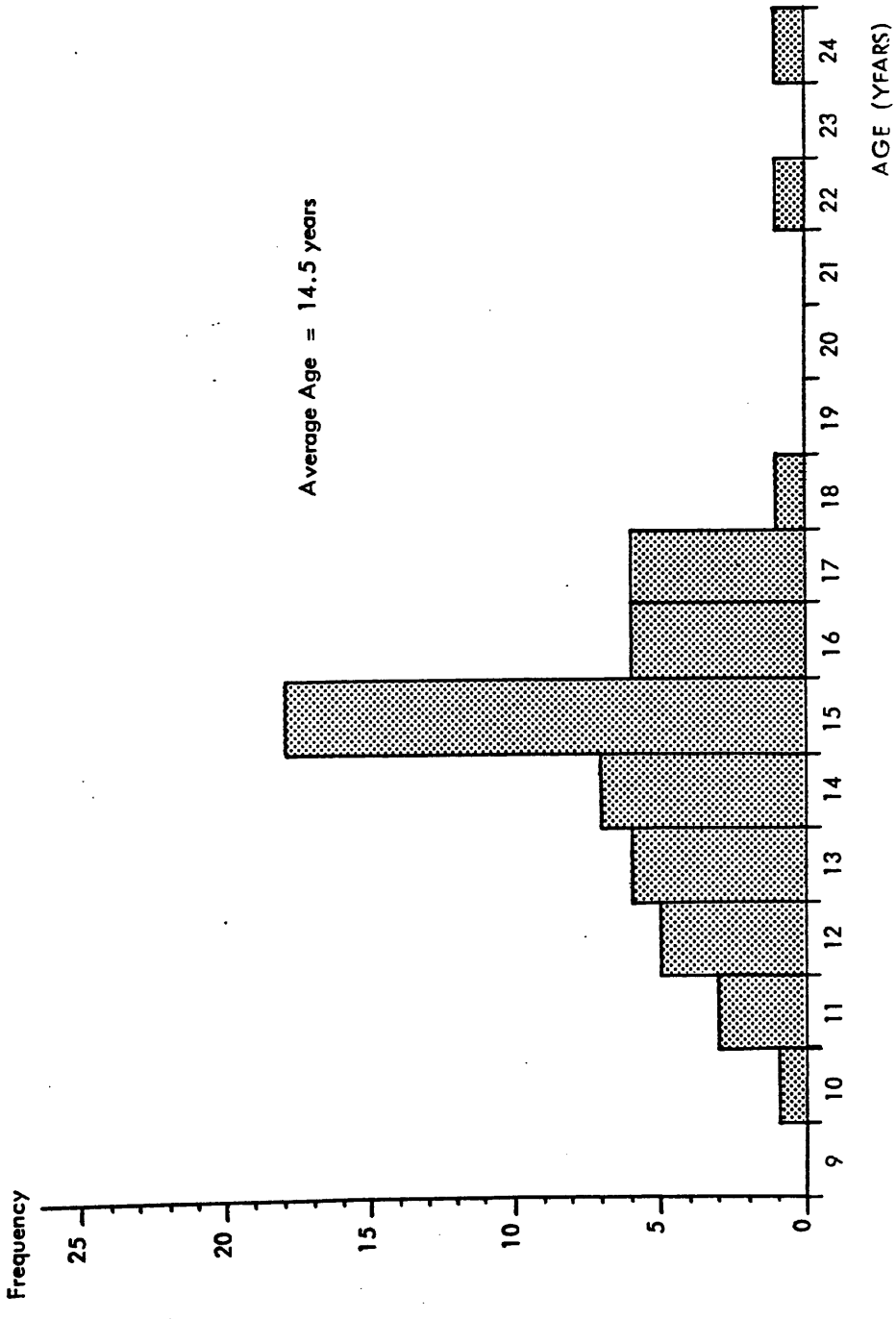
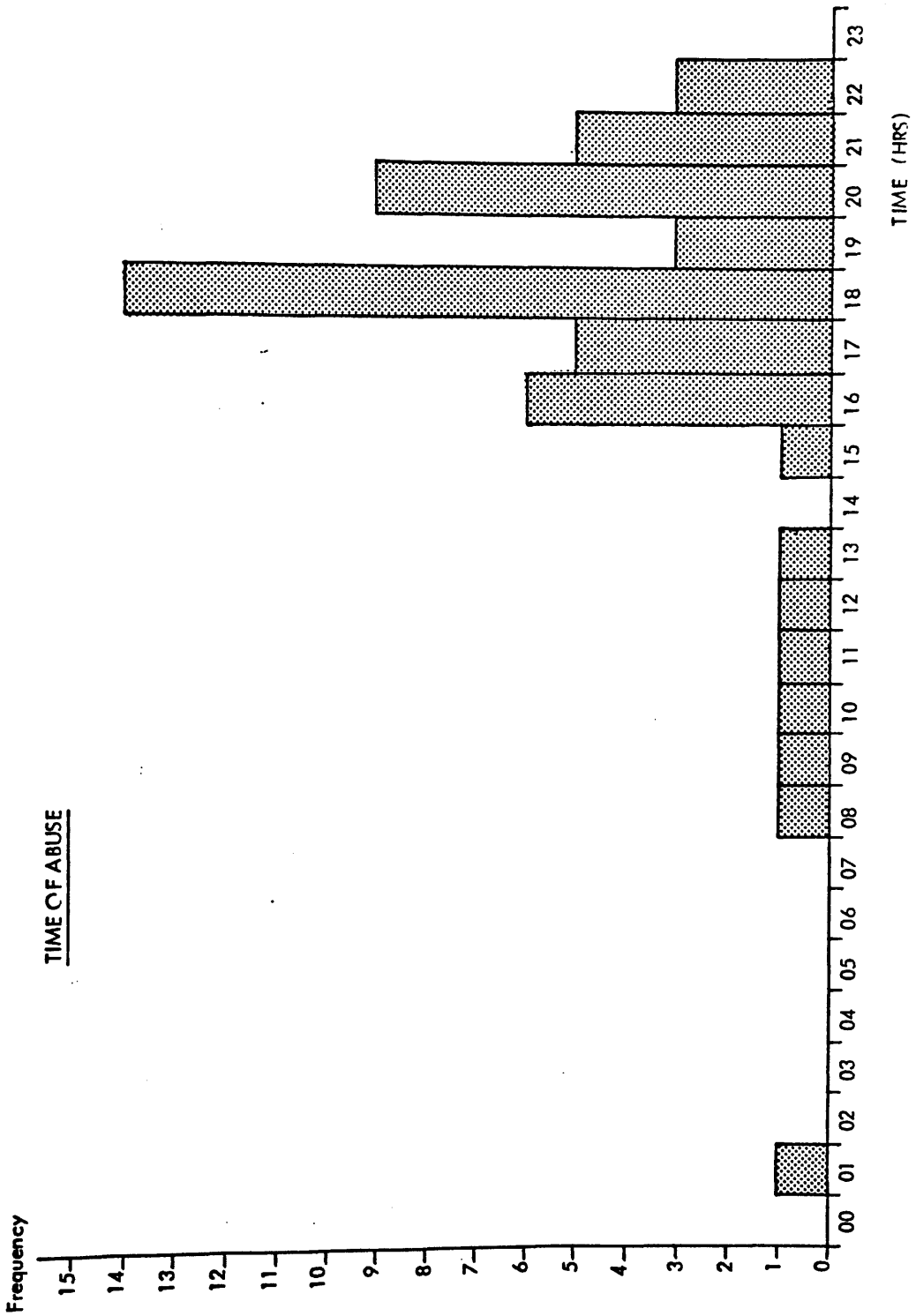


Figure 3.12.2 - The time of abuse distribution.



dependant on the honesty of the abuser or indeed his ability to give rational answers to the questionnaire.

Periods of between one and eight hours were given as the period of abuse and little can be deduced from this observation other than the expansive nature of the practice.

3.12.5 Conclusion.

Of the replies from fifty six questioned volatile substance abusers it has been calculated that the mean age of abuse is 14.5 years, that there are approximately six times as many male than female abusers, and that the most popular time of abuse is early evening.

These observations must be considered with the fact that the majority of abusers were referred by the police, were apprehended out-of-doors, and were of the age group most likely to be noticed causing minor misdemeanors.

3.13 Application of The Method Developed to Measure Toluene in Blood to Samples Obtained from Fatalities Resulting from the Abuse of Toluene.

3.13.1 Introduction.

Owing to uncontrolled abuse and often inability on the part of the abuser to discern dangerous levels of intoxication, it is inevitable that toxic and even fatal levels of the solvents concerned may be reached. This is of course to a great extent dependent on the solvent involved and the method of abuse.

Inhalation abuse complications can be expected from the volatile solvents and to a great extent are dependent on their lipid solubility. It is well known that high blood levels of ethanol cannot be reached by inhalation alone, however the levels reached with toluene, benzene, etc., may be significant. The simple fact that toluene appears to be the most popular solvent for inhalation abuse is evidence alone that levels attained by inhalation of this solvent can produce a pharmacological effect. As discussed in Section 3.11 there appears to be a level where the subject becomes intoxicated, similar to alcohol, and therefore it is only reasonable to suppose that the degree of intoxication could likewise progress through unconciousness to death.

For a variety of reasons the real fatal level may in fact never be reached. The simple method of inhalation abuse, that

of "sniffing" from an open container whose contents include the abused solvent, will result in the probable removal of the source of the solvent when a state of unconciousness is reached. Another reason may be that the subject becomes so intoxicated that he produces some other danger, for example stepping in front of a moving vehicle or falling from an elevated position. The method of abuse could itself involve some other significantly more dangerous practice, the use of a large plastic bag over the nose and mouth or perhaps just simply smoking in the presence of the solvent which in many cases is inflammable. All of these circumstances and many more have been encountered with cases involving solvent inhalation abuse. It is for these reasons that the definition of a fatal level is clouded.

The following four cases illustrate this point and give some indication of the levels obtained in fatal cases of toluene inhalation abuse. All of the cases involved known addicts whose deaths resulted directly from the fact that they were inhaling toluene.

3.13.2 Case Histories.

1. The deceased, a twenty-four year old male, had been addicted to the inhalation abuse of glue for several years. On the day of his death at about 1600 hours he was found in bed with a plastic bag over his head. He was found to be unconcious but in a state of severe intoxication. About two hours later he was once again found lying across the bed with

a plastic bag over his head. He was found to be dead on examination. Two empty bottles of "Britfix Liquid Poly 70" were found beside the body.

2. The deceased, a sixteen year old male, had been inhaling deeply from cans of "Timebond" glue during the week prior to his death. He was found suspended from a tree with his jacket acting as an improvised noose. Death was diagnosed to be from asphyxiation due to strangulation.

3. The deceased, a sixteen year old male, had been known to have been abusing glue, usually "Evo-Stick", for over four months prior to his death. He had been found unconcious three months prior to his death apparently from the effects of volatile substance abuse. On this occasion he had minor lacerations to his wrists which had been self inflicted with a razor blade. On the day of his death he was observed in a "dazed" state and smelling strongly of "adhesive fumes". Three hours later he was found in the loft of his home suspended by a length of electrical cable looped around his neck. His feet were resting on the ceiling and his knees were bent.

4. On the day of his death the deceased, an eighteen year old male, purchased two one pint tins of glue. He then proceeded with others to the banks of a local river to inhale the vapours from the glue after pouring into plastic bags. About four hours later he was seen to slip feet first into the river. A search for him was inconclusive and the body was

later recovered by the Police Underwater Unit submerged in less than five feet of water. The river current was not particularly strong and there was no entangling debris on the river bed.

3.13.3 Method.

Sample Preparation.

A series of standards of toluene were prepared in the range of one to ten micrograms per millilitre by serial dilution as described in Section 3.11.

Tissue specimens were homogenised and then weighed into six millilitre Hypo-vials in one gramme quantities.

To each of these were then added 2.5 micrograms of ethyl benzene, internal standard solution, in one millilitre water.

Sample Incubation.

As described in Section 3.11.

Gas Chromatographic Conditions.

As described in Section 3.11.

Concentrations of toluene in the samples were calculated from direct peak area ratio comparison with the standards. The quoted levels being the average of triplicate analyses.

3.13.4 Results.

The levels of toluene found in the samples submitted from the above cases are given in Table 3.13.1.

Sample	Toluene level (ug/ml)			
	Case 1	Case 2	Case 3	Case 4
Blood	5.9	39.6	52.0	15.0
Urine	0.23	7.6		5.1
Brain	22.5	98.6		13.3
Liver	24.6	115		10.5
Fat	0.73			
Kidney		77.5		

Table 3.13.1 - Toluene Levels Found in Fatalities Involving Toluene Inhalation Abuse.

3.13.5 Discussion.

There is very little available data in the literature on the possible fatal levels of toluene. Therefore these results illustrate the range of levels which may be observed. In each of the described cases death due to toluene poisoning is unlikely. In cases 1 and 2 toluene inhalation abuse had probably been occurring for several hours prior to death and this may well explain why in these two cases the levels of toluene found in the brain and liver were significantly higher than the level found in the blood. As expected from the low water solubility of toluene the levels in the urine were low.

3.13.6 Conclusion.

The method for the analysis of toluene in blood can also be used to measure levels in other tissues.

Although not fatal, the levels of 39.6 and 52.0 microgrammes per millilitre of blood must have resulted in at least partial anesthesia.

The level of 15.0 microgrammes per millilitre certainly brought about a severely intoxicated state a direct result of which was the abuser´s death.

The level found in case 1 was probably sufficient to produce significant intoxication the result of which was the anoxia produced by leaving the plastic bag over the abuser´s face. Death in this case was due to suffocation.

3.14 Development of a Method to Measure the Metabolites of Toluene, Xylene and Styrene.

3.14.1 Introduction.

As discussed in Section 3.11 the solvent most popularly abused appears to be toluene and although there is some suggestion of the choice of volatile substance follows a trend or fashion there has been no evidence of the problem spreading to other solvents in the data from this study. The other alkyl benzenes, xylene and styrene, are are not as readily available to the abuser as toluene and therefore do not seem to interest the abuser as an intoxicant. These compounds therefore are only of interest in cases of industrial over-exposure.

The detailed metabolism of toluene is complex and many compounds have been shown to be derived from inhaled doses. Principally biotransformation occurs as oxidation at the aromatic nucleus to give hippuric acid. The other metabolites, cresols and phenols, account for only a small fraction of the total metabolised toluene but as such may be of greatest significance considering the potential toxicity of these compounds. Of course a large proportion of the inhaled toluene is exhaled in the breath unchanged. For small doses of toluene, up to two hundred micrograms per litre per hour, and about seventy percent of the inhaled dose is excreted as hippuric acid (Masschelein & Veulemans 1979, Nomiyama & Nomiyama 1974).

The biotransformation of xylene undergoes a similar pathway to give 3-methyl and 4-methyl hippuric acids (Riihimaki 1979). Styrene undergoes side chain oxidation to give phenyl glyoxylic acid and mandelic acid, and also some hippuric acid by a similar route to toluene and xylene.

Hippuric acid is a major organic component of urine and arises from dietry sources such as quinic acid and of course the preservative sodium benzoate. This background level must be considered when use is made of high levels as an indicator of toluene exposure.

Owing to the poor retention of toluene in blood, and its high degree of volatility, the method developed to determine solvent levels in blood results in measurements which are limited in their value for the prediction of degree of exposure to the solvent. The measurement of hippuric acid levels therefore possibly provides a more practical marker of exposure.

There are several different methods available for the determination of these metabolites, the majority of which are colourimetric and involve a long extraction step or even pretreatment of the urine. These methods then usually involve the reaction of the extracted metabolites with pyridine and acetic anhydride to give a blue-violet coloured complex. However these colourimetric methods are non specific and are subject to interferences from naturally occurring compounds.

Other methods have been proposed using gas chromatography and these certainly are more specific, though most of them require different conditions for each metabolite and can be quite laborious, involving an extraction step followed by reaction under anhydrous conditions with a silanating reagent or diazomethane. It is also important to remember that these acids are difficult to extract into organic solvents and consequently require either the use of large samples or demand high sensitivity from the detection system.

An attempt to overcome these problems was made by the development of a high performance liquid chromatographic method.

3.14.2 Method.

Preparation of Samples.

An aqueous stock standard solution was prepared by accurately dissolving fifty milligrammes of hippuric acid in one hundred millilitres of deionised water. A series of aqueous standards were then prepared in concentrations in the range 0 to 5 microgrammes per millilitre by serial dilution.

One milligramme of the internal standard, benzoic acid, dissolved in one millilitre of methanol and water (1:4), was added to a one millilitre aliquot of sample urine or aqueous standard. This mixture was then diluted to ten millilitres with five percent sulphuric acid in a volumetric flask.

A system suitability test mixture was also prepared to qualitatively assess the chromatographic performance of the system conditions. This test mixture was composed of benzoic acid, hippuric acid, phenyl glyoxylic acid, mandelic acid, salicylic acid and acetyl salicylic acid.

Liquid Chromatographic Conditions.

A ten microlitre aliquot of this solution was then injected into the chromatograph. A 10cm. x 5mm.id. column packed with Hypersil 10 ODS was used as the analytical column. The mobile phase used consisted of a mixture of ethyl acetate, iso-propanol and 1.0M sodium acetate adjusted to pH 4.0 with acetic acid, in the ratio of 1:3:396. The column was operated at ambient temperature and with a flow rate of 1.5 millilitres of mobile phase per minute. Detection was by ultra-violet absorbance measurements at 245 nanometres. Standards were analysed in triplicate.

3.14.3 Results.

A typical chromatogram is shown in Figure 3.14.1. The peak area ratios for the standards are given in Table 3.14.1 and the resulting calibration graph is given in Figure 3.14.3.

Hippuric Acid (ug/ml)	Peak Height Ratio (Hippuric Acid / Benzoic Acid)			
	a	b	c	Mean
0.00	0.000	0.000	0.000	0.000
1.00	0.370	0.365	0.355	0.363
2.00	0.705	0.726	0.704	0.712
3.00	1.029	1.048	1.044	1.040
4.00	1.355	1.373	1.343	1.357
5.00	1.743	1.833	1.768	1.781

Table 3.14.1 - Peak Height Ratios for Three Series of Hippuric Acid Standards.

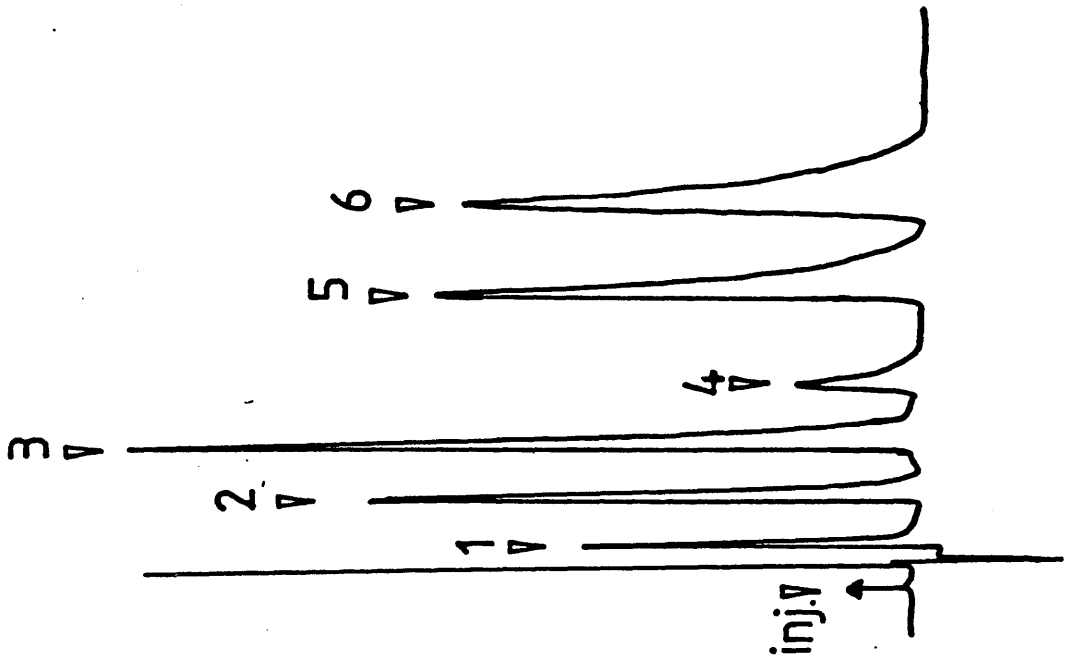
3.14.4 Discussion.

The test mixture chromatogram clearly demonstrates separation of the metabolites and internal standard from the two possible common interferants, salicylic acid and acetyl salicylic acid.

The observation was made that small changes in the organic concentration of the mobile phase, as little as 0.25%, produced a marked deterioration in the degree of resolution.

A total analysis time of fifteen minutes per sample was required. Though this may be reduced further by increasing the mobile phase flow rate to two millilitres per minute if it is known that salicylates are not present.

Figure 3.14.2 - A Typical Chromatogram Showing Separation of Hippuric Acid, Benzoic Acid, Phenyl Glyoxylic Acid, Mandelic Acid, Salicylic Acid and Acetyl Salicylic Acid.



1 - Mandelic Acid (1.1min)

2 - Hippuric Acid (2.3min)

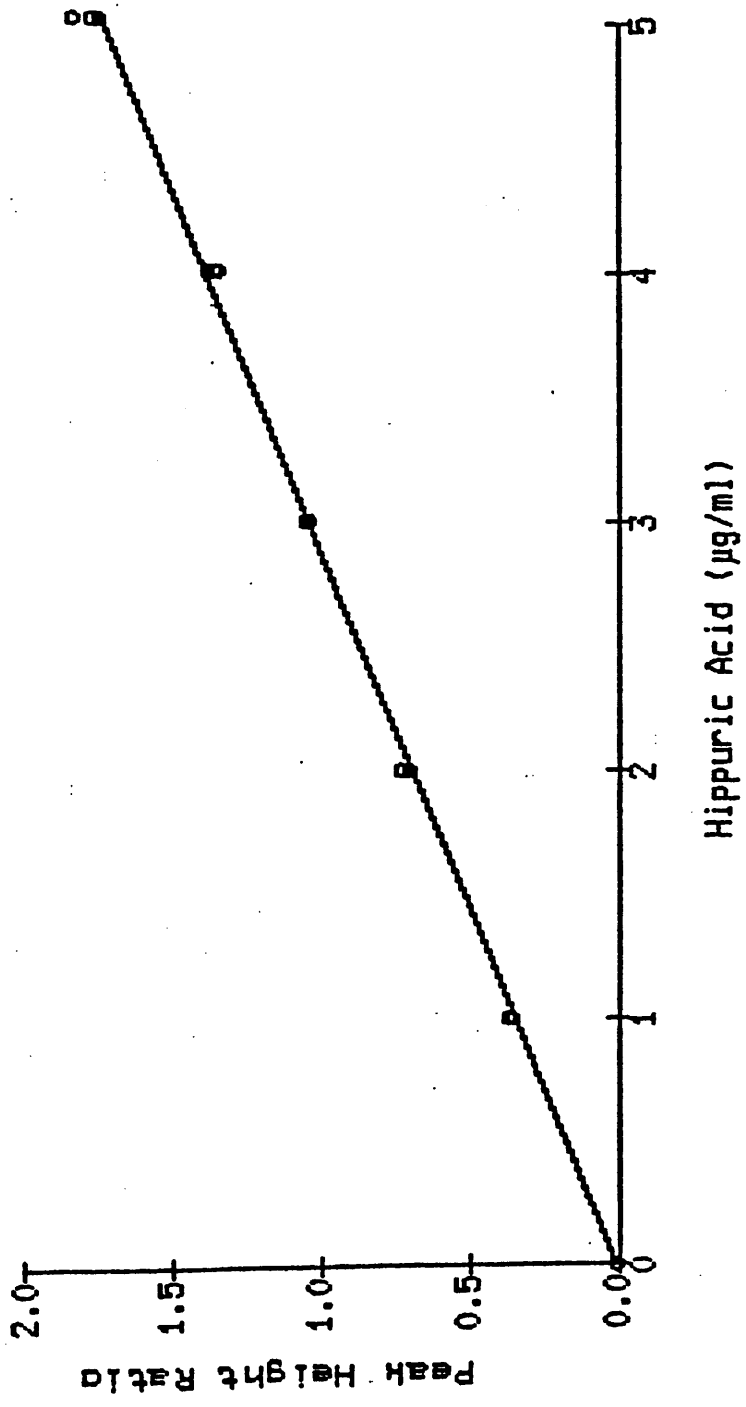
3 - Acetyl-Salicylic Acid (3.5min)

4 - Salicylic Acid (5.2min)

5 - p-(Toluoyl)-Glycine (7.5min)

6 - Benzoic Acid (10.1min)

Figure 3.14.3 - Calibration Graph for Hippuric Acid.



The graph obtained for the hippuric acid standards shows acceptable correlation (0.997) by linear regression analysis. This linearity was demonstrated over the range one to five microgrammes per millilitre which covers the range of levels expected from normal to high toluene dosage.

3.14.5 Conclusion.

Chromatographic separation of each of the metabolites was obtained. Separation of the salycilates was also obtained. This was intentional in the development so as to facilitate a quick method for the determination of salicylates and to demonstrate their separation from the metabolites in possible cases of interference.

The method described has been shown to be a quick and accurate method for the determination of hippuric acid in urine.

The application of this method to urine samples from volatile substance abusers should provide further information as to the degree of exposure to the alkyl benzenes and perhaps allow some estimation of the dose taken.

3.15 The Use of The Method for the Measurement of Hippuric Acid to Predict Exposure to Toluene.

3.15.1 Introduction.

In the last section the development of a method for the measurement of hippuric acid in urine was detailed. The measurement of this metabolite of toluene may offer some important advantages as a measurement of biological uptake compared to toluene blood levels. The relationship between this parameter and the time weighted absorbtion rate has been demonstrated to be independant of circumstantial factors such as varied exposure conditions and the influence of exercise (Masschelein & Veulemans 1979). This is probably not the case where direct measurement of toluene levels are concerned.

The average hippuric acid concentration associated with occupational and experimental exposure to one hundred microgrammes of toluene per litre of air for eight hours has been given in the literature as around 3.0 to 3.5 microgrammes per millilitre of urine (Lieberman & Pagnotto 1967, Ikeda & Otsuji 1969, Ogata, Takatsuka & Tomokuni 1970, Ogata, Takatsuka & Tomokuni 1971).

For the evaluation of hippuric acid as a monitor for toluene abuse and a predictor for degree of exposure it was decided that a baseline level should be obtained and that all six urine samples obtained during the study be examined.

3.15.2 Method.

Selection of Samples

In order to obtain a baseline level a random selection of fifteen urine samples was made from volunteers and post-mortem case samples and the hippuric acid levels measured.

The six urine samples obtained from toluene abusers, three post-mortem, were also assayed for hippuric acid.

Preparation of Standards.

As detailed in Section 3.14.

Liquid Chromatographic Conditions.

As detailed in Section 3.14.

3.15.3 Results.

The hippuric acid levels determined in the fifteen randomly selected urine samples from subjects not abusing toluene are given in Table 3.15.1. The hippuric acid levels determined in the six urine samples obtained from toluene abusers are given in Table 3.15.2 against their blood toluene levels (Section 3.11).

Sample	Hippuric acid (ug/ml)
1	0.286
2	0.379
3	0.147
4	0.081
5	0.363
6	0.063
7	0.121
8	0.244
9	0.053
10	0.066
11	0.025
12	0.352
13	0.403
14	0.181
15	0.061
Mean	0.188
Std.Dev.	0.132

Table 3.15.1 - Baseline Hippuric Acid Levels

Case Sample	Hippuric acid (ug/ml urine)	Toluene (ug/ml blood)
1 *	2.67	37.6
2 *	8.37	15.0
3	1.14	5.10
4	2.39	4.49
5	0.53	15.44
6 *	7.25	0.20

* Post-mortem

Table 3.15.2 - Toluene Abuser's Urine Hippuric Acid and Blood Toluene Levels.

3.15.4 Discussion.

The mean baseline hippuric acid level was found to be 0.19 microgrammes per millilitre. This level was observed to have a wide variation between subjects as predicted from the knowledge of the dietry influence on hippuric acid levels.

In cases 1 and 2 toluene was known to have been a significant contributory factor to the cause of death. This fact is reflected in the high hippuric acid levels, twenty to eighty times higher than baseline.

In case 6, however, the subject was a known abuser but did not die directly as a result of toluene abuse, although the hippuric acid level is high and may indicate that the subject had been abusing toluene to a considerable extent prior to death.

The subject in case 5 was found under the influence of toluene and the samples of blood and urine were taken soon after the onset of abuse. This probably accounts for the low hippuric acid level compared to toluene level.

In general it was found that significantly increased hippuric acid levels were observed in urine samples taken from known abusers although no direct relationship between blood toluene and urinary hippuric acid levels could be deduced.

3.15.5 Conclusion.

The baseline level for hippuric acid was found to be 0.19 microgrammes per millilitre with a maximum observed level of 0.40 microgrammes per millilitre.

No direct correlation was observed between blood toluene and urinary hippuric acid levels although there were certainly an

insufficient number of samples to establish this point statistically. In fact a total of only six urine samples were supplied over the entire study period. The absence of correlation is expected, however, and is probably a result of the high volatility of toluene and its rapid excretion in breath.

Increased hippuric acid levels were observed in cases of toluene abuse.

These facts may indicate that although the measurement of blood toluene is ideal for the identification of cases of toluene abuse it is perhaps not the best indicator of the extent of toluene abuse. It would therefore be of use if this property could be investigated further particularly with reference to cases of known abusers who have abstained from the practice for both short and extended periods. However, the ethical problems of an investigation of this nature and the apparent reluctance of the climicians monitoring volatile substance abuse to supply urine samples precluded any further examination of these points during the course of this study.

3.16 Development of a Method to Monitor Levels of Toluene in Breath.

3.16.1 Introduction.

In a previous Section it has been observed that an alternative method of monitoring volatile substance abuse was by the measurement of the metabolites of the volatile compounds. In the majority of cases this is less convenient than the direct measurement of the volatiles in blood, and is often more difficult. It does, however, have the important advantage in that it is non-invasive. Metabolites are usually measured in urine and the taking of urine samples is considerably less traumatic than the removal of samples of blood. However there is no immediate indication of the substance being abused and therefore the measurement of metabolite levels does not lend itself readily to the diagnosis of volatile substance abuse.

The majority of volatile substances abused are by their nature usually excreted predominantly in the breath. This is particulary true for the more lipid soluble, and therefore the more popular, of the abused volatiles. The measurement of these compounds in breath would thus provide an ideal non-invasive general diagnostic method for the clinical monitoring of the problem of volatile substance abuse.

3.16.2 Method.

Sampling Procedure.

Breath samples were taken by trapping the volatiles present in one litre of exhaled air on to charcoal chips in a glass tube. The tubes were standard commercial adsorbtion tubes and were attached to a one litre breathyliser plastic bag. The tubes were designed to be capped after use and then submitted to the laboratory where they were stored frozen until analysis.

Preparation of Samples.

The sample tubes were broken open and the charcoal chips transferred to a test tube. Specific care was taken to transfer all of the charcoal and to perform this step of the procedure in a solvent free atmosphere - not the working laboratory. To another clean test tube was added four hundred milligrammes of new charcoal chips. To this test tube and a third tube without charcoal chips was added one hundred microlitres of redistilled toluene. To each of these test tubes and a fourth blank tube was then added a two hundred and fifty microlitre aliquot of twice redistilled ethyl benzene and three millilitres of carbon disulphide.

The test tubes were then securely sealed with teflon lined caps and each vortexed for thirty seconds, before being transferred to a water bath maintained at forty degrees for three hours. After this period the contents were transferred to a five millilitre volumetric flask and the tubes washed with a further one millilitre of carbon disulphide, the

washings also being transferred to the respective volumetric flask. These solutions were then each made up to five millilitres with carbon disulphide

One microlitre aliquots of the samples were then injected into the gas chromatograph.

Gas Chromatographic Conditions.

A 1.5m x 4mm glass column packed with 10% free fatty acid phase was used as the chromatographic column. It was operated at a temperature of one hundred degrees and a nitrogen carrier gas flow rate of thirty five millilitres per minute. Flame ionisation detection was used, the peak heights being measured manually.

3.16.3 Results.

The peak height ratios were calculated for each tube as the ratio of height of toluene peak against the height of the ethyl benzene peak. The background toluene was then calculated as the ratio of the peak height ratios for the blank tube (without charcoal or toluene) against the method standard tube (without charcoal) and multiplying by a factor of one hundred. The sample toluene was then calculated from a similar ratio of the peak height ratios for the sample tube (with charcoal from breathyliser) against the standard tube (with unused charcoal) and subtracting the background level.

3.16.4 Discussion.

A total of two hundred and fifty kits were distributed to investigators who had previously supplied blood samples containing toluene. The kits consisted of a one litre breathyliser bag, an adsorption tube, blood collection syringe, a survey form and a simple set of instructions on how to use the kit. Of the total number of kits distributed only six breath tubes were returned for analysis. Of these six three arrived uncapped. Only one of the capped tubes was found to contain toluene by the above method.

This one sample showed a breath level of 200 microgrammes per litre and the blood sample taken at the same time showed a level of 0.33 microgrammes of toluene per gramme. The period between abuse and sampling was about fifteen hours and this would suggest that the level of exposure to toluene was quite high.

3.16.5 Conclusion.

A method was developed for the determination of levels of toluene collected on charcoal adsorbtion tubes used as a breathyliser.

Of two hundred and fifty tubes distributed only six tubes were returned, only one of which was found to contain a measurable level of toluene.

3.17 Extension of The Method to the Analysis of Blood for Methanol and Ethanol.

3.17.1 Introduction.

The abuse of methanol, usually in the form of methylated spirit or industrial alcohol, is a familiar problem to the analytical toxicologist. This observation is supported by the occurence, on a fairly regular basis, of post-mortem cases where the abuse of methanol has been thought to have had some influence on the cause of death. The cases are also interesting in themselves in that they do not involve the use of the more commonly encountered sources of methanol.

Six of the cases described in the following section involve the drinking of "Bellair Hair Lacquer". This solution is easily available in pharmacies and many high street stores. It is undoubtably this ease of availability that has given rise to its popularity among the vagrant population as a suitable substitute for ethanol based beverages. Administration is usually in diluted form, in which it becomes similar to milk in appearance owing to the colloidal suspension of the precipitated shellac. As such the drinking of it may be suitably disguised.

The solution contains by weight 93 % industrial methylated spirits, 6.4 % shellac, 0.4 % perfume, and 0.2 % benzyl-diethyl-N-2,6, xylylcarbamoyl methyl ammonium benzoate

(Bitrex). It is typically diluted about one in ten with water, milk or wine.

The seventh case involved the use of methylated spirits.

3.17.2 Method.

Preparation of Samples

A standard solution of methanol in water was prepared by diluting half a gram of AnalaR methanol in one litre of water. The blood or urine to be analysed and the standard methanol solution were then each diluted with n-propanol solution which was used as internal standard.

Gas Chromatographic Conditions

As detailed in Section 3.4.2. A temperature of one hundred degrees was chosen for the analysis based on the data obtained for methanol, ethanol and n-propanol in Section 2.3. One microlitre aliquots from each of the sample and standard solutions were injected on to the column in duplicate and the peak areas due to methanol, ethanol and n-propanol were measured. The concentrations of methanol and ethanol in the samples were then calculated from direct comparison with the standards.

Routine Toxicolgical Analysis.

Routine examination of the blood samples for presence of drugs was performed according to accepted standard procedures (Clarke 1975).

3.17.3 Results.

Case Histories.

1. The deceased was found dead on vacant ground in the centre of Glasgow at 22:00 hr. in December. The body was that of a male subject age 45 years and was in average state of nutrition. Post mortem examination did not reveal any significant injury or disease process contributable to his death. The deceased was found in possesion of an empty bottle of "Bellair Refill".

2. The deceased, a middle aged male, was suffering from severe liver disease consistant with chronic alcoholism. On the day of his death he had received treatment for vomiting of blood after having drunk a methanol based liquid.

3. Blood and urine were received from the Fiscal at Kilmarnock after the post mortem examination of the deceased who had last been seen drinking a bottle of "milk".

4. The deceased, a 30 year old female, was found dead in a derelict property in Glasgow. The body was that of a well nourished, adult female but was in a generally poor state of hygiene. Post mortem examination revealed a number of superficial injuries on the body, but they were not significant in her death.

5. The deceased, a 55 year old male subject, was a known chronic alcoholic and was receiving treatment at a local hospital. Relatives of the deceased attested that he had been drinking "Bellair Hair Laquer" for a number of years. On the evening prior to his death the deceased left his sister's home reportedly under the influence of "Bellair Hair Laquer". He was found the following day and an empty bottle of Bellair was lying nearby. He was found in conditions which suggested that his death was due to exposure. Post mortem examination revealed that he had sustained acute haemorrhagic pancreatitis and had a very fatty liver indicative of chronic alcoholism. There were some superficial injuries which could have been caused by him crawling around at the locus of his death.

6. This 60 year old male subject was admitted to hospital having deliberately drank almost a pint of methylated spirits. On admission he was reported to have smelled strongly of "meths" and was deeply unconscious with no response to painful stimuli. He was found to be hypothermic ($35^{o}C$), hypotensive (80/45), and had deep sighing respiration. He had metabolic acidosis (pH 7.25) with hypokalaemia and elevated urea. He was treated with plasma and an intravenous infusion of 1.26 % sodium bicarbonate. He was also given a solution of 10 % ethanol in normal saline by nasogastric tube for five days, and a total of twenty two cycles of peritoneal dialysis. Under this treatment the subject recovered fairly rapidly and did not appear to have any residual loss of visual acuity.

7. The deceased, a middle aged male, was a known alcoholic. He was one member of a group of four vagrants who had been drinking "Bellair Hair Laquer" diluted with water. He was found dead the following morning. Post mortem examination showed signs of chronic liver disease.

Analytical Results.

A summary of the results obtained is given in Table 3.17.1. A bottle labelled "Vodka" containing a milky suspension was also submitted for analysis in connection with case seven. The suspension was also analysed for methanol and ethanol after a 1 : 100 dilution and was found to contain 1.3 % by weight of methanol and 8.5 % by weight of ethanol. This is consistant with the dilution of the contents of a bottle of "Bellair Refill" with water. The white colloidal suspension being due to the precipitation of the shellac from solution.

Case No.	MeOH Blood	mg% Urine	EtOH Blood	mg% Urine	Other findings of significance
1	5.8		420	421	Known Bellair drinker
2	31.5		nil		
3	145	196	nil	nil	
4	1.8		4.9		3.7 mg% Phenobarbitone
5	66	84	292	398	Haemorrhagic Pancreatitis
6a	53		132		sample on admission
6b	10		55		15 hrs of treatment
7	40		274		

Table 3.17.1 - Methanol Levels Obtained from Cases of Suspected Methanol Abuse.

3.17.4 Discussion.

The deceased in case two died from inhalation of gastric contents, caused by methanol induced irritation of the stomach, and exacerbated by the liver condition.

The level of phenobarbitone found in case four has been known to be a fatal level.

The haemorrhagic pancreatitis in case five was suspected to have been brought about by the high level of methanol.

The results for case six demonstrate an effective rate of elimination of 3 mg % / hr. for methanol and 15 mg % / hr. for ethanol, assuming first order elimination kinetics for both alcohols.

Methanol and ethanol are known to compete for metabolism by liver alcohol dehydrogenase and it is generally accepted that the toxic effects of methanol are minimised by the ingestion of ethanol. These toxic effects are resultant from the action of the oxidation products of methanol rather than the alcohol itself. The formates, formaldehyde and methyl formate in particular are toxic especially the latter owing to its increased lipid solubility. The prevention of the formation of formaldehyde and hence methyl formate are considered to be important in the treatment of methanol poisoning. The reactions concerned can be used to explain how this presence of ethanol can reduce the rate of formation of formaldehyde:

a) $CoI + CH_3OH \xrightarrow{\text{alcohol deH}} HCHO + CoI.H_2$

b) $CoI + C_2H_5OH \xrightarrow{\text{alcohol deH}} CH_3CHO + CoI.H_2$

c) $CoI + HCHO + H_2O \xrightarrow{\text{aldehyde deH}} HCOOH + CoI.H_2$

This effective treatment was put into practice in case six, where by the infusion of ethanol what would, more than likely, have been a fatality was avoided. Obviously the quantity of ethanol given must be sufficient to depress the rate of formaldehyde production to less than the rate of equation (c) but without increasing the quantity of ethanol above its current or toxic level.

Other than these findings it is difficult to interpret anything conclusive from such a small sample size and there remains very little evidence for a fatal blood level. Indeed, as is the case with ethanol blood levels there is considerable subject variation and a level as low as 270 mg% ethanol might well be interpreted as a fatal level though many subjects have been known to survive levels much higher than this.

3.17.5 Conclusion.

Of the six fatalities which have been directly related to the ingestion of methanol death could have been caused by factors other than a fatal methanol level in two cases. The levels of methanol in the remainder of cases show considerable variation and there are too few cases to propose a fatal level. It must always be remembered that when a possible methanol fatality is being considered the level of ethanol will diminish the actual toxicity, and therefore fatal level, for methanol.

3.18 Extension of The Method to the Analysis of Chloroform.

3.18.1 Introduction.

An increase in sensitivity for the measurement of chlorinated hydrocarbons is obtainable by the use of an electron capture detector in place of the flame ionisation detector. It was therefore decided to examine the use of the previously developed method for abused solvents in conjuction with this detector for the analysis of chloroform.

3.18.2 Method.

Preparation of Samples.

A series of standards containing chloroform were prepared from a stock solution containing one millilitre (1.48 g) of AnalaR grade chloroform per litre of ten per cent methanol in water, by the dilution of 1,2,3,4 and 5 millilitre aliquots to one hundred millilitres with water in a volumetric flask. Five test samples were prepared from the same stock solution by the dilution of a three hundred microlitre aliquot to ten millilitres with whole blood obtained from outdated transfusion stock. One millilitre aliquots of each of these solutions were then added to individual six millilitre Hypo-vials and the vials sealed with butyl rubber septa and aluminium crimp seals.

Sample Incubation.

The vials were then heated in an aluminium heating block maintained at sixty degrees for a period of one hour. Five hundred microlitre headspace samples were then taken with a pre-heated gas-tight Hamilton syringe and injected into the chromatograph. Analysis was carried out in triplicate.

Gas Chromatographic Conditions.

A glass column (2m x 4mm), packed with Tenax G.C. was placed in a Pye GCD gas chromatograph equipped with a Nickel-63 electron capture detector connected to a Hewlett Packard CRS308 integrator. Argon with five per cent methane was used as a carrier gas, at a flow rate of forty millilitres per minute. The column oven temperature was maintained at 125^{o}C and the detector temperature at 200^{o}C with a detector current of sixteen milliamps. Tenax G.C. does not exhibit any column bleed. Therefore, as expected under these conditions, the column was found to give a steady output with no baseline drift.

The isothermal oven temperature of 125^{o}C was selected from the results obtained in Chapter 2.3 as being suitable for the separation of chloroform and ethanol. This was considered necessary as the presence of ethanol in post-mortem samples is common.

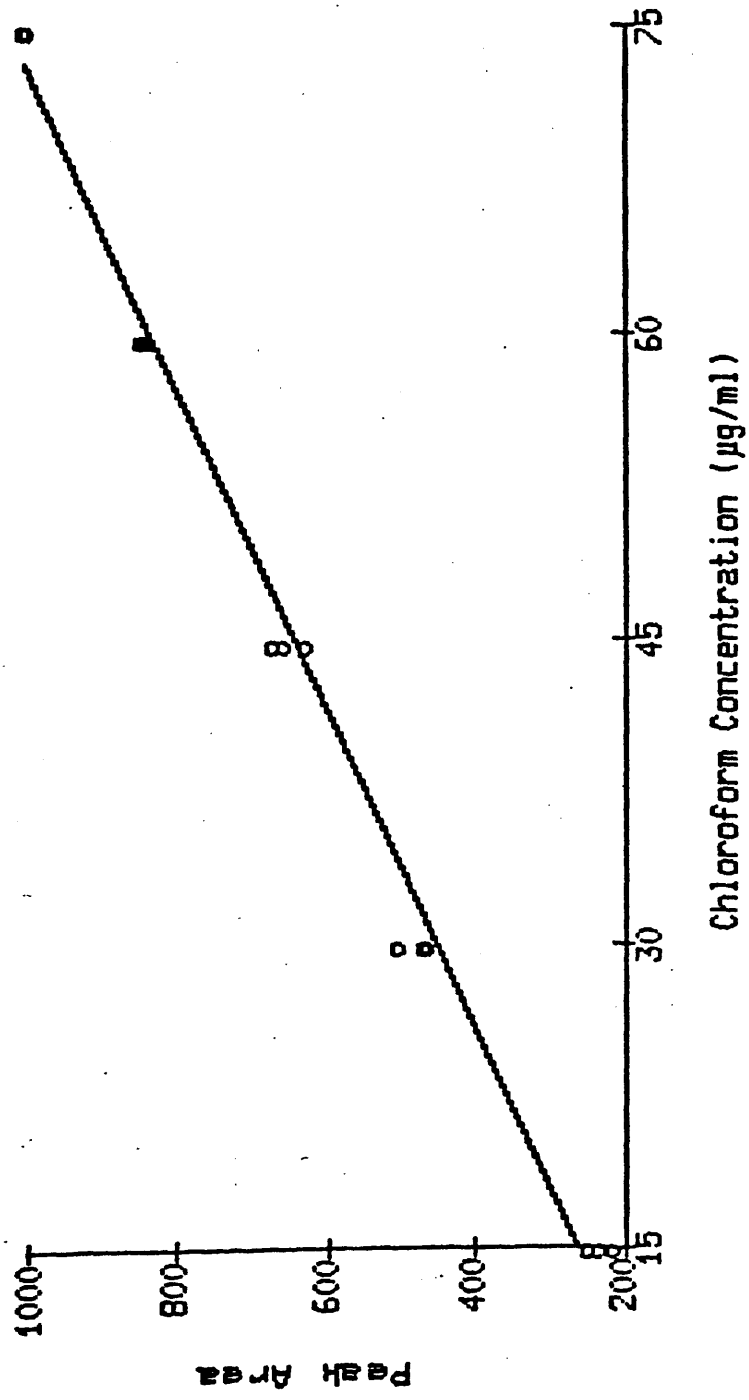
3.18.3 Results.

The results obtained from the headspace analysis of the chloroform standards and test samples are given in Table 3.18.1. The standard results are illustrated in the form of a calibration curve in Figure 3.18.1.

Sample	Area 1	Area 2	Area 3	Mean	c.o.v %	$CHCl_3$ ug/ml
Standard 1	234	249	212	232	8.0	14.8
Standard 2	468	506	466	480	4.7	29.7
Standrad 3	671	655	629	652	3.3	44.5
Standard 4	843	834	841	839	0.6	59.3
Standard 5	1000	998	996	998	0.2	74.2
Test Sample 1	639	631	650	640		44.5
Test Sample 2	667	640	636	648		45.1
Test Sample 3	609	609	624	614		42.5
Test Sample 4	659	635	613	636		44.1
Test Sample 5	635	634	670	646		45.0
correlation coefficient = 0.9955				Mean = 44.2 c.o.v.% = 2.4		

Table 3.18.1 - Peak Areas Obtained for Chloroform Standards and Test Sample.

Figure 3.18.1 - Chloroform Standards Calibration Curve.



3.18.4 Discussion.

The calibration curve obtained for chloroform demonstrates adequate linearity over the range of concentrations examined. This calibration curve was assessed as being sufficiently precise from the variation of results obtained for the test samples and to be reproducible from the coefficients of variation exhibited by the replicate standard curves.

3.18.5 Conclusion.

The method is adequate for the semi-quantitative measurement of chloroform.

3.19 The Analysis of Blood and Tissues for Chloroform in a Case of Suspected Poisoning.

3.19.1 Introduction.

Several post-mortem samples were submitted for analysis after a suspected chloroform poisoning. These samples included the tissue samples: brain, liver, lung, mesentry, muscle; and samples of urine, stomach contents and two samples of blood. One of these blood samples was in a badly clotted condition and the other contained heparin as anticoagulent but had been left open to the atmosphere for a considerable and unknown period prior to submission for analysis. All other specimens were received in air-tight containers.

The method described in Section 3.18 was applied to the analysis of these samples for levels of chloroform.

3.19.2 Method.

Sample Preparation.

A series of chloroform standards were prepared as described in Section 3.18.2.

One gramme of each of the samples was weighed into individual six millilitre Hypo-vials and the vials sealed in the usual manner. The vials were then subjected to ultrasonic vibration for ten minutes before incubation. Analysis was carried out

in triplicate and the calibration graph obtained from the standards was then used to determine the levels of chloroform in the samples.

In addition to the above method of sample preparation a one hundred gramme sample of brain was distilled in one hundred and fifty millilitres of triple distilled water and one millilitre of the resulting distillate was analysed using the above method.

Sample Incubation.

As described in Section 3.18.2.

Gas Chromatographic Conditions.

As described in Section 3.18.2.

3.19.3 Results.

The results obtained from the headspace analysis of the samples for chloroform are given in Table 3.19.1.

Sample	Mean Peak Area	$CHCl_3$ ug/ml
Standard 1	232	14.8
Standard 2	480	29.7
Standrad 3	652	44.5
Standard 4	839	59.3
Standard 5	998	74.2
Blood (clotted)	557	38.8
Blood (heparin)	421	28.7
Brain	263	17.0
Liver	227	14.3
Urine	6	trace
Muscle	24	trace
Mesentry	1087	78.2
Stomach Content	689	48.6
Lung	973	69.8
Brain distillate	172	10.2

Table 3.19.2 - Chloroform Levels in Samples Obtained in a Case of Suspected Poisoning.

3.19.4 Discussion.

One result of the chloroform levels is particularly outstanding - that of the brain sample. The major reference on chloroform poisoning (Bonnichsen & Maehly 1966) informs us that the brain level is usually higher than the blood level. This is as would be expected from the high fat content of the brain compared to that of bloodand from the high liposolubility of chloroform. The high level observed in the lungs of this case therefore suggests that the chloroform was administered by inhalation rather than ingestion and this

deduction is supported by the relatively low level found in the stomach contents.

The method of distillation yielded a lower result than direct analysis. The reason for this is probably due to the high volatility and low water solubility of chloroform resulting in unavoidable losses during distillation.

3.19.5 Conclusion.

Levels of chloroform found in biological fluids and tissues are presented for a case of suspected chloroform poisoning. The levels are indicative of inhalation as opposed to ingestion as the route of administration, and are similar to those levels quoted as fatal levels by other researchers.

3.20 Application of The Method to Two Fatal Cases of Industrial Over-exposure to Trichloroethylene and to One Case of Trichloroethylene Inhalation Abuse.

3.20.1 Introduction.

Trichloroethylene is a volatile, colourless, non-flammable liquid with an odour resembling chloroform. It is used in many industrial processes as a degreasing solvent. It is also used as a solvent base in many paints and some glues thus making it easily available for abuse. The Threshold Limit Value for trichloroethylene is one hundred part per million in air.

In high concentrations trichloroethylene acts as a powerful narcotic and anaesthetic. Loss of conciousness is preceeded by a brief period of confusion, dizziness and loss of muscular power. In less high concentrations this period is extended. Unless an anaesthetised subject is removed from the source of trichloroethylene quickly death may occur from respiratory failure. Sub-acute poisoning is more often encountered in industry.

During the three year period of this study there was only one case referred where trichloroethylene was the abused solvent.

There have also been two fatalities involving over-exposure to the vapours of this solvent. In both cases the workers had

been degreasing engineering components in an open bath of the solvent. They both collapsed and died during a rest break when they were remote from the source of the solvent.

3.20.2 Method.

Preparation of Samples.

A standard solution of trichloroethylene was prepared by dissolving one gramme of AnalaR trichloroethylene in a mixture of methanol and water (9:1) and by serial dilution in distilled water to give a final solution containing one microgramme of trichloroethylene per millilitre.

One millilitre of each of the blood samples and one gramme of each of the tissue samples were weighed into individual six millilitre Hypo-vials. Another vial was prepared containing one millilitre of the standard solution. All of the vials were then sealed with a teflon faced, butyl rubber septum and an aluminium crimp seal before incubation.

Sample Incubation.

As described in Section 3.11.

Gas Chromatographic Conditions.

As described in Section 3.11.

The trichloroethylene concentration in each of the samples was calculated from direct peak area ratio comparison with the standard.

3.20.3 Results.

The blood sample from the suspected trichloroethylene abuser was found to contain 1.24 microgramme of trichloroethylene per millilitre. The abuser was female and sixteen years of age. She was a typist and had apparently been inhaling solvents from a typing correction fluid. Subsequent analysis of this fluid showed it to contain trichloroethylene. The blood sample had been taken approximately two hours after last inhalation of the solvent. She had been admitted to the out patient department of a local hospital suffering from acute intoxication. Her recovery from this episode was uneventful.

The levels of trichloroethylene found in the two fatalities resulting from industrial over-exposure to trichloroethylene are given in Table 3.20.1. Both fatalities occurred at the same time and it would be reasonable to assume that both subjects had been exposed to similar levels of trichloroethylene vapour.

Levels of ethanol were also found in both cases and these are given in Table 3.20.2.

Sample	Trichloroethylene ug/ml	
	Case A	Case B
Blood	5.9	3.1
Urine	0.32	0.14
Brain	34.1	36.4
Liver	20.0	26.7

Table 3.20.1 - Levels of Trichloroethylene found in Samples from Two Industrial Fatalities.

Sample	Ethanol mg/100ml Case A	Case B
Blood	181	229
Urine	251	272

Table 3.20.2 - Levels of Ethanol found in Samples from Two Industrial Fatalities.

3.20.4 Discussion.

Interpretation of the result from the single case of trichloroethylene inhalation abuse is difficult when considered alone. In the case of the two fatalities both workers had high blood ethanol levels. It must also be remembered that collapse occurred in both cases during a rest break where alcohol was not being consumed therefore these levels of ethanol would probably have been higher at the start of the work period. The combined effects of the ethanol, itself sufficient in quantity to produce intoxication, and the trichloroethylene must have resulted in both workers being incapable of exercising their tasks with the required degree of safety awareness. This may have resulted in additional exposure to the solvent vapour than normally experienced during a typical shift.

3.20.5 Conclusion.

Trichloroethylene was identified and measured in blood in one case of abuse and in blood, brain, liver and urine in two fatalities resulting from industrial over-exposure. The

levels found in the blood of the fatalities were higher than that found in the blood from the abuser, and this level in itself was sufficient to cause intoxication. Alcohol was a significant contributory factor in the cause of death in both of the fatalities.

3.21 Application of Gas Chromatography with Mass Specrometric Detection to the Identification and Measurement of Solvents in a Fatal Case of Solvent Inhalation Abuse Involving Trichloroethane.

3.21.1 Introduction.

In the following case an analysis was performed using the data reference library established in Chapter 2.

1.1.1-Trichloroethane is used in many commercial products particularly as a replacement for the more toxic carbon tetrachloride. Despite this availability, often in the form of "pure" solvent as dry-cleaning fluids, there has been only one observed case involving its abuse during the three year period of this study. However, even in this case, when the circumstances of the abuse are considered it can be seen that the solvent's availability played an important part in its selection for abuse.

The solvent is very stable in the body and a large proportion is excreted unchanged through the lungs. A very small amount is metabolised to chloroethanol which is subsequently excreted as the glucuronide (Hake 1960). It has also been suggested that its high stability and rapid excretion account for its comparitively low toxicity, lethal dose approximately sixty microgrammes per millilitre of blood, and quick recovery from anaesthesia. The minimum narcotic dose has been suggested to

be about ten microgrammes per millilitre (Irish 1963).

Pure 1.1.1-trichloroethane is quite reactive and will readily react with aluminium. It is for this reason that tha pure form is rarely found outside the chemical industry where it is used in organic synthesis. The commercial 1.1.1-trichloroethane usually has a purity of 94 - 97 % with 3 - 7 % inhibitors. These inhibitors are often added after production but many impurities can be left after its manufacture which act as natural inhibitors. Butanol, dioxan and ethylene dichloride are three of the more common inhibitors used.

3.21.2 Case History.

A twenty-two year old inmate of a local prison had been found dead in his cell with a piece of flannel over his head. The flannel was reported to smell of "carbon tetrachloride", and the subsequent location of a treacle tin containing a similarly smelling, clear, colourless fluid on searching the cell, suggested a possible case of solvent inhalation abuse. The deceased had a previous history of volatile substance abuse. The prison authorities reported that the deceased worked in the laundry and in such position of trust had access to the cleaning fluid "Ezi-Klene" used there.

A post-mortem was carried out and a sample of blood along with the treacle tin of fluid were brought for analysis.

3.21.3 Method.

Preparation of Sample.

A solution of the solvent sample was prepared as a one part per thousand dilution in water. Ten millilitres of this solution were then sealed in a twenty-three millilitre Hypo-vial using a teflon faced, butyl rubber septum and an aluminium crimp seal. Another similar vial was prepared containing ten millilitres of the blood sample and another containing ten millilitres of a standard solution of 1.1.1-trichloroethane diluted to a concentration of one microgramme per millilitre in water.

Sample Incubation.

The vials were then placed in a water bath maintained at fifty degrees and allowed to equilibrate for thirty minutes.

After equilibration a fifty microlitre headspace sample was removed from each vial and injected on to Column A. The retention times were measured and the peak areas integrated using a Hewlett Packard 3370B integrator.

A second fifty microlitre headspace sample was then injected into Column B and the mass spectrum of each peak obtained. These spectra were then searched for in the solvent spectra library to confirm their identities.

Gas Chromatographic Conditions.

The analysis of the solvent sample was carried out on two

independant Pye 104 gas chromatographs. Column A was 10 % Apiezon L on Chromosorb W operated at an isothermal temperature of eighty degrees with nitrogen as carrier gas at forty millilitres per minute. Column B was 10 % Carbowax 400 on Chromasorb W operated at an isothermal temperature of seventy degrees with a helium as carrier gas at thirty millilitres per minute. Column A was connected to a flame ionisation detector and Column B was connected to the inlet of a VG 16F mass spectrometer which in turn was connected to a VG 2200 data system.

3.21.4 Results.

The retention times for the four component peaks observed in both the blood and the solvent sample are given for both Column A and Column B in Table 3.21.1.

A total ion current trace for the sample of solvent is given in Figure 3.21.1.

The mass spectrum taken on each of these peaks are given in Figures 3.21.2 to 3.21.3, and the corresponding library reference spectrum with the "best fit" to each of these spectra are given in Figures 3.21.4 to 3.21.8 respectively.

Figure 3.21.1 - Total Ion Current Chromatogram for the Solvent Sample.

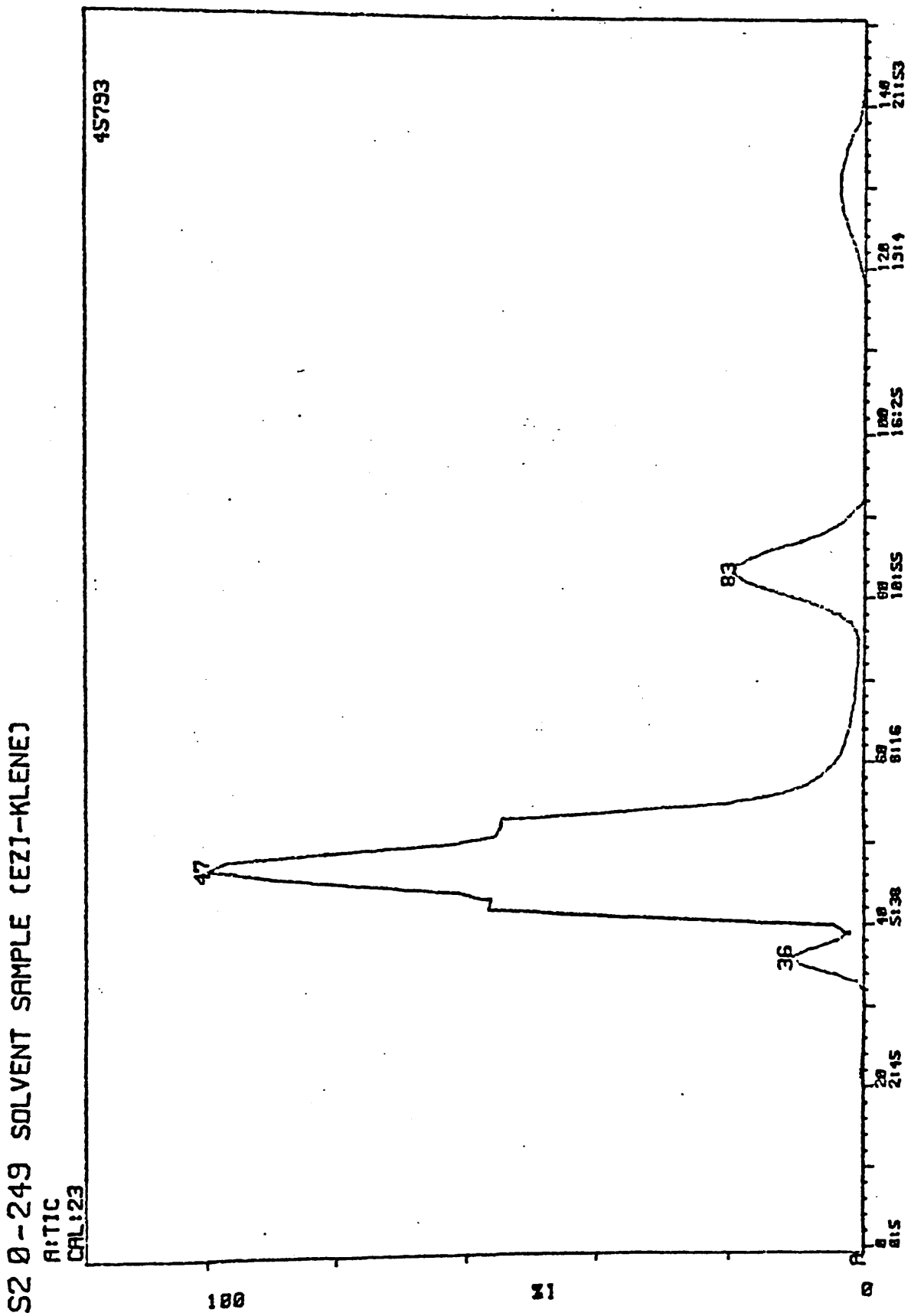


Figure 3.21.2 - Mass Spectrum of Peak No. 1 Scan 36.

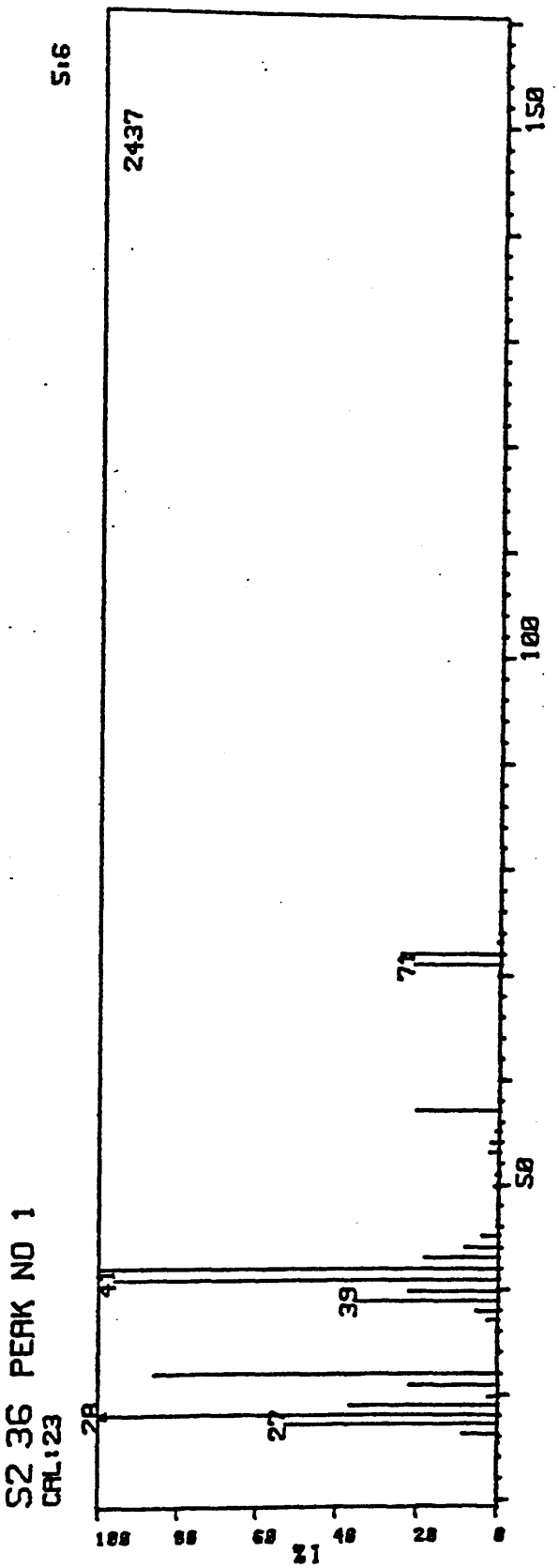


Figure 3.21.3 - Mass Spectrum of Peak No. 2 Scan 41.

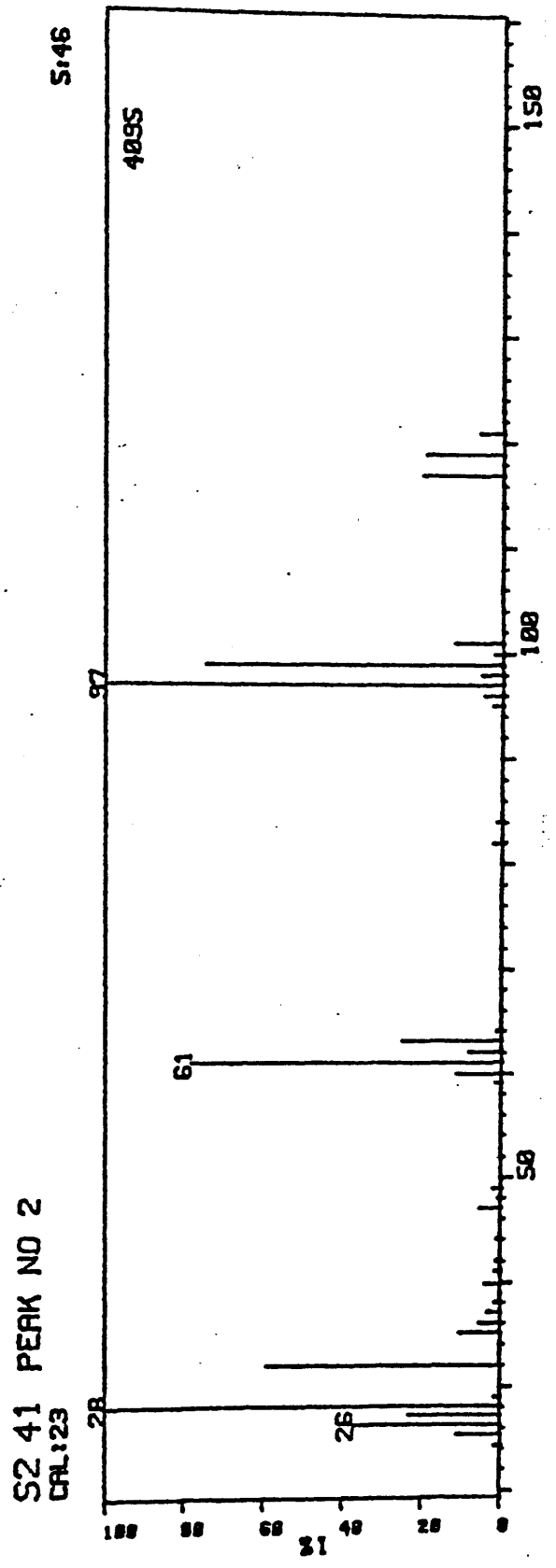


Figure 3.21.4 - Mass Spectrum of Peak No. 3 Scan 83.

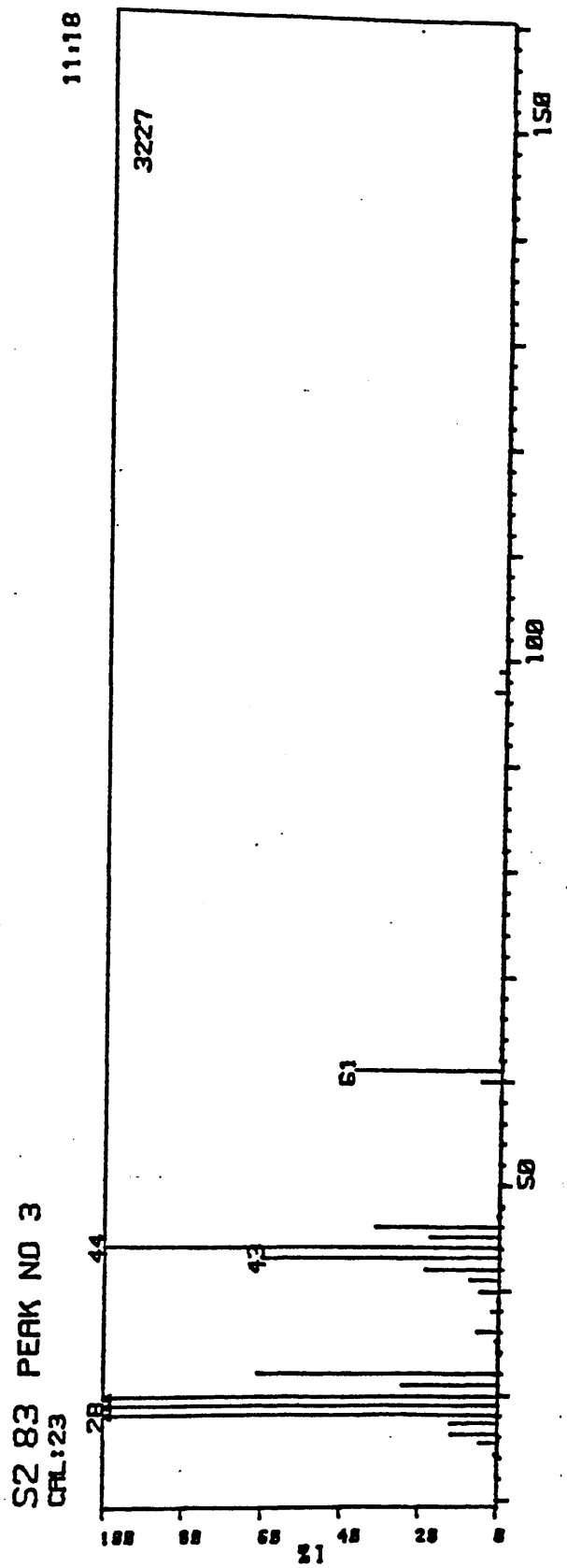


Figure 3.21.5 - Mass Spectrum of Peak No. 4 Scan 129.

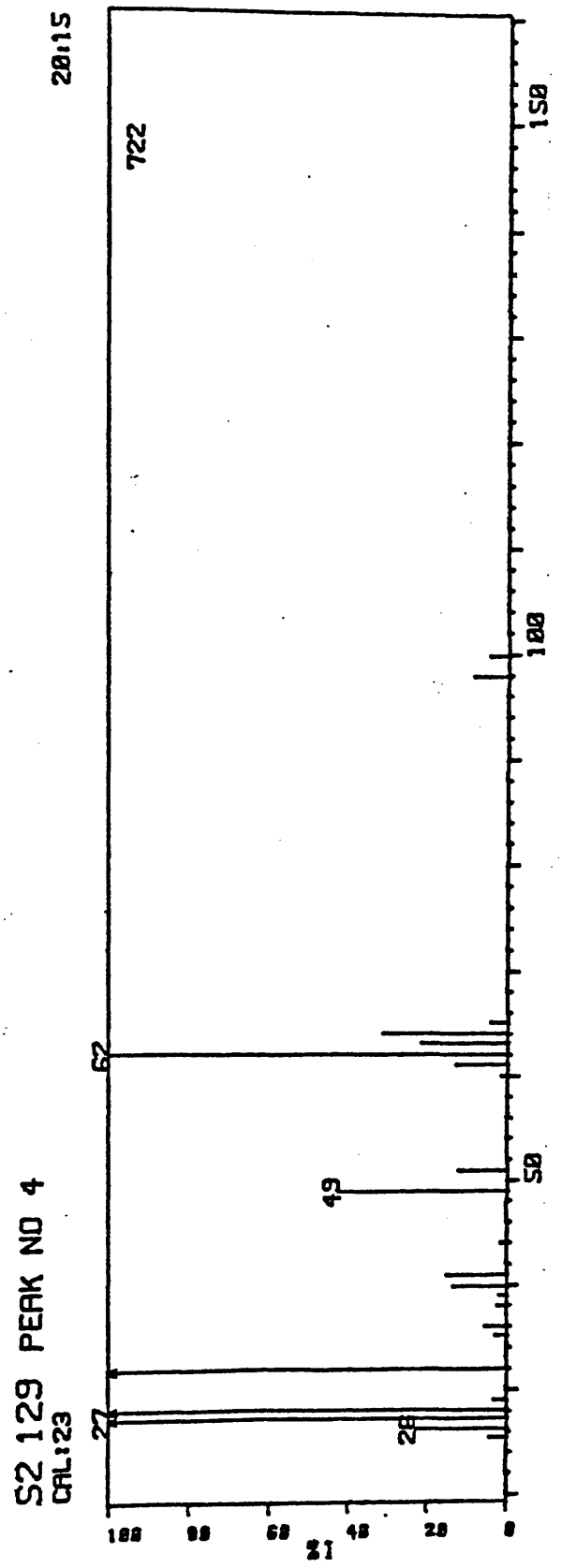


Figure 3.21.6 - Mass Spectrum of n-Butanal.

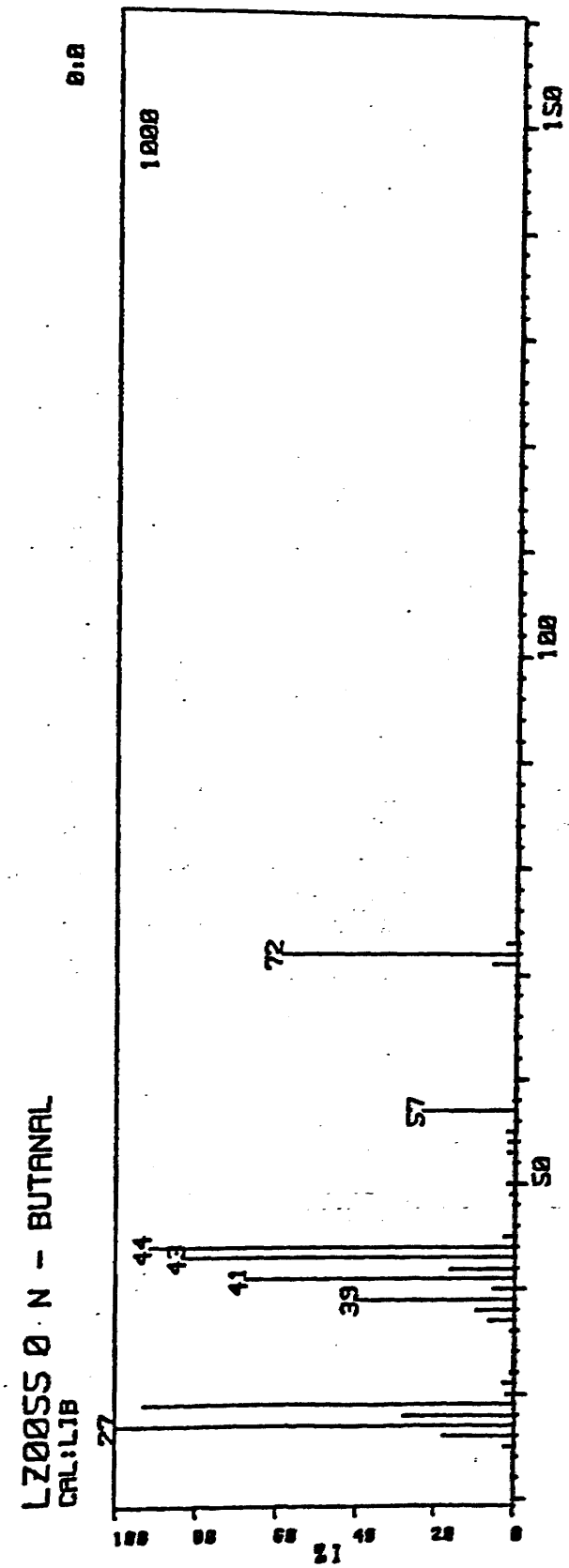


Figure 3.21.7 - Mass Spectrum of 1,1,1-Trichloroethane.

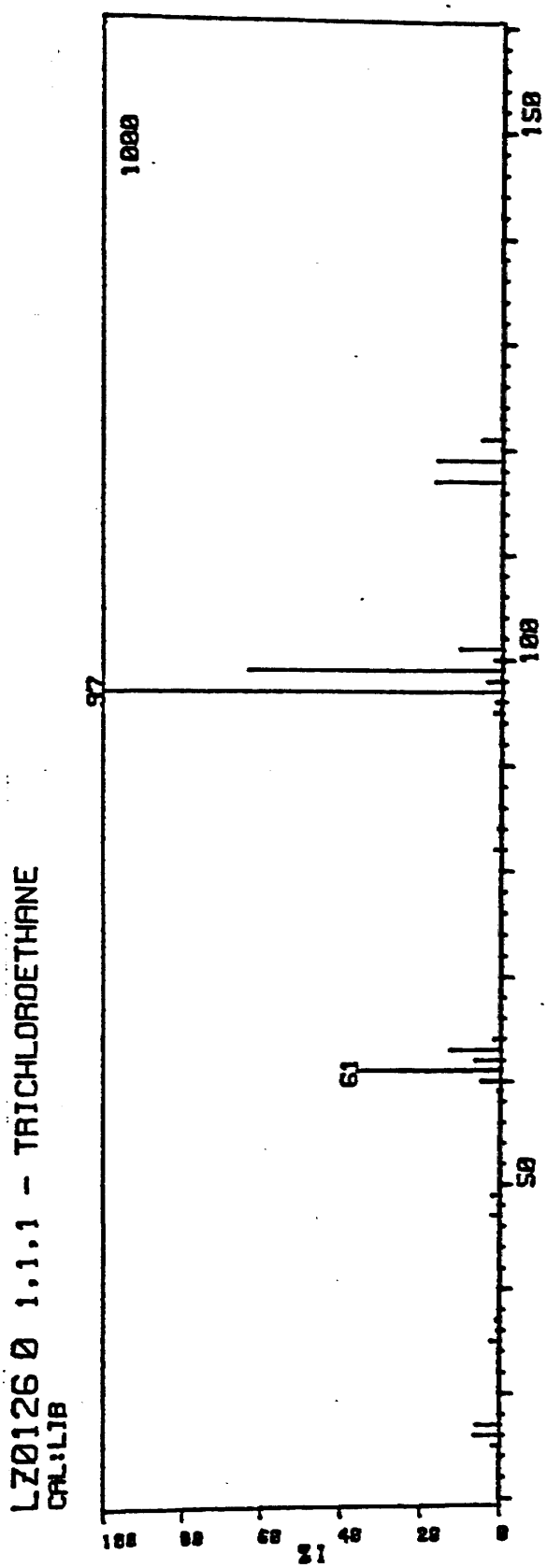
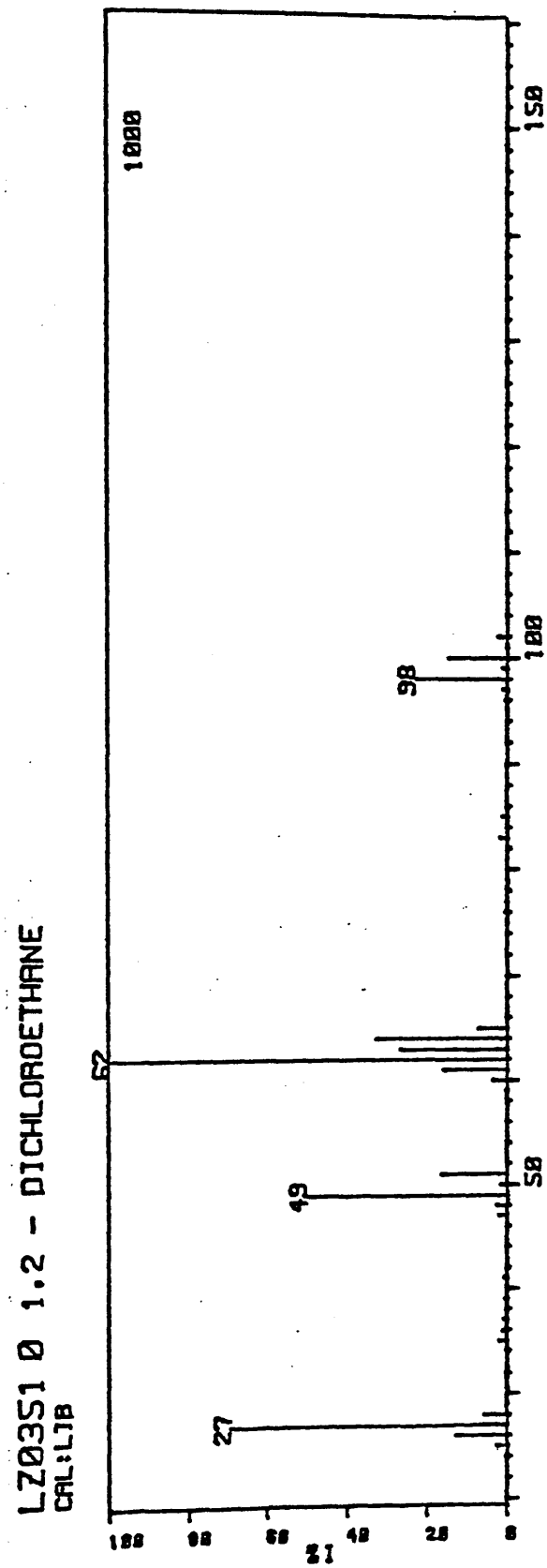


Figure 3.21.8 - Mass Spectrum of 1,2-Dichloroethane.



Peak No.	Column A		Column B	
	Rt.min.	% Nom	Rt.min.	% Nom
1	1.71	93.14	5.06	0.71
2	3.17	0.76	5.46	93.32
3	4.51	5.54	11.18	5.48
4	5.32	0.56	20.15	0.49

Table 3.21.1 - Retention Times of Peaks Observed in Sample of 1.1.1-Trichloroethane.

3.21.5 Discussion.

The identities of the four peaks were confirmed from their mass spectra. Peaks numbered two and four on Column B were identified as 1.1.1-trichloroethane and 1.2-dichloroethane respectively with a good correlation fit factor with the standard library spectra for these compounds. Peaks numbered one and three were not conclusively identified although the library search gave best correlation fit factors for chloroethylene and butanol respectively.

The same peaks that were identified in the solvent sample were also identified in the blood sample confirming the supposition that the deceased had been inhaling or drinking the solvent.

No quantitation of the levels of the solvents in blood were made.

3.21.6 Conclusion.

Although no quantitation was performed it was clear from the circumstances involved and other available information that

the deceased died as a direct result of exposure to 1.1.1-trichloroethane, probably arising from the inhalation abuse of a product containing this compound as its main ingredient.

The identity of the abused product was confirmed by the identification of its constituents and their presence in the sample of blood from the deceased. This identification was performed by mass spectroscopic comparison with a library of known standard solvent spectra.

3.22 The Toxicology of Halothane.

3.22.1 Introduction.

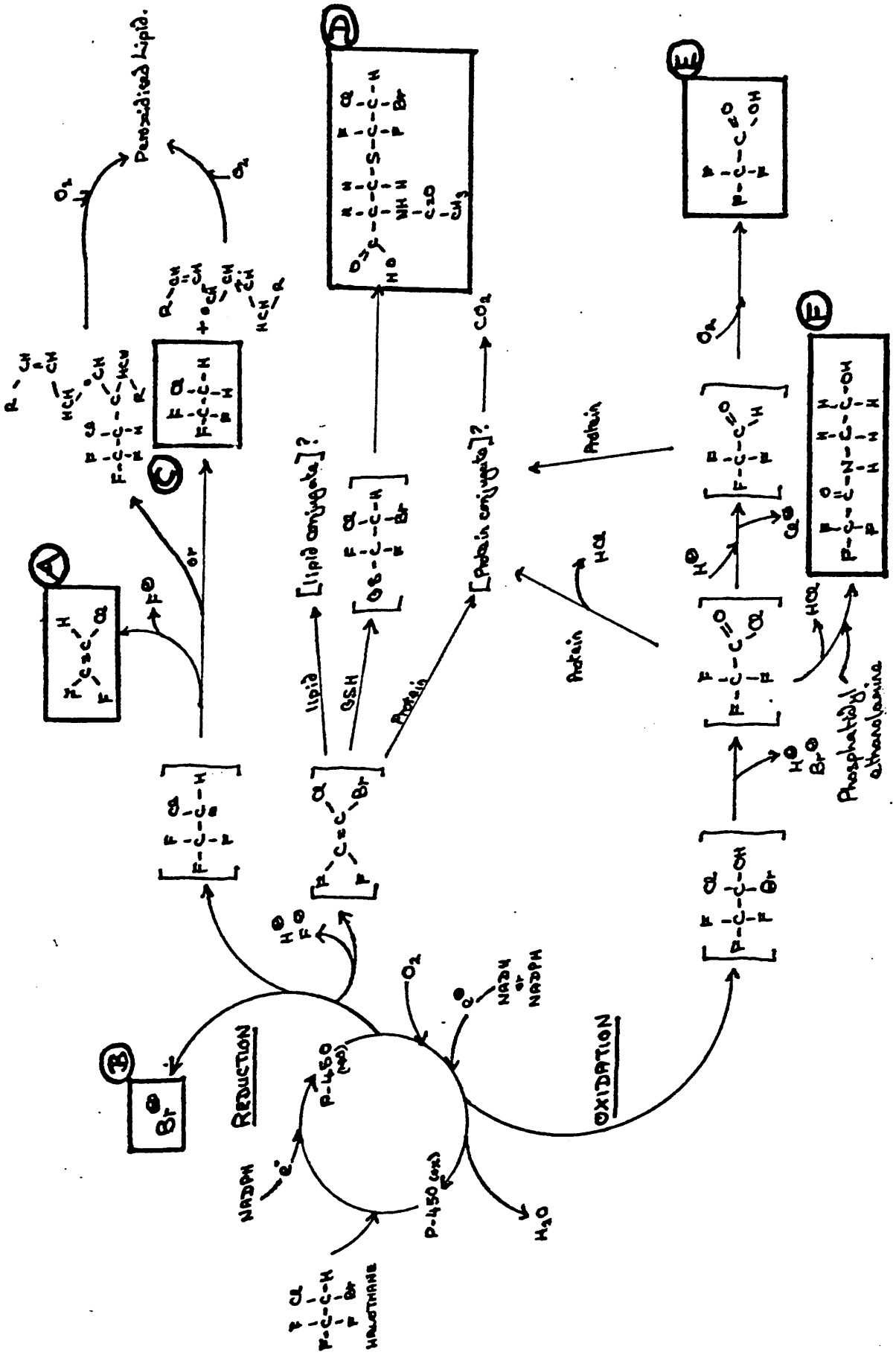
Since its introduction in 1956 Halothane has gained widespread popularity as a safe anaesthetic with almost unlimited use. Being nonflammable, volatile, easy to administer and for a while considered to be unaffected by biotransformation it has become one of the most popular anaesthetics, competing favourably with nitrous oxide.

By 1963, however, there became increasing evidence of possible hepatic injury associated with its use (Tygstrup 1963). This possibility was and still is by many anaesthetists totally rejected, although their rationalisation may be due to the fact that the use of Halothane is very popular and easy to administer. There still appear at regular intervals reports both for (Sherlock 1978) and against (Lancet Editorial 1969). The case against Halothane as a direct toxin now seems to be proven and hepatic lessions can only be induced in laboratory animals under extremes of exposure (Fabian, Margolis & Stephen 1958), and adverse reactions in humans are rare, are not dose related and often the hepatic injury is not immediate (Griner 1966).

3.22.2 Metabolism of Halothane.

It is now known that the metabolism of Halothane Figure 3.22.1

Figure 3.22.1 - Metabolic Pathway of Halothane.



is complex and is a factor of critical concern in relationship to its toxicity as it is higher in humans than in any other examined species (Van Dyke 1973). This establishment of a complex metabolic fate for Halothane was made by the use of radiotracer studies with which it was shown that Halothane is metabolised to a number of potentially toxic compounds (Cohen 1975). More recent work has shown no less than twelve stable metabolites being produced over a period of several days after exposure (Moslen & Reynolds 1977). However, most of these compounds have been shown to be only transient.

1. 2-Chloro-1-1 difluoroethylene. ($Cl.CH:CF_2$)

This is the most controversial of the proposed metabolites. Its existance was demonstrated by Mukai (1977) and is present in only small concentrations, less than five microgrammes per millilitre, for a short period after exposure. It was probable that this very volatile metabolite was missed by previous work simply by the fact that a large quantity of halothane is eliminated in the breath during the same period. Halothane itself is exhaled for up to four days after exposure but usually decreases to about one hundred nanogrammes per millilitre during the first twenty-four hours.

2. Bromide. (Br^-)

The evidence for bromide as a metabolite was at fist contradictory. The presence of bromide was first demonstrated by Stier but his initial work was refuted by Raventos (1959) whose work with rats showed no increase in bromide levels after exposure to Halothane. However later work by Stier

(1964) and a more recent study, (Tinker 1976) has shown considerable elevation of bromide levels after exposure to Halothane. Levels ranged between fifty and one hundred and eighty microgrammes per millilitre at the peak of excretion. The excretion of bromide is known to be dependant on the chloride intake and half life measurements give a period of between twelve and fourteen days. Therefore elevated bromide levels, or more usefully bromide : chloride ratios, may be used to indicate the degree of exposure to Halothane. The peak plasma bromide levels occurring between forty eight and seventy two hours after exposure. For comparison the mean control bromide levels were quoted to be about two microgrammes per millilitre.

3. 2-Chloro-1,1,1-Trifluoroethane. ($Cl.CH_2.CF_3$)

This is another very volatile metabolite which was found to be excreted in exhaled air for up to fortyeight hours after exposure (Mukai 1977). It was reported to be present in only low concentrations and like 2-chloro-1,1-difluoroethylene is not a major metabolite. This may be due to the possible conjugation with lipid molecules resulting in the formation of a peroxidised lipid.

4. Methyl-N-Acetyl-S-(2-Bromo-1,1-Difluoroethyl) -L-Cysteinoate.

This metabolite, a mercapturic acid, has been isolated from the urine of subjects exposed to Halothane (Cohen 1975). Its presence is interesting as mercapturic acids are common metabolites of halogenated hydrocarbons. It was at first

thought that a mercapturic acid would be formed in the usual way, in this case by the displacement of bromine from halothane by glutathione resulting in the formation of 1-chloro-2,2,2-trifluoroethane mercapturate, however this is not the metabolite found. Instead it appears that an unusual displacement of fluoride occurs resulting in fluoride concentrations in blood and urine. There is now strong evidence that this is the major route of biotransformation of Halothane as the appearance of fluoride occurs almost immediately after administration of halothane, rapidly decreases in concentration with a half life of less than two hours and it is almost entirely excreted within twelve hours.

5. N-Trifluoroacetyl-2-Aminoethanol.

($CF_3.CO.NH.CH_2CH_2CH_2OH$)

This is a very interesting metabolite and an unusual metabolic conjugate, the most likely source of the ethanolamide being phosphatidyl-ethanolamine, a normal constituent of the cell membrane. The occurence of this metabolite therefore suggests conjugation of the trifluoroacetyl radical with the cell wall and this may well lead to the interference with normal cell function. This metabolite accounts for about fifteen percent of the total urinary metabolites of Halothane, about one percent of the inhaled dose, by approximately six hours after exposure (Cohen 1975).

6. Chloride and Fluoride. (Cl^- and F^-)

Inorganic fluoride is present in such large quantities in the body that the small increase brought about by Halothane

metabolism is easily obscured by normal dietry and metabolic fluctuations.

Inorganic fluoride has been shown to be present after Halothane administration (Cohen 1975) but very little reliability can be placed on the use of this metabolite as an indicator of Halothane exposure, again due to the increasing dietry intake of fluorides.

7. Trifluoroacetic Acid. ($CF_3.CO.OH$)

This is the major metabolite of Halothane and can be found in levels of about two milligrammes per millilitre of urine. It reaches its peak concentration between twenty-four and forty eight hours after exposure and has been demonstrated to be still present in measurable quantities as long as thirty days post-exposure. Trifluoroacetic acid is very reactive and is most likely present in the urine as esters and salts.

The route of biotransformation to trifluoroacetic acid appears to be the subject of debate. It was initially thought that it proceeds via the trifluoroethanol and the aldehyde in the same manner as trichloroethylene and trichloroethane. However, the presence of the intermediate alcohol has not been proved and as this would be stabilised by the formation of its glucuronide it would be expected to be observed. The alternative hypothesis is that all of the trifluoroacetic acid is produced via the other metabolites and by the biotransformation of their protein conjugates. This provides an acceptable explanation as to why this metabolite can be found

in the urine for such long periods after exposure and is perhaps a more satisfactory explanation than that of the slow release of Halothane from stores in fatty tissue.

3.22.3 Discussion.

It has been suggested that the so called "Halothane hepatitis" is due to an allergic response or sensitisation to one or more of its metabolites. The fact that reported cases are rare, although it must be appreciated that all but the most severe cases go unnoticed, seemingly suggests an individual response to Halothane or its metabolites. Due to the large number of pathways for biotransformation, accumulation of the more toxic metabolites may occur to a greater extent in some individuals than in others. A study of the levels of some of these metabolites might be useful to determine the degree of variation of metabolism between individuals.

As far as the use of metabolite levels to indicate the degree of exposure to Halothane or even the differences in the quantity absorbed and metabolised is concerned, the obvious preference must fall on the measurement of trifluoroacetate and bromide levels. As there is a natural bromide level present, and this quantity is largely dependant on the intake of chloride, the specific nature of the trifluoroacetate levels are to be preferred. This is supported by the fact that trifluoroacetate is present in measurable quantities many days after exposure.

3.22.4 Conclusion.

Trifluoroacetate levels in urine should be a useful indicator of the degree of exposure to the anesthetic liquid Halothane. These levels should also be of some use in the study of "Halothane hepatitis". However, the major study of total metabolites, in a large population of patients and controls exposed to Halothane, which is required for a full appreciation of this problem, is beyond the directive of this thesis.

3.23 The Determination of Trifluoroacetate Levels in Urine by Gas Chromatography.

3.23.1 Introduction.

Owing to the high reactivity of trifluoroacetic acid it will occur in urine as salts or as esters. Even if the acid itself was present it would be difficult to chromatograph. To overcome this problem a suitable derivative must be made. A solvent extraction procedure followed by the methylation of the free acid has been employed (Furukawa 1976). However the author was the first to highlight the fact that this method was not acceptable for the quantitative evaluation of urine levels owing to the large errors and losses occuring in the extraction steps.

To establish the possibility of using a similar method for the determination of trifluoroacetate levels in patients after exposure to halothane, and to provide a method for the investigation of the possible metabolism of other fluoroalkanes, particularly those which are thought to be popularised by the abuser, the freon aerosol propellants, the following method was examined.

Heptafluorobutyric acid was used as an internal standard.

3.23.2 Method.

Preparation of Samples.

One millilitre of a 0.001M solution of sodium trifluoroacetate in methanol in a six millilitre Hypo vial was evaporated to dryness under a stream of nitrogen at sixty degrees. A similar vial was prepared containing a one millilitre of a dilute solution of sodium heptafluorobutyrate in methanol, approximately 0.001M, and one millilitre of the 0.001M solution of sodium trifluoroacetate. To the residue in each vial was added a two hundred microlitre aliquot of AnalaR grade trichloroethanol using a Hamilton syringe.

Sample Incubation.

The vials were then sealed with teflon lined septa and a one hundred microlitre aliquot of concentrated sulphuric acid was added through the septa using a Hamilton syringe. The vials were then heated to sixty degrees for twenty minutes after which a one hundred microlitre aliquot of the headspace in the vials were removed in a preheated gas tight syringe and injected into the gas chromatograph.

Gas Chromatographic Conditions.

A Pye 104 gas chromatograph equipped with a flame ionisation detector was used. A 1.25m. x 6mm. glass column packed with 3% OV-17 on Chromasorb W (100-120 mesh) was used for the separation. The column was operated at eighty degrees and with a zero grade nitrogen carrier gas flow rate of twenty millilitres per minute.

3.23.3 Results.

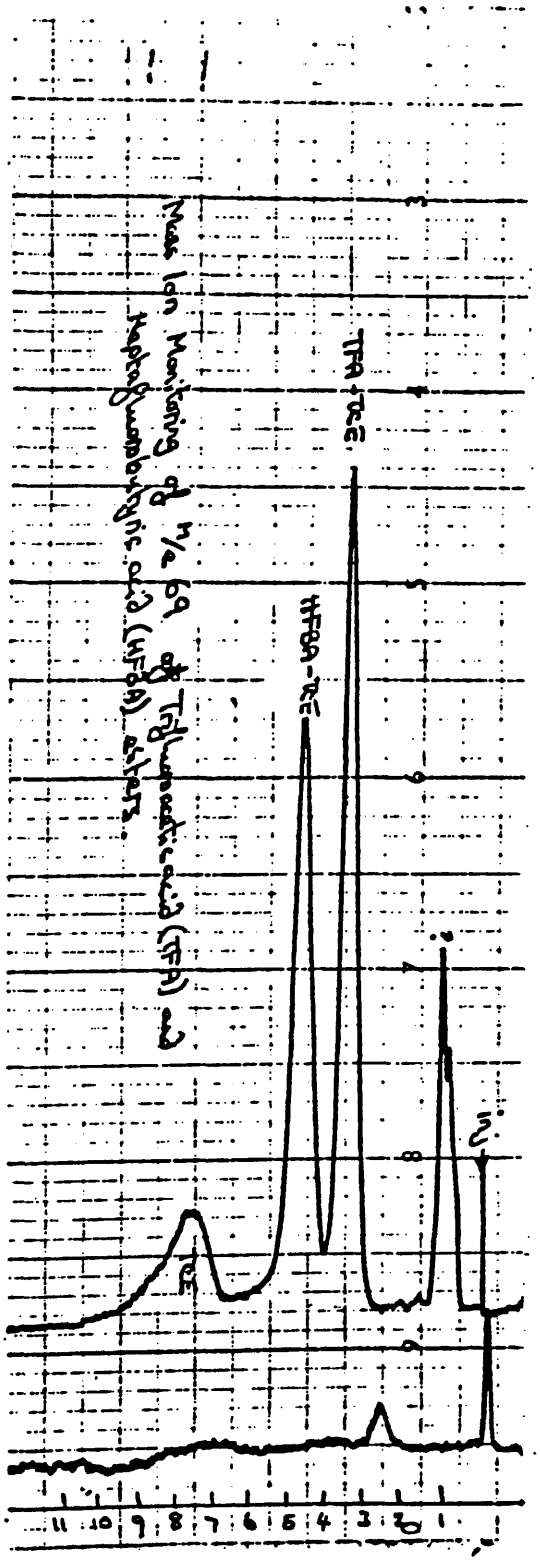
Chromatography of the derivatised acids revealed three major peaks. A chromatogram of the mixture of the two acids after esterification with trichloroethanol is shown in Figure 3.23.1. The peaks at retention times of 2.4 and 2.9 minutes are due to the trichloroethyl esters of trifluoroacetic acid and heptafluorobutyric acid respectively. The larger peak at retention time 5.5 minutes being due to the unreacted trichloroethanol.

3.23.4 Discussion.

The conditions described are sufficient to separate the esters of the two acids for possible use as a quantitative method. However the chromatographic run time was increased by the large quantity of excess reagent trichloroethanol present.

The method does not involve a concentration step and therefore the total trifluoroacetate can be measured. This would be a problem with actual samples as the expected concentrations of trifluoroacetates would be lower than 0.001M. The required decrease in attenuation of the detector response would make the problem of excess tricloroethanol even more significant producing totally unacceptable chromatogram clean up periods of over one hour.

Figure 3.23.1 - Chromatogram of trifluoroacetic and heptafluorobutyric acids after esterification with trichloroethanol.



3.23.5 Conclusion.

The chromatographic method described has been shown to provide adequate separation of the products of the esterification of trifluoroacetic acid and heptafluorobutyric acid, a proposed internal standard, with trichloroethanol. However the sensitivity was found to be inadequate for use as a practical method. It may be possible to circumvent this problem by the use of a backflushing system, though the priority of a requirement for this analysis and the absence of available equipment does not permit the examination of this technique.

3.24 The Determination of the Mass Spectra of the Trichloroethyl Esters of Trifluoroacetic Acid and Hetafluorobutyric Acid.

3.24.1 Introduction.

In an attempt to improve on the method examined in Section 3.23 by using the equipment available the possibility of using a mass selective detector was considered. To ascertain if the use of this detection system would be feasable the mass spectra of the esterification reagents and products had to be obtained.

3.24.2 Method.

Preparation of Samples.

Samples of 2,2,2-trichloroethyl trifluoroacetate and 2,2,2-trichloroethyl heptafluorobutyrate were prepared in individual sealed Hypo vials by the addition of the respective anhydride to 2,2,2-trichloroethanol. The vials were heated to sixty degrees until homogenous solutions were obtained, about thirty minutes.

Gas Chromatographic and Mass Spectrometer Conditions.

A VG MM16F mass spectrometer was used to obtain the mass spectra and was interfaced with a VG 2000 data system for control and for the interpretation of the spectra. A 1.25m. x 6mm. glass column packed with 3% OV17 on Chromasorb W (100-120

mesh), operating with a helium carrier gas flow rate of thirty millilitres per minute and at a temperature of eighty degrees, was used to separate the products of the esterification reactions.

The mass spectrometer was calibrated before use over the mass range 20 to 410 mass units with a sample of standard perfluorokerosene calibrant.

Fifty microlitre aliquots taken from the headspace above the 2,2,2-trichloroethyl trifluoroacetate and 2,2,2-trichloroethyl heptafluorobutyrate were then separately chromatographed. The total ion current for each sample was retained by the data system.

3.24.3 Results.

A total ion current trace for the chromatographed sample of 2,2,2-trichloroethyl trifluoroacetate is given in Figure 3.24.1. The retention time of the most intense peak was found to be 2.4 minutes and one of the spectra taken during this peak is given in Figure 3.24.2.

A total ion current trace for the chromatographed sample of 2,2,2-trichloroethyl heptafluorobutyrate is given in Figure 3.24.3. The retention time of the most intense peak was found to be 4.0 minutes and one of the spectra taken during this peak is given in Figure 3.24.4.

A spectrum taken during the second peak, occurring in each chromatogram at a retention time of 5.3 minutes, corresponds to the spectrum of 2,2,2-trichloroethanol given in Figure 3.24.5.

The fact that molecular ion peaks, expected at 344 amu and 244 amu, were not observed suggested the possibility that the 2,2,2-trichloroethyl trifluoroacetate and 2,2,2-trichloroethyl heptafluorobutyrate were decomposing on the column and that the spectra obtained were in fact these decomposition products. This suggestion was refuted in both cases by assigning identities to the major ions in each spectrum. The suggested identities of these ions are given in Tables 3.24.6a and 3.24.6b.

Figure 3.24.1 - A TIC trace for the sample of 2,2,2-trichloroethyl trifluoroacetate.

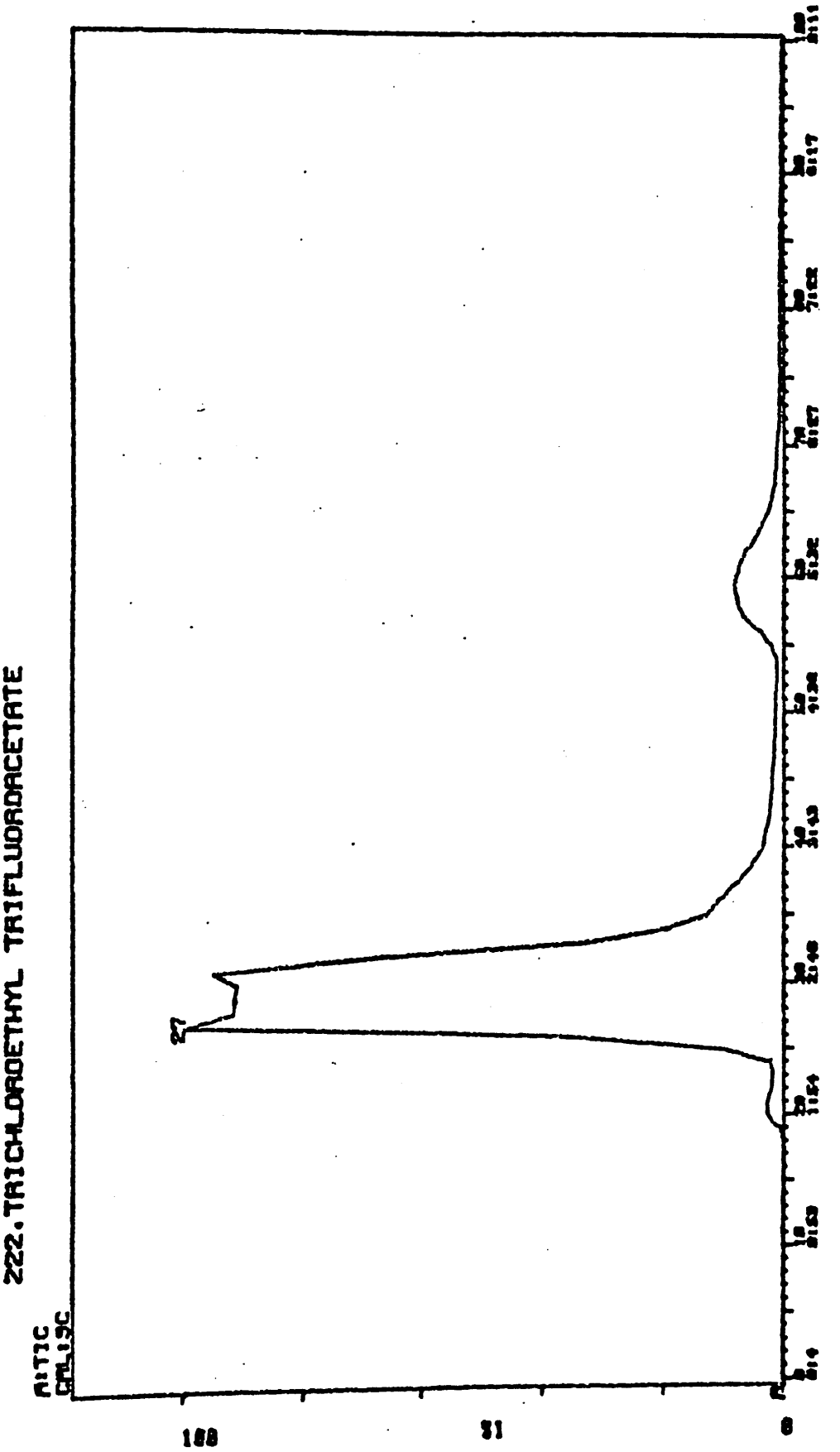


Figure 3.24.2 - Mass Spectral Scan taken during the Peak at 2.4 minutes.

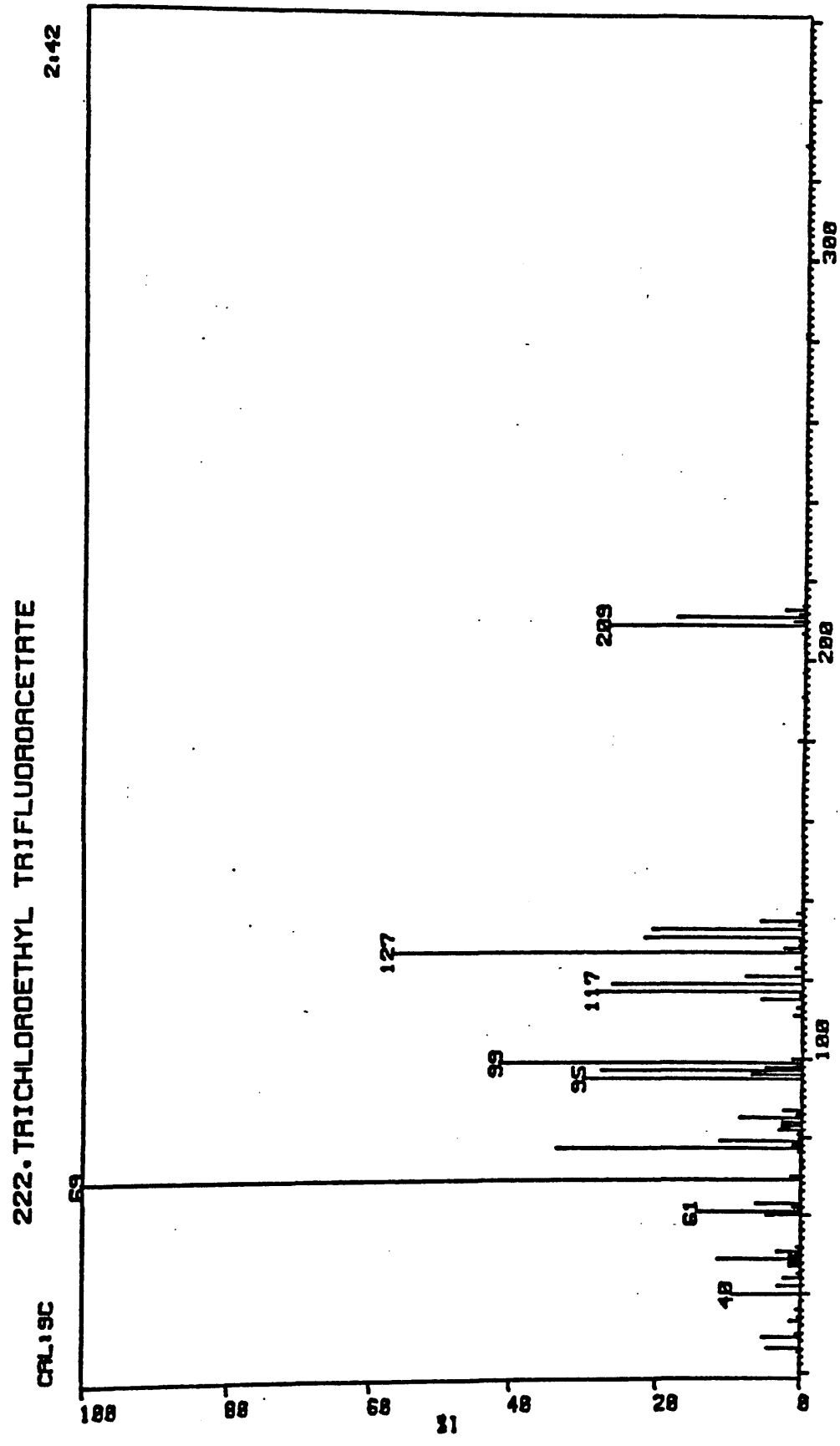


Figure 3.24.3 - A TIC trace for the sample of 2,2,2-trichloroethyl heptafluorobutyrate.

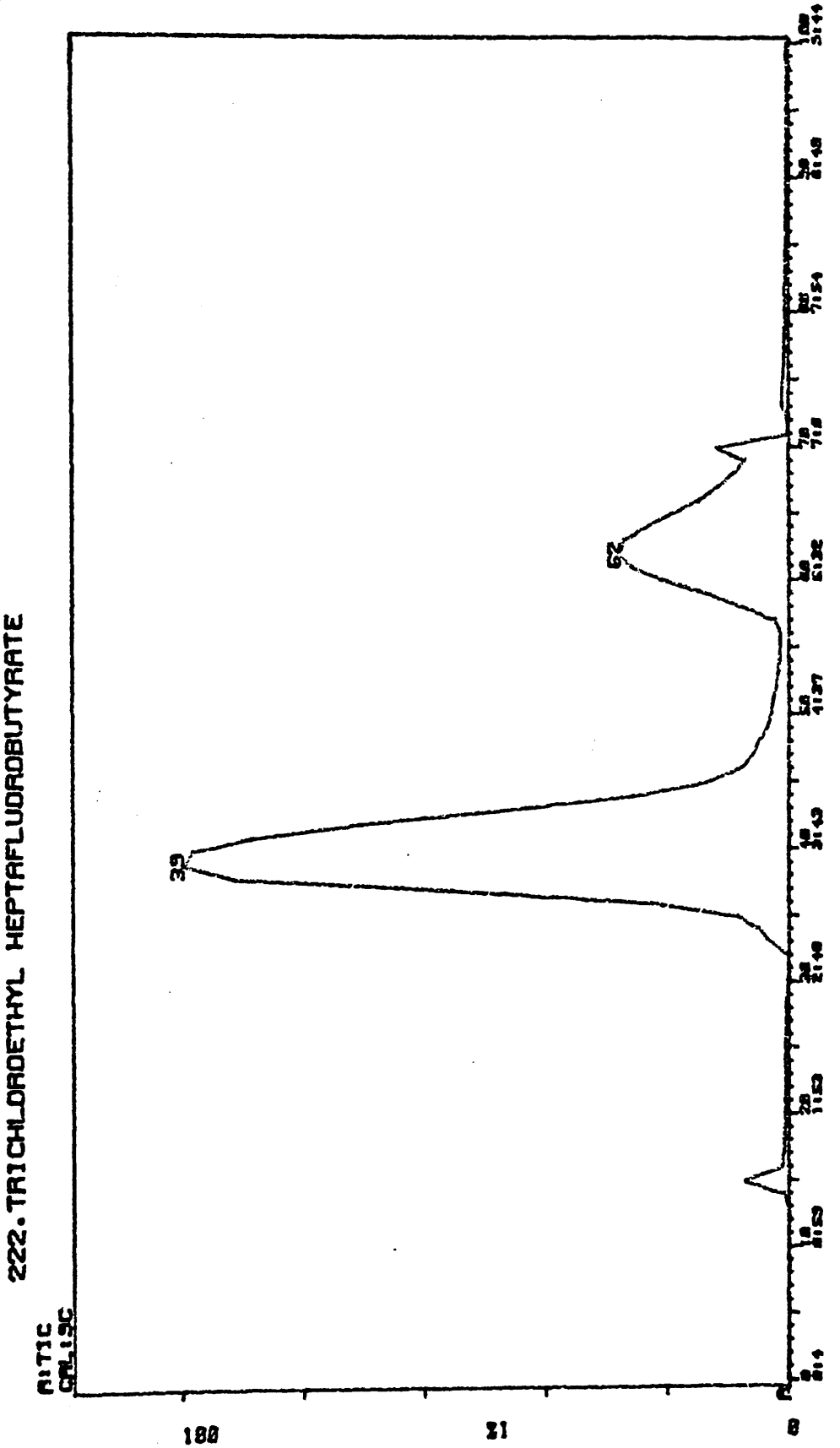


Figure 3.24.4 - Mass Spectral Scan taken during the Peak at 4.0 minutes.

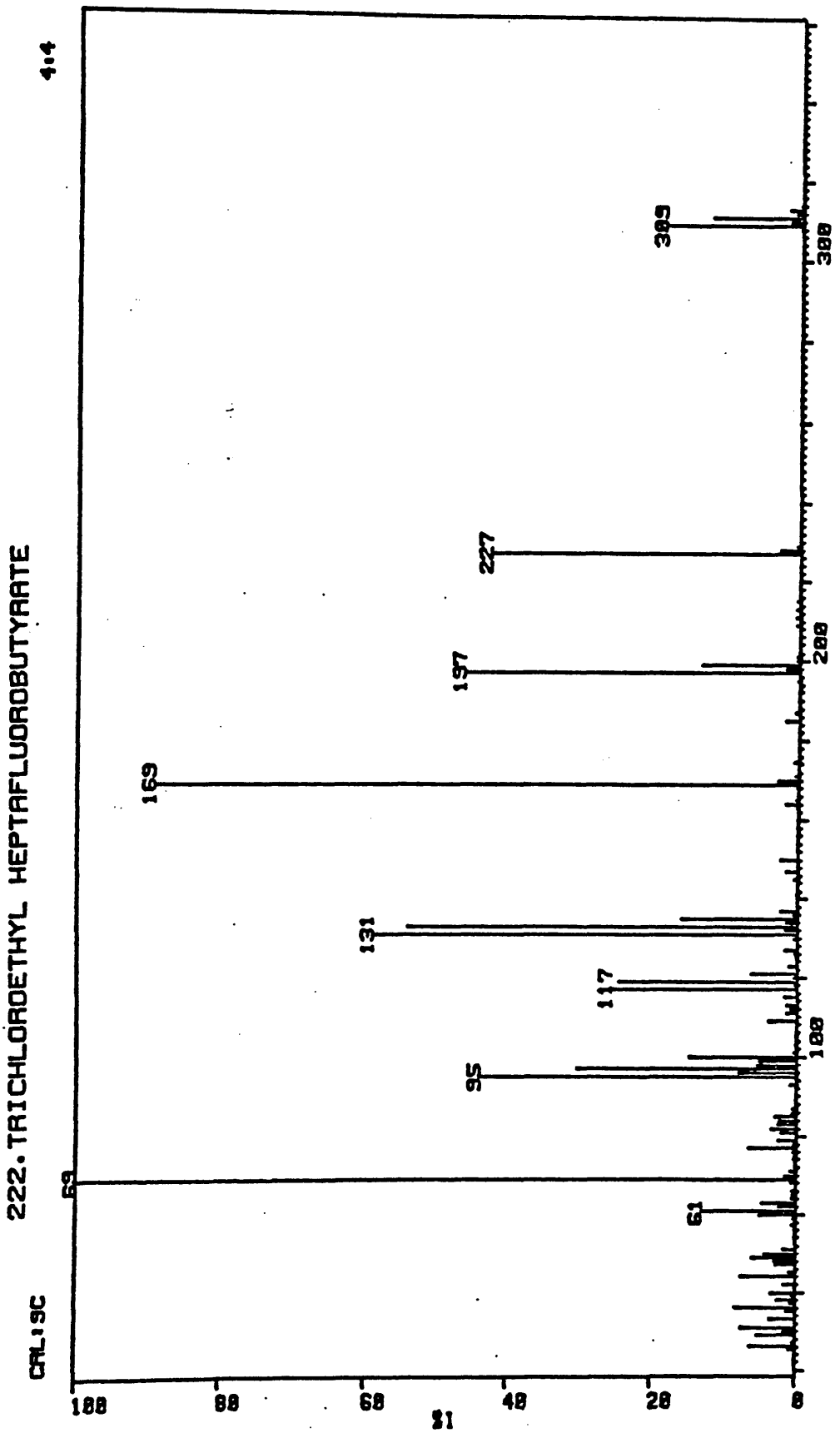
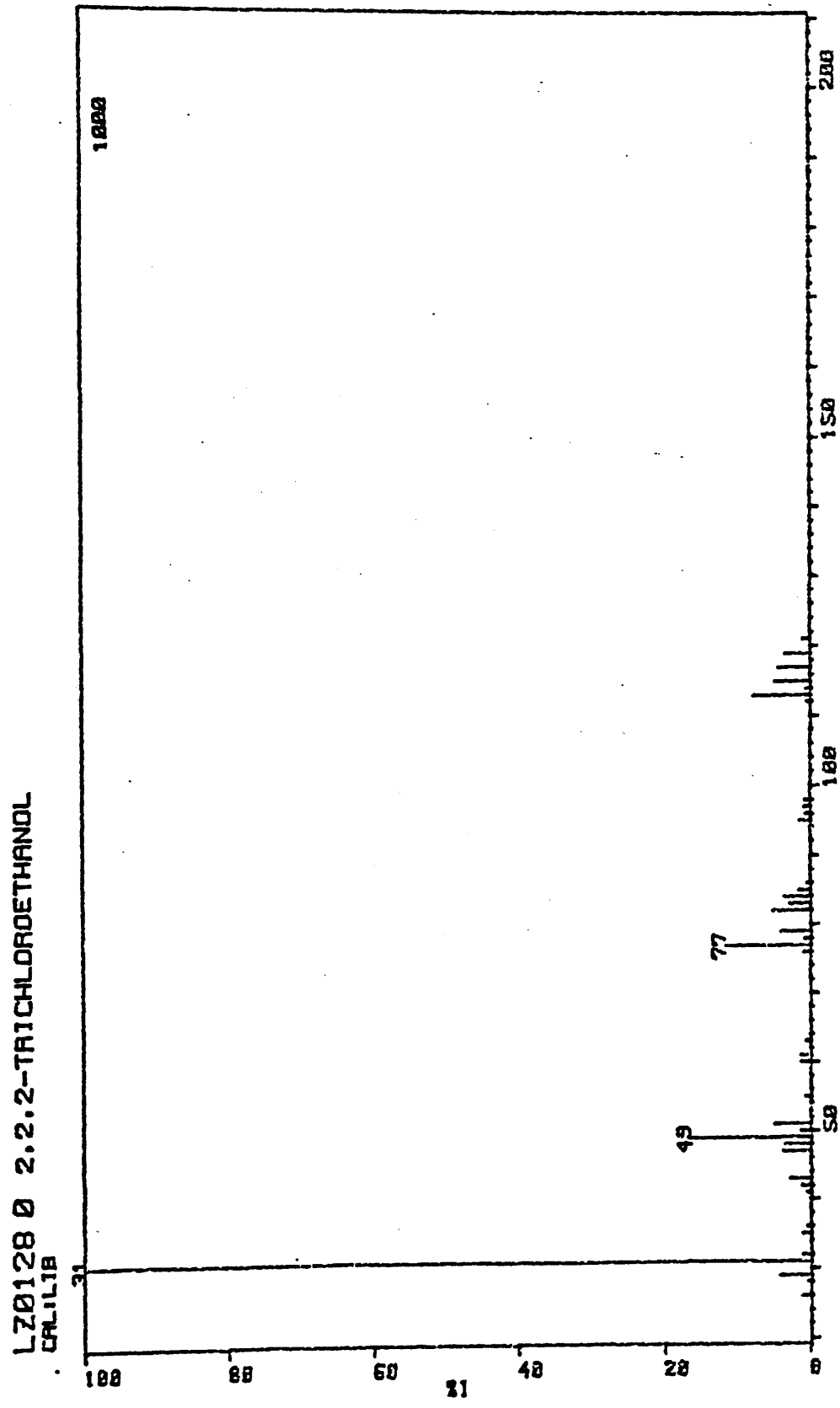


Figure 3.24.5 - Mass Spectral Scan taken during the Peak at 5.3 minutes corresponding to 2,2,2-trichloroethanol.



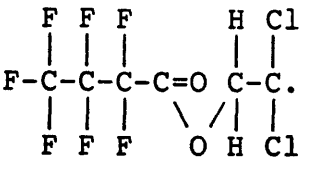
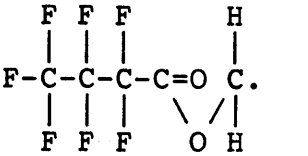
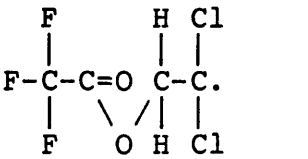
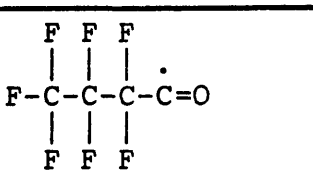
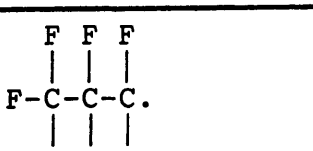
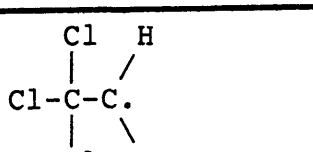
amu	Structure of ion	Formula	%I occurrence in TCETFA	%I occurrence in TCEHBFA
309		C 6 H 2 O 2 F 7 Cl 2		18.6
227		C 5 H 2 O 2 F 7		42.5
209		C 4 H 2 O 2 F 3	26.8	
197		C 4 O 1 F 7		46.2
169		C 3 F 7		89.2
131		C 2 H 2 Cl 3	21.7	59.0

Table 3.24.6a - Suggested Identities of the major ions present in the mass spectra of TCETFA and TCEHFBA.

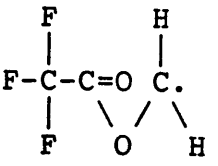
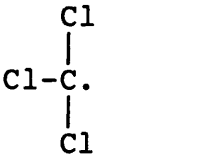
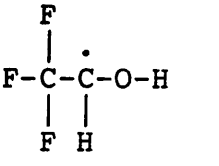
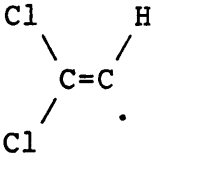
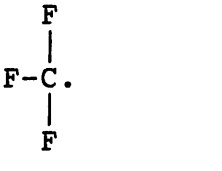
amu	Structure of ion	Formula	%I occurrence in TCETFA	%I occurrence in TCEHBFA
127		C 3 H 2 O 2 F 3	56.9	1.7
117		C 1 Cl 3	28.0	25.5
99		C 2 H 2 O 1 F 3	28.4	5.1
95		C 2 H 1 Cl 2	8.6	43.8
69		C 1 F 3	100	100

Table 3.24.6b - Suggested Identities of the major ions present in the mass spectra of TCETFA and TCEHFBA.

3.24.4 Discussion.

The total absence of molecular ion peaks in the mass spectra of compounds is unusual and it is often indicative of a very unstable molecule. In both of these molecules there is a very high molecular weight to carbon number ratio and the stability of both esters is very poor.

The base peak for both esters, m/e 69, was expected and as a

first choice this ion would prove to be most suitable for single ion monitoring. However, this ion is not specific and peculiar to these two molecules alone or indeed to the mass fragment ion (CF_3^+).

3.24.5 Conclusion.

The mass spectra of the 2,2,2-trichloroethyl esters of trifluoroacetic and heptafluoroacetic acids were obtained. Neither spectrum demonstrated a molecular ion. There were considerable similarities between the two spectra as predicted from their similar structures. Both spectra showed a base peak at m/e 69 and a psuedo-molecular ion due to the loss of chlorine, m/e 209 and m/e 309.

3.25 Improved Sensitivity and Selectivity in the Determination of Trifluoroacetate Levels using Mass Selective Detection.

3.25.1 Introduction.

In Section 3.23 a gas chromatographic method was decribed for the separation of the 2,2,2-trichloroethyl esters of trifluoroacetic and heptafluorobutyric acids. The method itself was shown to be inadequate owing to the large quantity of unreacted 2,2,2-trichlorooethanol remaining, swamping the chromatogram of the esters. In Section 3.24 the mass spectra of these esters were shown to have some similarities and in particular the presence of the mass fragment at m/e 69. Although it was noted that this ion is not specific it is sufficiently rare for the purpose of single ion monitoring and, of greatest importance is in very low abundance in the spectrum of 2,2,2-trichloroethanol.

This section will demonstrate that the use of mass selective detection provides an acceptable and available alternative to the column switching solution proposed in Section 3.23.

3.25.2 Method.

Preparation of Samples.

A series of fortified urine samples were prepared by spiking a pooled blank urine sample with known quantities of

trifluoroacetic acid. These samples were prepared to contain 0, 25, 50, 75, 100, 125, 150, 175 and 200 ug/ml. On four separate occasions these samples were used to obtain a calibration curve by duplicate analysis.

Approximately five hundred milligrammes of sample urine was accurately weighed into a vial. To each vial was then added fifty microlitres of each of heptafluorobutyric acid, a two milligramme per millilitre solution in a mixture of water and methanol (9:1), as internal standard, and 0.1M sodium hydroxide.

The samples were then frozen in liquid nitrogen and freeze dried for four hours. After slowly allowing the samples to reach ambient temperature, avoiding any condensation, a two hundred microlitres of 2,2,2-trichloroethanol was added. The vials were then sealed with a teflon lined septum and aluminium crimp seal. A one hundred microlitre aliquot of concentrated sulphuric acid was then added through the septum.

Sample Incubation.

The vials were then placed in a heating block at 50^{o}C and allowed to equilibrate for twenty minutes. After this period a one hundred microlitre aliquot of the vapour phase in the vials was removed and injected into the gas chromatograph.

Gas Chromatographic and Mass Spectrometer Conditions.

As detailed in Section 3.24.

The mass spectrometer was tuned to monitor the ion at 69 m/e.

3.25.3 Results.

The data for each of the four sets of standards was subjected to linear regression analysis and these results are presented in Table 3.25.1.

Day	slope	intercept	correlation
1	0.758	6.3	0.9614
2	0.684	9.0	0.9866
3	0.717	7.0	0.9946
4	0.620	1.0	0.9965
Mean	0.695	2.3	0.9848

Table 3.25.1 - Results of Linear Regression Analysis of Relative Peak Areas (TCETFA:TCEHFBA) vs. Concentration for each Standard Curve.

3.25.4 Discussion.

Each set of data produced a straight line calibration plot with a reasonable value for correlation, although several points had considerable error. This can in part be explained from the following points:

a) A gas tight syringe was not used for the sampling of the vials and although small fluctuations in the quantity injected would be corrected by the use of the internal standard, this may not be the case in larger volume deviations. It has also been found necessary to preheat syringes in other work to prevent condensation in the syringe and this may have also been a critical factor.

b) Peak height measurements were made rather than peak areas and this can be considered as unsatisfactory as the peak shapes are far from ideal.

c) The addition of the concentrated sulphuric acid is an important stage in the reaction. The esters produced are very volatile and so the reaction must be carried out after sealing the vial. This is difficult and the method used, dropwise addition of the acid from a large volume disposable syringe, illustrates the problem of handling small volumes of a very corrosive liquid.

3.25.5 Conclusion.

The use of mass selective detection provides a solution to the problem of detecting the 2,2,2-trichloroethyl esters of trifluoroacetic and heptafluobutyric acids.

The monitoring of ion m/e 69 from fortified urine samples resulted in calibration curves with acceptable precision. The accuracy of the method could probably be improved further by the use of a preheated gas-tight syringe for sampling, peak area measurements and more accurate addition of the concentrated sulphuric acid.

The method is capable of monitoring trifluoroacetic acid levels down to twenty-five microgrammes per millilitre of urine.

3.26 Carbon Monoxide Levels as a Measure of Exposure to Dichloromethane.

3.26.1 Introduction.

The use of chlorinated hydrocarbons is widespread in industry. With particular reference to chlorinated methane derivatives both carbon tetrachloride and dichloromethane have been used extensively. However, since the ability of carbon tetrachloride to produce liver damage is well documented, the use of this solvent has been severely restricted. It has in most instances been replaced with dichloromethane, trichloroethane or trichloroethylene. The latter two compounds have been dealt with in previous sections of this chapter.

It has been understood for many years that the toxicity of dichloromethane is substantially lower than carbon tetrachloride, although it is noted that in one report the toxicity of these two compounds was considered similar (Alford, Highman, Oettingen & Sviberly 1947).

Of particular interest to volatile substance abuse is the fact that dichloromethane is the major ingredient in most paint removers, and is also included in the formulation of many household aerosols. The first suggestion that dichloromethane is metabolised to carbon monoxide, a recognised toxic substance, was reported by Fisher and Stewart (Fisher 1972,

Stewart 1972). Their discovery and subsequent investigations showed that exposure to paint remover for a two hour period produced an elevated carboxyhaemoglobin level eight hours later. They also showed that exposure to one thousand parts per million of dichloromethane resulted in carboxyhaemoglobin levels in excess of those permitted in industry from carbon monoxide alone. Perhaps even more significant was the finding that the half-life for the elimination of carbon monoxide produced by the metabolism of dichloromethane was considerably greater than that for inhaled carbon monoxide. As this level of one thousand parts per million of dichloromethane can easily be achieved in using products containing this compound in poorly ventilated areas such as a garage or workshop, or by the misuse of these products by inhalation abuse, these findings have even greater significance.

Subsequent studies on rats (Anders & Kubric 1974) and on humans (Elkins, Ratney & Wegman 1974) confirmed the metabolism of dichloromethane to carbon monoxide. The study involving rats was performed using labelled carbon and thus proved unequivocably the metabolic conversion of dichloromethane to carbon monoxide. However, their work also demonstrated that dichloromethane is also metabolised to formaldehyde, a compound of significant toxicity as discussed in Section 3.17. Formaldehyde itself is to some extent metabolised to carbon monoxide (Neely 1964) and is a plausible metabolic pathway from dichloromethane to carbon monoxide (Heppel & Porterfield 1948).

This mechanism which converts dichloromethane and possibly other chlorinated methanes to carbon monoxide is of considerable importance as the only precedent for the production of carbon monoxide in mammalian systems is the degradation of haeme by the haeme oxygenase pathway (Marver, Schmid & Tenhunen 1969).

The work by Ratney confirmed the extension of the half-life for elimination of carbon monoxide from approximately five hours to about thirteen hours. This is very significant as it suggests that dichloromethane is stored in the body and then is slowly released and metabolised after removal from exposure, the implications of this are considerable. Exposure to a level of one thousand parts per million of dichloromethane for two hours will result in an elevated carboxyhaemoglobin level for up to twenty-one hours after exposure. An individual exposed to the recognised Threshold Limit Value of five hundred parts per million on five consecutive working days will have a "normal" carboxyhaemoglobin level for only one day out of each week.

Toxicology textbooks and many recent publications have stated that the toxic properties of carbon monoxide are the result of its combination with haemoglobin, the direct diminishing of the oxygen carrying capacity of the blood and the altered dissociation characteristics of the remaining oxyhaemoglobin. Typical signs and symptoms of carbon monoxide poisoning are given in Table 3.26.1. This demonstrated that the toxic symptoms are present at levels of ten to twenty per cent

carboxyhaemoglobin, but lower levels have been suggested (Freimuth & Gettler 1940). It has also been suggested that carboxyhaemoglobin measurement, although useful as measure of carbon monoxide exposure, is a poor estimate of the degree of toxicity (Dergal, Goldbaum & Orellano 1976). This is mainly due to the supposition that the mechanism of toxic action is by its combination with cytochrome oxidase, thus restricting cell respiration and therefore producing stress on the cardiovascular system.

Symptom	%COHb	
None	0	10
Tightness across forehead,possible slight headache, dilation of cutaneous blood vessels.	10	20
Headache; throbbing in temples	20	30
Weakness, severe headache, dizziness, dimming vision, nausea, collapse.	30	40
Collapse, increased respiration.	40	50
Coma with intermittent convulsions.	50	60
Depressed heart action, and possible death.	60	70
Weak pulse, respiratory failure and death.	70	80

Table 3.26.1 - Symptoms of Carbon Monoxide Poisoning Correlated with Percentage Blood Saturation.

The occurrence of a fatality from normal use of a paint thinner based on dichloromethane showed that an elevation of up to fifteen per cent in the carboxyhaemoglobin level could be expected after only three hours exposure in a well ventilated room. Elevation of the carboxyhaemoglobin to levels above five per cent can adversely effect subjects with angina pectoris or cardiovascular disease, their exercise tolerance is reduced and the anginal pain is of longer

duration (Aronow & Isbell 1973, Anderson 1973, Sargent, Scharf & Thames 1974). This is of even further importance where smokers are concerned as their carboxyhaemoglobin levels are usually well in excess of five per cent and often even ten per cent, therefore an increase of five to fifteen per cent becomes very significant.

The storage of dichloromethane is most likely to occur in tissue with high lipid content due to its lipid solubility. As this implies that brain tissue would also be susceptible to this concentration of the solvent it is not suprising to find reference to the possibility of brain damage from dichloromethane (Barrowcliffe & Knell 1978).

Although in the survey of volatile inhalation abuse there were no reported instances of the abuse of paint remover, its possible abuse cannot be ignored. The typical paint remover contains dichloromethane, methanol and toluene and as such may prove to be a popular source of the solvent toluene as the sales of adhesives become more restricted. Dichloromethane exposure should also be considered in any case of sudden unexplained fatalities.

Ten blood samples taken from workers exposed to dichloromethane based paint thinner were supplied for analysis in order to establish if the level of carboxyhaemoglobin in the blood gave any indication of their exposure to the solvent.

3.26.2 Method.

An Instrumentation Laboratory IL182 CO-Oximeter was used for the analysis. This instrument is a spectrometer which uses three fixed wavelength filters in the green spectrum (548, 568 and 578 nanometers) for the measurement of blood oxygen, carbon monoxide saturation and total haemoglobin concentration respectively. Determinations are made in sequence on the same sample, the digital servo readout being switched in turn to read all three parameters. Carbon monoxide saturation is measured in the range 0 to 100 per cent. The haemoglobin is compared to a fixed reference solution and the oxygen and carbon monoxide are compared to the haemoglobin.

Each of the samples analysed were in a good condition and duplicate analyses were performed on each of the ten blood samples without any pretreatment.

Each of the samples was routinely analysed for solvents and in particular dichloromethane using the method established for abused solvents.

3.26.3 Results.

No measurable level of dichloromethane was observed in any of the ten blood samples.

The carbon monoxide level for each of the samples is given in Table 3.26.2. The samples were categorised into two groups,

non-smokers and smokers owing to the normal differences that exists between the expected baseline levels of these two classes of subjects.

Statistical analysis of the results (t-test) show that there is a significant difference between the results and the normal levels for both the non-smokers and the smokers. The results show an average elevation of about five per cent in the blood carboxyhaemoglobin levels.

Sample No	Non Smokers Blood COHb (%)	Sample No	Smokers Blood COHb (%)
1	3.3	5	10.9
2	4.8	6	6.6
3	8.7	7	11.1
4	9.9	8	11.3
		9	16.2
		10	13.0
Mean	6.7	Mean	11.5
Standard Level	2.7 ($\pm$ 1.3%)	Standard Level	5.3 ($\pm$ 2.6%)

Table 3.26.2 - Carbon monoxide levels in blood of workers exposed to dichloromethane.

3.26.4 Discussion.

The results of this experiment show that the workers have been exposed to a source of carbon monoxide. In all probability this source was the direct result of exposure to dichloromethane which in turn was metabolised to the carbon monoxide. Unfortunately very little additional information was given about the samples and factors such as duration of exposure, levels of exposure and time of sampling after exposure were unobtainable. However, it must be considered

significant that all samples showed an elevated carboxyhaemoglobin level with a deviation of as much as nearly eleven per cent in one case.

To produce this increase from carbon monoxide alone the Threshold Limit Value for carbon monoxide (50ppm) would have to have been exceeded and there is no other reason to suspect that this had occurred. There is no suggestion that the Threshold Limit Value for dichloromethane had been exceeded either. The results therefore show a highly significant and possibly dangerous increase in the carboxyhaemoglobin levels probably resulting from an "acceptable" level of exposure to dichloromethane.

3.26.5 Conclusion.

Examination of blood samples from ten workers exposed to dichloromethane at levels below the Threshold Limit Value showed significant increase in their carboxyhaemoglobin levels. An average increase of about five per cent was noted and confirms other work in this area (Hake & Stewart 1976).

3.27 A Case of Suspected Status Epilepticus Due to Solvent Inhalation Abuse.

3.27.1 Introduction.

Previous sections of this chapter have discussed both fatal and non-fatal cases of solvent inhalation abuse. In non-fatal cases no indication was given of any long term effects from the solvents. In Chapter One the medical implications of long term volatile substance abuse were highlighted along with the problems of following individual abusers during the post abuse period.

The following section illustrates the case of an adolescent with severe status epilepticus which has been assigned as a direct complication of solvent inhalation abuse.

3.27.2 Case History.

The subject, a fifteen year old male subject, was presented to a local District Hospital having had a single, apparently unheralded Grand Mal seizure. He then had several more seizures which were not controlled by the usual treatment. He was referred to the Regional Neurosurgical Unit for investigation and management of a suspected focal lesion. On examination there was no evidence of infection, the concious level was slightly depressed, he was localising painful stimuli, obeying commands but not vocalising. There were no

focal neurological signs. Relevant past history included two febrile convulsions as a young child and a mild upper respiratory tract infection two weeks prior to admission.

Routine biochemical, haematological, radiological and bacteriological examinations were carried out by the hospital and were found to be normal, as was the examination of the cerebrospinal fluid. The EEG record was grossly abnormal and suggestive of a diffuse disorder. Histological examination of two frontal lobe biopsies taken five days apart showed non-specific hypoxic features.

During the first biopsy the boy had a further seizure, and at this time it was decided that he should be given muscle relaxants and be ventillated. Various combinations of phenobarbitone, phenytoin, carbamazepin and sodium valproate, chlormethiazole, diazepam and paraldehyde were used. Despite this course of treatment after eight days on the ventilator he was found to be having fits on average six times daily. These were both Grand Mal and Focal, modified by muscle relaxants. Continuous EEG monitoring, however, indicated a seizure activity of around six per hour and concurrent intracranial pressure monitoring showed that there was a marked rise in pressure associated with seizure activity. By this time he had developed focal neurological signs which varied from day to day. His general condition had deteriorated rapidly, he had become markedly catabolic requiring nasogastric, and later, intravenous feeding, and he had developed broncopneumonia.

At this stage samples from the first brain biopsy and a blood sample were submitted for solvent analysis.

3.27.3 Method.

Preparation of Samples.

The brain biopsy sample (51 milligrammes) was received in a thirty millilitre container from which it was entirely transferred to a six millilitre hypo-vial and sealed with a butyl rubber septum and aluminium crimp seal. One gramme of the blood sample, which was in a severe state of clotting, was weighed into another vial and similarly sealed.

After qualitative analysis and identification of one of two solvent peaks as toluene a standard was prepared. This standard was made in the usual manner by serial dilution to a concentration of one microgramme of toluene per millilitre. One millilitre of this standard was sealed in a vial.

Sample Incubation.

As described in Section 3.11.

Gas Chromatographic Conditions.

As described in Section 3.11.

3.27.4 Results.

The qualitative chromatograms of the samples each showed two peaks with retention times of 1.3 minutes and 5.7 minutes. These peaks corresponded to acetone and toluene respectively. The peak areas of the toluene peaks from each sample and standard are given in Table 3.27.1.

The concentrations of toluene in each sample were calculated by direct comparison with the standard and found to be 14.5 microgrammes of toluene per gramme of brain sample and 40 nanogrammes per millilitre of blood sample.

Sample	Peak Areas 1	2	3	Mean Area	Toluene ug/ml
Std. A	2218	2256	2192	2222	1.0
Brain	1677	1620	1632	1632	14.5
Blood	70	88	75	78	0.04

Table 3.27.1 - Peak Areas of Toluene Peak in Samples from Status Epilepticus Case.

3.27.5 Discussion.

The level of toluene found in the brain sample was above the levels found for acutely intoxicated abusers. This finding was despite the fact that the sample size was small and in a container of large comparitive volume. Post mortem brain levels of 22.5, 98.6 and 13.3 microgrammes per gramme have been reported in a previous section and these levels do not differ significantly from the level observed in this case. In these fatalities death was not due to toluene poisoning but by

3.28 Conclusion.

The method developed for the quantitative determination of toluene in blood has been applied both to other solvents and to other biological fluids. The results obtained from a number of fatalities and from a larger group of abusers has also been detailed.

From the results illustrated in this Chapter it appears that the problem of solvent inhalation abuse is restricted to only a few solvents. Of these there seems to be a distinct preference for toluene and subsequently toluene based products. By the use of a headspace method on a Tenax G.C. packed column, after the appropriate selection of column temperature for the solvent mixture involved, levels may be determined with sufficient accuracy.

A series of twenty-three samples from industrial workers exposed to toluene showed blood concentrations up to two hundred and fifty nanogrammes per millilitre of blood, and with two cases where exposure was known to have exceeded the Threshold Limit Value the blood concentrations were under four hundred nanogrammes per millilitre of blood. In all of these cases sampling occurred within one hour of removal from the contaminated area. The cut off level for the diagnosis of toluene inhalation abuse may therefore be presumed as four hundred nanogrammes per millilitre of blood. This assumption does not preclude that levels below this are not as a result

some other means whereby toluene was an important precipitative factor. Normally in cases of toluene inhalation abuse levels are not detected after a period of forty eight hours due to the rapid elimination of toluene from the body. It is therefore even more significant that a high level was found in the sample taken over forty eight hours after the last possible time of abuse.

Four days after diagnosis of toluene poisoning the subject began to make what appeared to be a spontaneous recovery. The seizure frequency decreased considerably without any major change in therapy, and over the following week it became possible for him to be removed from the ventilator. He was discharged from hospital four months after the onset of the attacks. One month after discharge he had one more fit. Although there was only mild intellectual impairment, the major conclusion was that of marked behavioural difficulties and problems with social adjustment.

3.27.6 Conclusion.

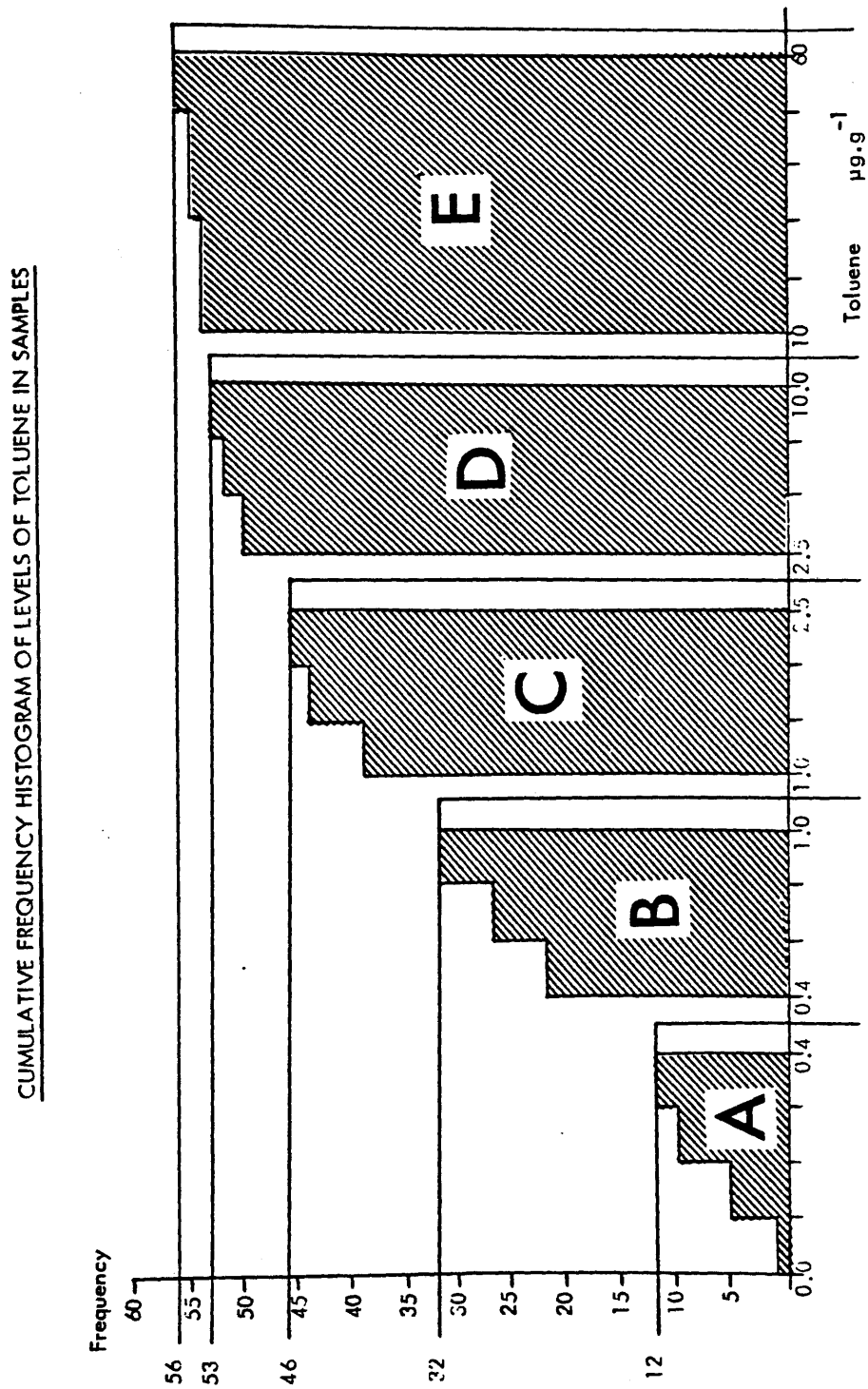
The level of 14.5 microgrammes of toluene per gramme of the brain sample probably does not reflect the true level at the time of the biopsy due to sample handling and small size. Neither does this level represent the level at the time of abuse. However, considering these points and the medical notes on the case it can be proposed with reasonable confidence that this abuser was suffering from complications probably resulting from acute toluene poisoning.

of toluene abuse, but that they might be obtained from normal exposure to toluene at levels that are considered safe.

It was found that abusers with blood toluene levels in the range four hundred to one thousand nanogrammes per millilitre all exhibited signs of solvent abuse and in particular the smell of solvent on their breath. Those abusers with levels of between one to two and one half microgrammes of toluene per millilitre of blood indicated symptoms of increasing intoxication. Fifty per cent of the abusers with blood concentrations in the range two and one half to ten microgrammes per millilitre were hospitalised and showed signs of marked intoxication with some reporting hallucinations. The abusers with levels above ten microgrammes of toluene per millilitre of blood were either unconcious or deceased at the time of sampling. The one post-mortem sample with a level of less than ten microgrammes per millilitre also had a relatively high blood level of ethanol. These statements are illustrated graphically by the cumulative frequency histogram given in Figure 3.28.1.

The above levels were present at the time of sampling and as often a period of up to eight hours elapses between the time of abuse and the time of sampling many of these levels would have been higher at the time of abuse. The original dosage is always unknown due to the inconsistant method of inhalation of the solvent fumes and the intoxicated state of the abuser.

Figure 3.28.1 - Cummulative Frequency Histogram of Levels of Toluene Found in Abuser's Samples.



The problem of monitoring the period between exposure and sampling is a difficult one to tackle. Solvents are usually eliminated from the body very rapidly particulary in the breath and consequently any drug kinetics are difficult to evaluate. It is almost certain however that these solvents cause some physiological changes to the system and therefore may present themselves in the form of metabolites or metabolic abnormalities.

The use of dichloromethane in many paint removers and aerosols presents the opportunity for abuse of this solvent. The metabolism of this compound to carbon monoxide is well established. It is therefore with some concern that significantly elevated carboxyhaemoglobin levels have been observed in workers exposed to levels of dichloromethane below the recommended Threshold Limit Value. This highlight of a potential problem of occupational exposure really warrents further investigation, however the complexity of the issue is beyond the scope of this thesis.

Measurement of Halothane metabolites may provide further insight into the problem of halothane hepatitis and the provision of methods for their measurement was an important step in this direction. Despite interest made by several people in this area, the supply of clinical samples was not forthcoming and therefore no practical use was made of these developed methods.

The measurement of the trichloroethylene metabolites should

have been more practical, however despite the common occurence of this volatile, its frequency of abuse was observed to be very low. In fact no incidence of its abuse occurred after the investigation into its metabolites. Once again the provision of methods for what at first appeared to be a useful purpose failed in the supply of clinical material, although in this case it may be perceived that future trends of volatile substance abuse may favour trichloroethylene.

Hippuric acid is a well known metabolite of toluene and its presence in the urine of abusers has been demonstrated. The method developed was also found to be applicable to the metabolites of xylene and styrene. It was used to establish baseline levels and to investigate the levels from some toluene related deaths.

Owing to the fact of the high volatility of the substances abused, it is common to find that they are not metabolised to any great extent and in fact the greater proportion of the dose is exhaled in the breath. The option to study breath levels was made available to several of the groups involved in the study of volatile substance abuse but the idea was received with a general feeling of apathy. Indeed the number of samples submitted for analysis was insignificant. However, the use of breath to monitor the problem must not be so lightly ignored. It has questionable value in the quantitative monitoring of the severity of abuse and it may be difficult to produce any correlation between toxic symptoms and breath levels, but its use as a qualtitative tool could be

of prime importance. The sampling of breath is totally non-invasive and requires the minimum of skill. Samples could be taken by teacher, police, social helpers and even parents with simple instruction and the results available on substance abused within a short space of time. The collection method described in this chapter is applicable to a wide range of volatiles and a major increase in the qualitative power of the method could probably be achieved by the desorption of these tubes into a mass spectrometer equipped with an extensive library facility.

CONCLUSION

The work detailed in the previous Chapters of this thesis describes the evaluation and development of several analytical techniques for the measurement of volatiles, and in particular the solvents which commonly feature in the problem of solvent abuse, in biological samples. These methods have been used practically to evaluate the extent of volatile substance abuse in the Glasgow area.

A careful and in-depth survey of the literature on the phenomenon and a review of the medico-sociological aspects have also been presented to attempt to clarify the causes, characteristics and effects of the problem.

Having established the requirement to monitor volatile substance abuse a new method was presented for the analysis of of the most commonly abused solvent, toluene, in plasma. This method was validated and subsequently extended for the analysis of other volatiles. This method was applied to plasma samples obtained as part of a survey of the solvent abuse problem in the Glasgow region and was subsequently extended for use on post-mortem tissue samples.

A library of data has been produced on potential volatiles of abuse in the form of retention index parameters and has been demonstrated to be a valuable instrument in the qualitative

analysis and identification of solvents. In addition mass spectroscopy has been illustrated in the provision of further information on the identification of components in complex solvent mixtures.

Blood level results have been obtained from a number of fatalities and from a larger group of known or suspected abusers. It has become apparent from these results that the problem of solvent inhalation abuse is currentlty restricted to only a few solvents and evidently the most popular has been identified as toluene.

Based on levels observed in samples from workers exposed to toluene the baseline level for the diagnosis of toluene inhalation abuse was establised as four hundred nanogrammes per millilitre of blood. Though levels below this value may be found resulting from toluene abuse they might equally be obtained from normal exposure to toluene at levels that are considered safe. The signs of solvent abuse and in particular the smell of solvent on the breath were shown to correlate well with blood toluene levels in the range four hundred to one thousand nanogrammes per millilitre. Levels in excess of one microgrammes of toluene per millilitre of blood indicated a state of intoxication, with hospitalisation being required when levels were in excess of five microgrammes per millilitre. Blood levels above ten microgrammes of toluene per millilitre occurred only when the abuser was either unconcious or dead. The interpretation of post-mortem samples can be made more complex with the presence of other solvents

or drugs.

The problem of solvent abuse does not show any signs of abating and the attention which it has drawn in the media over other significant problems such as alcoholism or drug abuse has been out of all proportion to its potential harm to the abuser. The point of most concern is that this form of abuse may lead to more harmful substance abuse although the evidence so far for this is inconclusive. We now have the tools available for the scientific monitoring of the extent of volatile substance abuse though there are many ethical problems in implementing any form of controls or discipline on the abuser population.

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APPENDIX I

CAPILLARY GAS CHROMATOGRAPHY DEVELOPMENT IN EXAMINATION OF SUITABLE COLUMNS FOR RESOLVING OF MULTI-COMPONENT MIXTURES.

I.0 Introduction.

As has been illustrated in this thesis the abuse of solvents presents many potential challenges to the chromatographer in that the identities of the solvents which have been abused are not always known. Ideally it would be preferable to be able to separate all of the possibly abused solvents from each other and give some degree of qualitative and quantitative assessment in one analysis. Although some chromatographic columns do approximate to this ideal as demonstrated in Chapter Two, total separation of all mixtures is not possible on packed columns.

Over past years the use of open tubular gas chromatography has gained increasing popularity mainly due to the larger number of possible theoretical plates and therefore considerably increased separation power that may be obtained from these columns.

This Appendix describes investigations into the manufacture, quality and selection of open tubular columns for solvent separations.

I.1 Preparation of Glass Capillary Blanks.

I.1.1 Introduction.

Open tubular columns also known as high efficiency columns, or capillary columns have a separating power well in excess of that required for the usual chromatographic work of separating a mixture where the number of components is small. In such cases, unlike in packed column gas chromatography, it is often not necessary to select a particular stationary phase to suit the particular separation problem.

There exists four main types of capillary column as described below. Each in turn offering advantages in efficiency but each with progressively more complications with regard to their use and preparation.

a) Support coated open tubular columns, the nearest relatives to packed columns, consist of an open tube of glass, stainless steel, or in a few cases plastic, where the internal wall has been coated with a support material of the same type as used in packed columns. The support has then been coated with a stationary phase as with packed columns.

b) Porous layer open tubular columns are support coated open tubular columns in which the support has been selected because of its greater porosity and they are mainly used for gas solid chromatography.

c) Wall coated open tubular columns consist of an empty tube where the internal wall has been directly coated with the

stationary phase.

d) Whisker walled or surface modified open tubular columns are a deviant of the wall coated open tubular columns but with an average eight fold increase in efficiency they require special mention. They are prepared by "growing" silica whiskers on the inner wall of the capillary tube and thus greatly increasing the surface area in contact with the carrier gas without increasing the column diameter or significantly decreasing the column permeability. These are the most difficult of all the open tubular columns to manufacture and indeed the variability between columns really precludes them from being supplied commercially.

The open tubular column is dependant primarilly on its emptyness or permeablitiy for its high efficiency. The permeability is expressed by the specific permeability coefficient which is a direct measure of the openness of the cross section. Typical values for specific permeability coefficients are given in Table I.1.1.

Column Type	Typical Value
Micropacked	100000000
Packed	10000000
Support Coated Open Tubular	10000
Porous Layer Open Tubular	10000
Wall Coated Open Tubular	0.001

Table I.1.1 - Typical values of specific permeability coefficient for different column types.

It is this emptiness which permits the use of low mobile phase flow rates, very long columns and gives correspondingly high efficiencies. The choice of stationary phase is then no

longer as much of a problem as with packed columns but the efficiency is now very much dependant on the column construction and preparation. The ideal column is a straight capillary with a smooth bore and therefore the stationary phase will give best results if coated evenly and uniformly over the entire inner surface of the tube. This has not yet been achieved or is likely to with the restrictions imposed by column material and the necessary coiling of these very long columns.

Open tubular columns are in theory much less dependant on flow rates and temperature than packed columns but in practice it is found that they are even more dependant owing to the high sensitivity of the detection system with the low levels of solutes allowable. This dependance is only an effect of the detector and the efficiency of open tubular columns remains effectively unchanged by relatively large changes in flow rate or temperature.

At the time of the work of this thesis there were few open tubular columns available commercially, indeed the manufacture of good columns existed as much of an art form.

As described above one of the most important parameters in obtaining open tubular columns of high efficiency is the preparation of tubing of uniform and smooth internal bore. Typical dimensions of the open tube are of the order of half a millimeter internal dismeter and of some twenty-five to one hundred metres in length. This length requires that the open

tube is formed into a helical coil, the least disturbing arrangement to the ideal of lamina flow of mobile phase through the column,and of a convenient diameter to enable it to be located in the oven of the gas chromatograph.

For the purpose of manufacturing open tubular columns a considerable portion of the grant had been used to purchase a glass drawing machine, Shimadzu GDM1, so that glass capillary blanks could be prepared. The choice of glass tubing was limited to soda glass by the requirement to produce capillary of internal diameters suitable for open tubular columns and still retain a sufficiently high ratio of outer to inner diameters to provide the column with physical strength. This feature becomes even more critical when further treatment of the column blanks is required. The specifications of the glass which was used are given in Table I.1.2.

Requirement	Specification
Material	Glass with a maximum softening tempreature of 820°C
Cross section	Circular
Outer diameter D	6 mm < D < 8mm
Inner diameter d	1 mm < d < 5mm
Relatio D to d	Approximately D = 2 * d
Length L	250 cm < L < 1500 cm

Table I.1.2 - Material Specifications for Shimadzu GDM1 glass drawing machine.

I.1.2 Operation of the GDM1.

A schematic of the Shimadzu GDM1 is given in Figure I.1.1. A glass tube is softened in the furnace and is drawn mechanically by differentiating the feeding rate and the

drawing rate controlled by two motorised rollers. The drawing ratios (16, 20, 25, 32, 40, 50, 64, 80, 100) are obtained by the selection of gear wheel combinations. The drawing ratio governs the diameters of the finished tube in relation to the initial diameters by the following expression.

Final Diameter = SQR (1 / Draw Ratio) * Initial Diameter

From information given in Tables I.1.3 and I.1.4 the temperature of the furnace and coiling tubes were selected. Adjustments were made during a run as required.

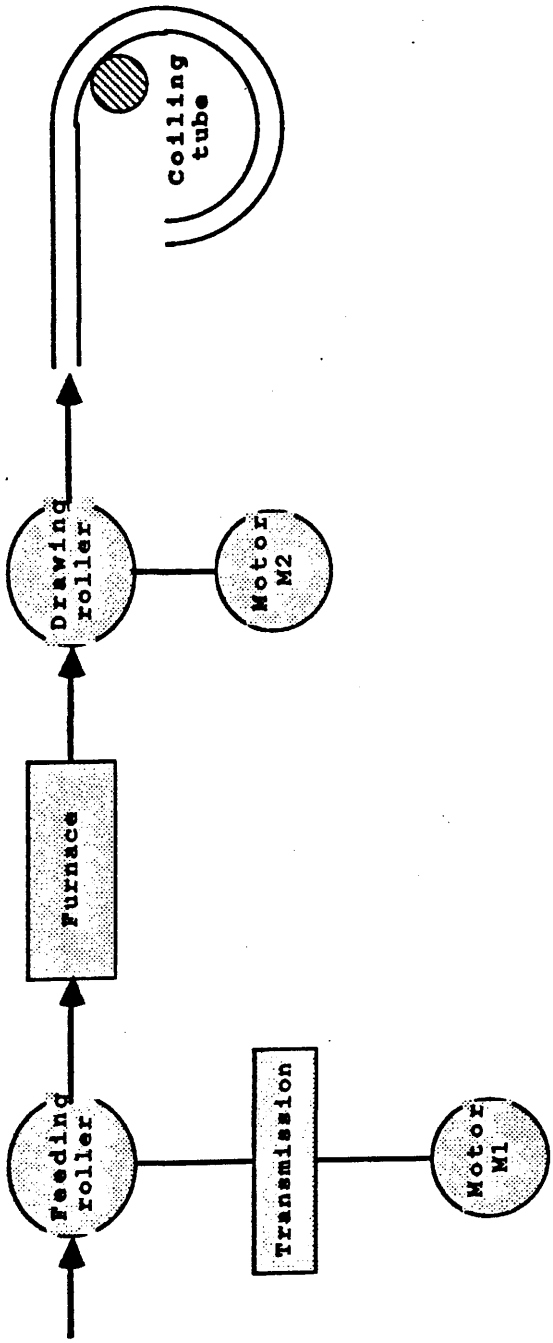
Material of Glass Tube	Furnace Temperature ^{o}C
Soda Glass	650 - 750
Boro silicate Glass	700 - 800
Pyrex Glass	750 - 850

Table I.1.3 - Appoximate Furnace Temperatures Required To Aneal Different Types of Glass.

Outer Diameter of Drawn Tube	Voltage
Below 0.8 mm	33 - 50
0.8 - 1.2 mm	35 - 55
1.2 - 1.4 mm	40 - 60
1.4 - 1.6 mm	50 - 70

Table I.1.4 - Approximate Coiling Tube Voltage Settings Required for Different Final Outer Tube Diameters.

Figure I.1.1 - Schematic of the Shimadzu GDM1.



I.1.3 Method.

Preparation of Glass Tubing.

The glass was washed in concentrated hydrochloric acid, water, methanol, and hexane before use.

GDM Operating Conditions.

The capillaries were drawn from tubing having an internal diameter of 3.5 millimetres using a drawing ratio of 32 (gears 2,3,4,4) to give an estimated internal diameter of 0.5 millimetres. The furnace temperature required for this glass was found to be equivalent to a setting of 74 volts and the coiling tube temperature equivalent to a setting of 45 volts. All columns were coiled using the 110 millimetre diameter coiling tube.

Measurement of Internal Diameter and Volume.

Ten coils were removed from the end of the column and were weighed accurately (w). These coils were then filled with deionised water and accurately reweighed (W). The number of coils remaining were counted (N). The column length, volume and internal diameter were then calculated by substituting the values in the following equations:

Volume of one turn (ml) = v = (W - w) / 9.9707

Total volume (ml) = V = v * N

Column Length (m) = L = N * 0.11 * PI

Internal Diameter (mm) = SQR ((400 * v) / (PI * PI * 11))

I.1.4 Results.

A total of seventeen column blanks were succesfully drawn. The dimensions of these capillaries were measured and are given in Table I.1.5.

Column No.	Total Coils	Length (m)	Total Column Volume (ml)	Internal Diameter (mm)
1	83	28.68	4.399	0.442
2	75	25.92	3.639	0.423
3	100	34.56	7.207	0.515
4	165	51.02	10.538	0.485
5	100	34.56	8.918	0.573
6	175	60.48	11.381	0.490
7	100	34.56	12.593	0.681
8	135	46.65	11.305	0.555
9	135	46.65	10.564	0.537
10	143	49.42	12.797	0.574
11	140	48.38	12.525	0.574
12	130	44.92	12.986	0.607
13	134	46.31	9.942	0.523
14	133	45.96	11.051	0.553
15	127	43.89	13.314	0.621
16	139	48.03	10.260	0.521
17	73	25.23	6.655	0.580

Table I.1.5 - Physical Dimensions of the Prepared Capillary Blanks.

I.1.5 Discussion.

The drawing of capillary blanks on the Shimadzu GDM1 was found to be an unexpectedly difficult task. The machine was found to be very suceptible to environmental conditions and often tediously unstable. In particular the coiling tube was found to have different temperature settings on each attempt to draw glass from the same material stock. It was also found that the combination of friction, poor quality glass, and poorly controlled coiling tube temperature caused numerous restarts

from breaking drawn tube.

However a total of seventeen columns were successfully drawn and their physical dimensions determined. The wide variation of the internal diameters and total volumes of these columns, despite the fact that they all originated from the same stock material and were manufactured under the same instrumental conditions, demonstrates an important problem in the manufacture of open tubular columns.

I.1.6 Conclusion.

Seventeen capillary column blanks were prepared from a single stock batch of soda glass tubing using the Shimadzu Glass Drawing Machine GDM1. These columns were found to have a wide variation of physical dimensions.

The machine was found to be both difficult and inconsistant in use. The greatest problem being the coiling tube.

I.2 Surface Treatment of Capillary Blanks to Improve Coating Efficiency.

I.2.1 Introduction.

Stationary phases exhibit poor wettability on a glass surface and demonstrate a measurable contact angle. The process of anealing and drawing the glass also has the effect of fire-polishing the inner surface resulting in a comparitively smooth surface (Alexander & Rutten 1974). To promote spreading of the stationary phase chemical etching of the inner wall of the capillary is performed despite the fact that this action may result in increased activity of the column. Even if only a small proportion of the total surface area is occupied by active sites a considerable peak broadening and loss of efficiency could be expected. Deactivation of these sites must therefore be carried out before the column can be ready for coating.

There are several chemical treatments which have been used in conditioning columns. Of the more successful to have been demonstrated are: silylation with polar silyl reagents (Bartle 1973); chlorination followed by reaction with an alcohol (Grob 1968); reaction with a organo-lithium compound (Beisel, Schier & Unger 1973); in-situ formation of a polymeric layer (Grob 1968); carbonisation of the wall (Grob 1968, Liberti 1966); and the application of surface active agents (Martin & Metcalfe 1967). However the major problem with the treatments

which depend on the production of intermediate layers is their low thermal stability. At elevated temperatures the organic interlayers can decompose, surface active agents desorb and swelling may develop with the polymer films. The stationary phase inside the capillary column has a concave cylindrical surface and is under contractional forces due to surface tension resulting in the tendancy to accumulate into droplets.

Column conditioning by the use of gaseous hydrogen fluoride or hydrogen chloride has been shown to produce very regular etching patterns (Alexander & Rutten 1974). The composition of the glass is an important factor influencing the results that can be achieved from this method of column conditioning and best results are obtained from the use of soda glass.

Surface deactivation by the producion of a mono-molecular layer of the polymer Carbowax 20M is the method which has been shown to be the most efficient in masking the adsorbtion sites produced by the surface conditioning (Blomberg 1975).

The capillary blanks were therefore conditioned by etching with gaseous hydrogen chloride, the use of hydrogen fluoride was excluded owing to the increased safety risks involved in using this extremely corrosive gas, and then subsequently deactivated with heat-treated Carbowax 20M.

I.2.2 Method.

Preparation of Reagent Gas.

Dry hydrogen chloride was prepared by the reaction of sodium chloride with concentrated sulphuric acid. The gas was then dried by passage through anhydrous calcium chloride.

Column Filling.

It was observed that by timing the period taken for the hydrogen chloride gas to pass through the capillary blank, measured at the exit end of the column by moistened blue litmus paper, and continuing filling for five times this period resulted in the column being filled in excess of ninety percent by volume.

Reaction Conditions.

The capillary blank was sealed at both ends in a microburner flame and placed in an oven. The temperature of the oven was programmed up to 380^{o}C at a rate of one degree per minute and held at that temperature for a period of two hours before being allowed to cool slowly to room temperature. The column was then opened and flushed of unreacted hydrogen chloride with dry nitrogen. The reactions which take place in the column result in the formation of chloride salts.

Deactivation.

Deactivation was carried out immediately by dynamic coating with a 2.5 percent by weight solution of Carbowax 20M in freshly distilled dry dichloromethane by drawing twenty-five

millilitres of this solution through the column under vacuum. All moisture contact with the inner surface of the column was avoided at each stage to reduce possible damage to the chloride salt layer. The column was then dried with a flow of dry nitrogen and the ends resealed in a microburner flame. The column was then placed in an oven and was programmed up to 280^{o}C at a rate of two degrees per minute and held at that temperature for a period of twenty-four hours before being allowed to cool slowly to room temperature. The extractable part of the polymer film was then removed by washing out the column with seventy five millilitres of dichloromethane under vacuum. The column was then dried by flushing with dry nitrogen.

I.2.3 Results and Discussion.

All fifteen capillary blanks were successfully conditioned and deactivated by the above method. The columns showed an opaque appearance after conditioning due to the formation of crystaline salt deposits. This opaqueness was reduced considerably after deactivation and was probably a direct result of traces of moisture in the solvents dissolving the salts. To what extent this dissolution of the chloride salt layer occurs was unknown but as some opaqueness remained after deactivation it may be assumed that this dissolution was not complete.

I.2.4 Conclusion.

Fifteen conditioned and deactivated columns were prepared from the capillary blanks by hydrogen chloride gas etching of the glass surface and by producing a non-extractable polymer layer of heat-treated Carbowax 20M.

I.3 Examination of The Methods of Wall Coating Open Tubular Columns.

I.3.1 Introduction.

There are three main coating techniques available and an attempt was made to prepare a series of columns with different stationary phases in order that a comparison of of each technique could be made.

1) Dynamic coating was first described by Dijkstra and De Goey and used in the early work of Golay (De Goey & Dijkstra 1958). A solution of the stationary phase in a suitable solvent is forced through the capillary with the aid of an inert gas. In the process the wall is wetted by the solution and by the stationary phase. One of the variables in this method is the volume of stationary phase solution used and that the linear velocity of the solvent plug remains constant throughout the coating process. Linear velocities of ten centimetres per second were suggested, requiring very low flow rates of gas, typically less than one millilitre per minute for columns of half a millimetre internal diameter. Another variable is the stationary phase concentration which is a major problem with this methods as at flow rates above five millimetres per second the thickness of the coated film increases with linear velocity, the concentration of stationary phase and the solvent viscosity (Kaiser 1963). The polarity of the solvent also affects coating, the more polar the solvent the thicker the coating. The film thickness is

also inversely proportional to the column diameter. Additional to these variables the polarity of the stationary phase has itself has a direct effct on the wetting ability of its solutions. There is therefore a comprehensive list of problems wich may be encountered when using dynamic methods of coating.

2) Dynamic plug coating is a variation of the dynamic coating method, has similar disadvantages and tends to produce a more uneven film thickness owing to the difficulty of maintaining a constant linear velocity. The method involves pushing the solution of the stationary phase through the column in front of a plug of mercury. Columns can be produced with better film thickness and also produces better results with the more polar stationary phases.

3) Static coating involves filling the column with the coating solution, sealing one end of the tubing, and slowly evaporating the solvent from the other end. The method was first described by Golay, who evaporated the solvent by passing the column slowly into an oven (Golay, 1958). The disadvantages to this method of evaporation include: the requirement for a special oven, the use of short columns, and the stationary phase stability at the solvent boiling point. Considerable improvement was made following the work carried out by Bouche where the solvent was evaporated under vacuum at ambient temperature (Bouche 1968). This procedure provided a solution to these disadvantages but created a new one, in that extreme care was required when filling and sealing the column in order that no air was permitted to enter. Exhaustively degassed solutions were used and sealing of the column end was

carried out with sodium silicate. Evaporation of the solvent using this method typically takes days or even weeks depending on the length of the column, solvent used, temperature, and a good reliable vacuum system.

Fifteen of the columns prepared, deactivated and conditioned as described in Sections I.1 and I.2 were divided into three groups of five and each column was coated by one of the above methods.

I.3.2 Method.

In coating Columns II - X a buffer volume, in the form of a ten metre length of PTFE tubing, was added to one end. Addition of this buffer volume was an attempt to maintain some control over the linear velocity of the coating solution as it neared the end of the column.

Group 1.

Column I was filled with a degassed solution of 219.9 milligrammes of Carbowax 20M in dichloromethane using a vacuum. One end of the column was sealed by drawing a plug of sodium silicate into the column. The column was then allowed to stand overnight to allow the sodium silicate to set. The open end of the column was attached to a vacuum and the solvent evaporated. After approximately three hours evaporation of the solvent was observed to have stopped and that a plug of stationary phase had formed preventing further evaporation. This plugging resulted from an air bubble

forming further along the column. This column was then shortened by breaking at the plug, the remaining solution forced out under pressure and dried in a flow of nitrogen. The column was then assumed to have been dynamically coated by the air plug method.

Columns II, III, IV and IX were coated by the air plug dynamic method using a five per cent solution of the selected stationary phase, Table I.3.1, prepared in its recommended solvent and filtered through a two micron filter before use. Ten millilitres of the solution were forced through the column at a flow rate of one hundred microlitres per minute using a syringe pump. After the solution had passed through the column and into the buffer volume the remaining solution was forced out and the column dried with a flow of nitrogen.

Group 2.

Columns V, VI, VII, VIII and X were coated by the mercury plug dynamic method by forcing through the column ten millilitres of a ten per cent solution of the selected stationary phase, Table I.3.1, followed immediately by a five centimetre plug of mercury at a flow rate of one hundred microlitres per minute. After the solution had passed into the buffer volume the remaining solution and mercury plug were expelled and the column dried with nitrogen.

Group 3.

Columns XI, XII, XIII, XIV and XV were coated using the static coating method by filling the column with the selected

staionary phase solution, Table I.3.1. One end of the column was then sealed by drawing a ten centimetre plug of sodium silicate into the column by the application of a low vacuum to the other end. Twenty-four hours was allowed for the sodium silicate to harden before the application of a high vacuum to the open end of the column. This vacuum was maintained until all of the solvent had been evaporated. This evaporation step was complete for columns XI and XIII within two days, column XII took over a week and both columns XIV and XV required gentle heating in a water bath at 35^{o}C to assist evaporation of the solvent, taking over two weeks for to complete. The appearance of unevenly coated coils evident in both columns XIV and XV probably resulted from the difficulty in sustaining a constant temperature during the evaporation period.

I.3.3 Results.

The columns were coated according to the scheme given in Table I.3.1.

I.3.4 Discussion.

Dynamic coating was observed to be a much faster method of column stationary phase coating than the static method, an important factor when selecting the method of choice. Static coating required considerable care and a level of skill particulary in the elimination of air from both the solvent and the column. The presence of even a minute amount of air

causes the loss of all previous work expended on the preparation of that column by uneven evaporation of the solvent and plugging of the column. The dynamic coating method was found to be very straightforward and although care was required in obtaining a good column, it was possible to use this method quickly to produce columns for chromatographic use.

No.	Phase	Solvent	Method
I	Carbowax 20M	Dichloromethane	Air Plug
II	Hallcomid M18	Acetone	Air Plug
III	Emulphur ON870	Dichloromethane	Air Plug
IV	Apiezon L	Benzene	Air Plug
V	Carbowax 20M	Dichloromethane	Mercury Plug
VI	Hallcomid M18	Acetone	Mercury Plug
VII	Emulphur ON870	Dichloromethane	Mercury Plug
VIII	Apiezon L	Benzene	Mercury Plug
IX	SF 96	Chloroform	Air Plug
X	SF 96	Chloroform	Mercury Plug
XI	Carbowax 20M	Dichloromethane	Static
XII	Hallcomid M18	Acetone	Static
XIII	Emulphur ON870	Dichloromethane	Static
XIV	Apiezon L	Benzene	Static
XV	SF 96	Chloroform	Static

Table I.3.1 - Scheme for Wall Coating Fifteen of the Open Tubular Columns.

I.3.5 Conclusion.

Fifteen columns were coated with one of five different stationary phases by one of the three different coating procedures.

I.4 Determination of Optimum Nitrogen Carrier Gas Velocity for a Wall Coated Open Tubular Column.

I.4.1 Introduction.

The optimum gas velocity can be determined by plotting a H.E.T.P. curve given by the following equation:

$$h = \frac{2*Dg}{u_m} + \frac{(1+6k+11k^2)*r^2*u_m}{24*(1+k)^2*Dg} + \frac{2*k*d_f^2*u_m}{3*(1+k)^2 Dl}$$

where d_f - film thickness,
Dg - diffusion coefficient of the solute in the gas phase,
Dl - diffusion coefficient of the solute in the liquid phase,
k - partition ratio
u_m - mean gas velocity.

In this equation the flow through the column is assumed to be lamina. The flow pattern is considered to be independant of gas velocity and therefore the eddy diffusion in an open tubular column is zero. Band broadening occurs due to longitudinal diffusion which is greater in open tubular columns than in packed columns but is only significant at very low gas velocities, and is also dependant on the diameter of the column; the smaller the diameter the lower the gas velocity will have to be before longitudinal diffusion has any contribution. The major contribution is therefore from the rates of mass transfer in the mobile and stationary phases.

I.4.2 Method.

A solvent mixture of acetone, toluene and xylene was prepared to represent the mixtures common in abused solvent analysis. The headspace sample above this mixture was injected on to the column at its operating temperature of 50°C and the injections were repeated at various carrier gas flow rates.

I.4.3 Results.

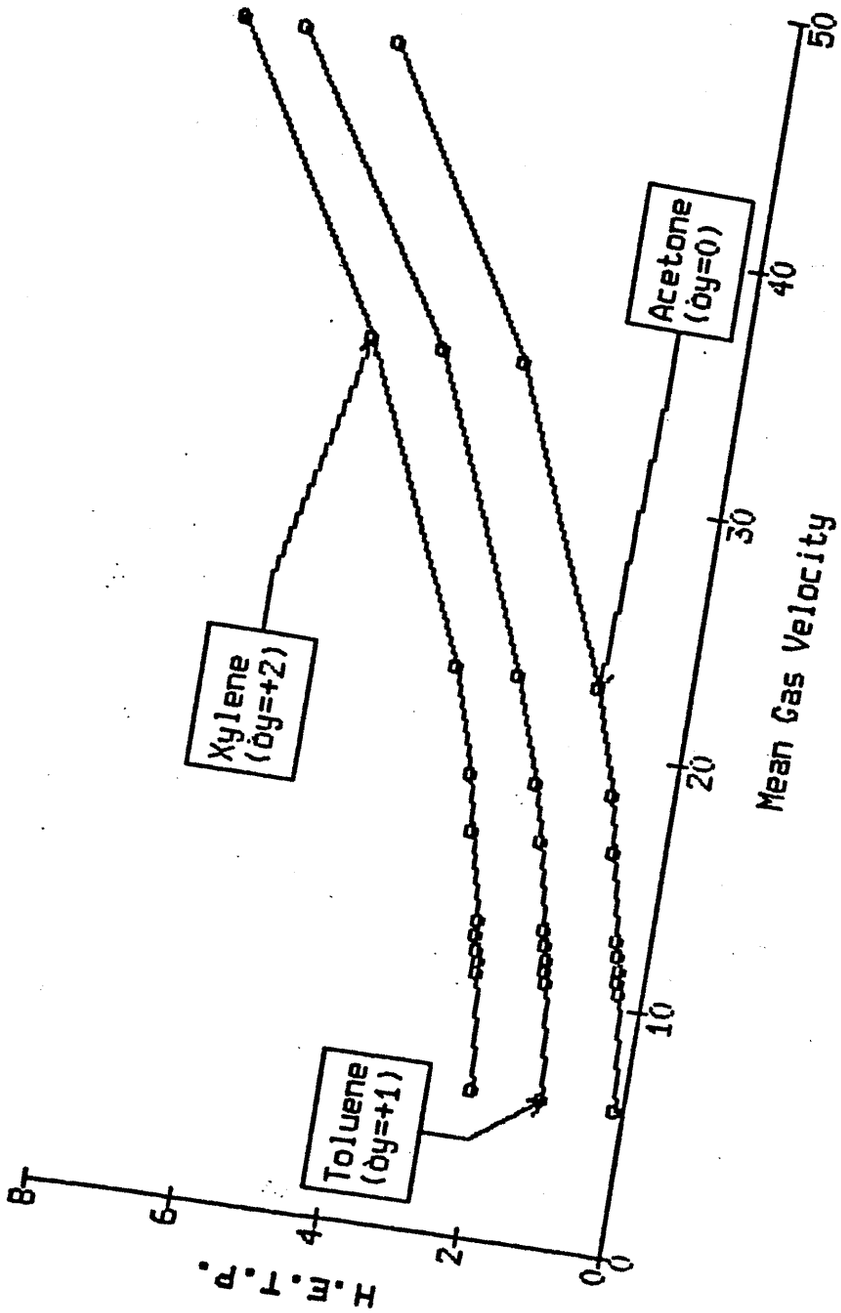
The flow rates were calculated from the retention time of the air peak using the following equation:

$$u_g = a * u_m / q = L / t_A$$

where (a) and (q) are both approximated to unity for open tubular columns and u_g represents the carrier gas velocity.

The values calculated for (u_m) and (h) are presented in Table I.4.1. These values were plotted and the resulting curve is illustrated in Figure I.4.1.

Figure I.4.1 - HETP Curves for Acetone, Toluene and Xylene on an Emulphur ON870 Mercury Plug Dynamically Wall Coated Open Tubular Column.



Stationary Phase Emulphur ON870 (Hg plug)
Oven Temperature 50°C
Column Length 49.4 m.
Column i.d. 0.574 mm.
Column Volume 12.797 ml.
Detector Tempreature 200°C
Injector Temperature 150°C

Mean Gas Velocity	Acetone	Toluene	Xylene
5.94 cm.sec.$^{-1}$	0.09	0.10	0.09
10.6	0.30	0.32	0.30
11.1	0.32	0.35	0.32
11.5	0.34	0.37	0.35
12.1	0.38	0.41	0.39
12.7	0.42	0.46	0.43
16.2	0.67	0.73	0.69
18.4	0.85	0.94	0.85
22.6	1.27	1.43	1.27
34.9	3.06	3.15	3.16
46.5	5.44	5.74	5.57

Table I.4.1 - Heights Equivalent to Theoretical Plate for Acetone, Toluene and Xylene at Different Mean Carrier Gas Velocities on an Emulphur ON870 Mercury Plug Dynamically Wall Coated Open Tubular Column.

I.4.4 Discussion.

The determination of optimum linear gas velocity by this method is more difficult than with packed columns. The gas flow rates used are very small, in the region of one millilitre per minute, which is difficult to maintain constant and to reproduce between columns. The gas velocity values are, however, similar to those obtained with packed columns and this is brought about by the great increase in column length.

The graph in Figure I.4.1 illustrates that the optimum gas velocity for this column is in the region of five centimetres

per second. A gas velocity of up to double would be acceptable and would result in a reduction of the analysis time by almost half, eg. from thirty five minutes to twenty minutes for xylene. The graph does not show the curve expected and this is in part due to the difficulty of measuring peak widths accurately. The peaks are very sharp in comparison to peaks obtained in packed column gas chromatography and typical values of peak width are of the order of thirty seconds. The variations in band width are only a fraction of this value. Therefore it is generally accepted that owing to the large number of theoretical plates available with open tubular columns it is probably more advisable to adjust the flow rate to suit the analysis time available.

I.4.5 Conclusion.

The measurement of optimum gas velocity for an open tubular column was more difficult than for a packed column.

The contribution to band broadening from longitudinal diffusion is much greater than for packed columns although this fact is a direct result of the increased column length. However the contribution from eddy diffusion is zero. This resulted in a different H.E.T.P. curve than the typical U-shape that is observed for packed columns. The curve observed was shallow and represented only relatively small changes in height equivalent to a theoretical plate for any change in carrier gas velocity.

I.5 Performance Evaluation of a Commercial Support Coated Open Tubular Column.

I.5.1 Introduction.

A commercially prepared support coated open tubular column, coated with SE30 (E301 silicone oil) stationary phase, had previously been purchased for examination of the chromatographic separation of drugs. It was proposed to use this column to gain the practical experience of open tubular column installation and use in an adapted Varian gas chromatograph. It was also intended to use this column as a base for the evaluation of column performance.

I.5.2 Method.

The column was connected into an adapted Varian 2800 gas chromatograph. This instrument had one injection port equipped with a simple needle valve sample splitter and a similar make-up needle valve. The glass column was connected between these two valves with heat shrinkable PTFE tubing. Dead volumes were avoided as much as possible although in open tubular chromatography they produce little interference providing they do not present active sites or a large cross sectional area to the solute. It was also decided not to pack the injection port and detector inlet with glass wool or inert packing as this would have also affected column performance by increasing the pressure drop across the column,

by providing active sites for peak broadening and drastically increasing the column permeability. A flow rate of two millilitres of zero grade nitrogen was established through the column and measured at the column outlet using a soap film flow-meter. The column was conditioned at 120^{o}C for a period of sixteen hours before connection to the detector. The flame ionisation detector was that normally used for packed column work and the make-up gas flow rate was adjusted to produce thirty five millilitres per minute at the detector outlet. One microlitre samples of the headspace were removed from above a series of pure solvent standards in sealed vials. The syringe was flushed twice with air and a one microlitre sample of air injected from the syringe in to the chromatograph. The sample split ratio was set to approximately 1:4.

I.5.3 Results.

For comparison of the performance of this column with other open tubular columns and between individual solvents the results are presented in Table I.5.1 with the solutes listed in order of increasing retention time. The resolution (R) of the listed solute and the preceeding solute is also given along with theoretical plate estimates. (NB. R value >1.5 approximates to 99.7% separation, and a R value >1.0 to 98% separation)

Oven Temperature 100°C Detector Temperature 200°C Injec or Temperature 150°C	Column Length 28.6 metres Carrier gas flow rate 2 millilitres / minute Nitrogen Inlet Pressure 4 p.s.i.

Solute	t	n	h	k	N	H	R
Air	201						
Dichloromethane	210	144	19.90	0.045	0.27	10700	
Diethyl ether	210	144	19.90	0.045	0.27	10700	0.00
n Hexane	216	400	7.15	0.075	1.95	1470	0.14
Chloroform	219	516	5.54	0.090	3.52	813	0.08
Cyclohexane	225	256	11.20	0.120	2.94	973	0.11
Benzene	225	256	11.20	0.120	2.94	973	0.00
Trichloroethylene	231	400	7.15	0.150	6.81	420	0.13
Carbon Tetrachloride	231	400	7.15	0.150	6.81	420	0.00
Methanol *	235	53	53.90	0.165	1.06	2700	0.02
Acetone *	237	64	44.70	0.180	1.49	1920	0.03
Ethanol *	243	49	69.40	0.210	1.48	1930	0.04
2 Propanol *	246	225	12.70	0.225	7.59	376	0.05
Toluene	246	900	3.17	0.225	30.4	94	0.00
n Propanol *	261	400	7.15	0.300	21.3	134	0.29
2 Butanol *	267	215	13.3	0.328	13.1	218	0.08
Xylene 1	282	2920	0.98	0.403	241	12	0.72
Xylene 2	288	3360	0.85	0.433	307	9	0.30
M.I.B.K. *	294	427	6.69	0.463	42.8	67	0.11
n Butanol *	318	380	7.53	0.582	51.4	56	0.37

Key: tr Absolute retention time of solute. (sec.)
n Plate number.
h Plate height (cm).
k Solute partition ratio.
N Effective plate number.
H Effective plate height (cm).
R Resolution from preceeding solute.

Table I.5.1 - Retention Parameters and Column Operating Conditions for Commercial Support Coated Open Tubular Column.

Equations used to calculate parameters given in Table I.5.1.

$$tr = tx - ta \quad (1)$$

where tr - absolute retention time of solute,
tx - measured time to solute peak,
ta - measured time to air peak.

$$n = 16 * (tr / w)^2 \quad (2)$$

where n - plate number, w - peak width.

$$h = L / n \quad (3)$$

where h - plate height, L - column length.

$$k = (tx - ta) / ta \quad (4)$$

where k - partition ratio.

$$N = n * (k / (1 + k))^2 \quad (5)$$

where N - effective plate number (Brenner 1962).

$$H = L / N \quad (6)$$

where H - effective plate height.

$$R = ((r' - 1) / (4 * r') * SQR(N) \quad (7)$$

where R - resolution, r'- relative retention time, r' and N refer to the more slowly moving solute.

I.5.4 Discussion.

The ability to use small quantities of solute and still obtain an adequate response from the detector demonstrates that open tubular columns require only a small sample size to prevent overloading the stationary phase. However, this decrease in allowable sample size is more than compensated by the increase in sensitivity. As observed with the packed SE30 column (see Chapter 2.2) the peak shape of the polar solutes (* in Table I.5.1) was found to be very poor and to exhibit severe tailing. The retention times were found to be very short. Although this column does not solve the separation problem which was set before it, it does demonstrate the order of magnitude of values to be expected for the various column parameters. The results were in general much as to be expected from the data obtained from experience with packed columns using SE30 stationary phase, in that the resolution was lost by the poor shape of many of the peaks.

I.5.5 Conclusion.

The commercial SE30 support coated open tubular column was shown to be inadequate for the purpose of separation of complex solvent mixtures.

The column and collection of above data was found to be useful in gaining the practical experience in converting a gas chromatograph equipped for packed columns to one suitable for use with open tubular columns. Observations on the importance of reducing dead volumes, obtaining steady carrier gas flow rates, and the ajustment of injection volume or split ratio in order to prevent overloading the column were made. These observations would be of greater importance when a similar examination was made of the wall coated open tubular columns produced as described in earlier sections.

I.6 Performance Evaluation of Different Stationary Phases on Wall Coated Open Tubular Columns.

I.6.1 Introduction.

Wall coated open tubular columns have expected efficiencies much higher than the support coated open tubular column examined in Section I.5 and are to some extent easier to manufacture. The performance of fifteen wall coated open tubular columns prepared, deactivated, conditioned and coated as described in earlier sections of this appendix were evaluated by their ability to separate a selection of solvents.

I.6.2 Method.

The columns were each connected in turn into the same adapted Varian 2800 gas chromatograph which was used in the evaluation of the support coated open tubular column. All column to valve connections were made with heat shrinkable PTFE tubing. A flow rate of two millilitres per minute of zero grade nitrogen was established through the column. The columns were each conditioned before use by heating up to a temperature twenty degrees above the intended operating temperature for a period of sixteen hours, before connection to the detector. A flame ionisation detector was used in each case and the make-up gas flow rate was adjusted to make thirty five millilitres per minute at the detector outlet.

Headspace samples from above the pure solvent standards were then injected as described in Section I.5.

I.6.3 Results.

The results are presented in the form of a series of tables with the solutes listed in order of increasing retention time. Table I.6.1 summarises these tables and Tables I.6.2 through to Table I.6.16 list the actual results obtained.

No.	Stationary Phase	Table No
I	Carbowax 20M	I.6.2
II	Hallcomid M18	I.6.3
III	Emulphur ON870	I.6.4
IV	Apiezon L	I.6.5
V	Carbowax 20M	I.6.6
VI	Hallcomid M18	I.6.7
VII	Emulphur ON870	I.6.8
VIII	Apiezon L	I.6.9
IX	SF 96	I.6.10
X	SF 96	I.6.11
XI	Carbowax 20M	I.6.12
XII	Hallcomid M18	I.6.13
XIII	Emulphur ON870	I.6.14
XIV	Apiezon L	I.6.15
XV	SF 96	I.6.16

Table 5.4.1 - Index Table of the Parameter Results Tables.

Oven Temperature 100oC Detector Temperature 200oC Injector Temperature 150oC			Column Length 28.6 m. Column Volume 4.399 ml. Column i.d. 0.442 mm.				
Solute	t	n	h	k	N	H	R
Air	171						
n-Hexane	9	57600	0.049	0.053	144	19.90	
Diethyl ether	9	57600	0.049	0.053	144	19.90	0.00
Cyclohexane	18	15880	0.181	0.105	144	19.90	1.49
Acetone	27	17420	0.165	0.157	324	8.86	2.10
Methanol	45	20140	0.142	0.263	900	3.19	1.88
Ethanol	48	21320	0.134	0.281	1020	2.80	0.51
Dichloromethane	51	5480	0.524	0.298	289	9.93	0.24
2-Propanol	63	24340	0.118	0.368	1160	2.47	1.62
Chloroform	66	6240	0.459	0.386	484	5.93	0.26
Benzene	78	12250	0.234	0.456	1200	2.39	1.33
Trichloroethylene	105	15050	0.191	0.614	2180	1.32	3.00
M.I.B.K.	114	9020	0.318	0.667	1440	1.99	0.75
n-Propanol	123	9600	0.299	0.719	1680	1.70	0.74
Carbon Tetrachloride	126	4360	0.659	0.737	184	15.60	0.08
2-Butanol	129	40000	0.072	0.754	7390	0.39	0.48
Toluene	150	11450	0.251	0.877	2500	1.15	1.75
Xylene 1	270	9600	0.299	1.579	3600	0.80	6.67
n-Butanol	276	7150	0.401	1.614	3160	0.90	0.30
Xylene 2	294	6000	0.478	1.719	2400	1.19	0.75

Key: tr Absolute retention time of solute (sec).
n Plate number.
h Plate height (ml).
k Solute partition ratio.
N Effective plate number.
H Effective plate height (ml).
R Resolution from preceeding solute.

Table I.6.2 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No I.

Oven Temperature 80oC Detector Temperature 200oC Injector Temperature 150oC			Column Length 51.0 m. Column Volume 10.538 ml. Column i.d. 0.485 mm.				
Solute	t	n	h	k	N	H	R
Air	543						
Diethyl ether	45	17070	0.299	0.083	100	51.00	
Acetone	72	18620	0.274	0.133	256	19.90	1.50
n-Hexane	99	11450	0.446	0.182	212	24.10	0.98
Methanol	111	5280	0.965	0.204	152	33.50	0.33
Dichloromethane	174	5350	0.952	0.320	275	18.50	1.50
Ethanol	177	4700	1.090	0.326	284	17.90	0.08
Cyclohexane	180	4740	1.070	0.332	294	17.30	0.08
2-Propanol	219	5270	0.968	0.403	435	11.70	0.92
Carbon Tetrachloride	231	4160	1.230	0.425	371	13.70	0.25
Benzene	270	8160	0.625	0.497	900	5.67	1.09
n-Propanol	426	2900	1.760	0.785	560	9.11	2.17
Chloroform	432	3490	1.460	0.796	685	7.45	0.09
Trichloroethylene	438	5280	0.966	0.807	1050	4.85	0.11
2-Butanol	516	2950	1.730	0.950	700	7.28	1.00
M.I.B.K.	528	3540	1.440	0.972	860	5.93	0.17
Toluene	642	2774	1.830	1.182	814	6.26	1.27
n-Butanol	1044	2800	1.820	1.923	1210	4.21	3.35
Xylene 1	1296	3410	1.490	2.387	1690	3.01	2.00
Xylene 2	1464	1990	2.560	2.696	1060	4.02	0.93
Key: As for Table I.6.2							

Table I.6.3 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No II.

Oven Temperature 50°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 25.9 m. Column Volume 3.369 ml. Column i.d. 0.423 mm.				
Solute	t	n	h	k	N	H	R
Air	141						
Acetone	18	11240	0.230	0.128	144	17.80	
Methanol	30	13000	0.200	0.213	400	6.40	1.99
Dichloromethane	36	3480	0.736	0.255	144	17.80	0.49
Ethanol	42	14880	0.172	0.298	784	3.27	1.01
2-Propanol	45	15380	0.166	0.319	900	2.84	0.49
Chloroform	75	5180	0.494	0.532	625	4.09	2.50
n-Propanol	93	24340	0.105	0.660	3840	0.67	3.00
2-Butanol	165	10400	0.246	1.170	3030	0.85	6.00
n-Butanol	180	5090	0.503	1.277	1600	1.60	0.84
Key: As for Table I.6.2							

Table I.6.4 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No III.

Oven Temperature 65°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 34.5 m. Column Volume 7.207 ml. Column i.d. 0.515 mm.				
Solute	t	n	h	k	N	H	R
Air	423						
Acetone	12	84100	0.041	0.028	64	53.90	
Methanol	12	84100	0.041	0.028	64	53.90	0.00
Ethanol	12	84100	0.041	0.028	64	53.90	0.00
2-Propanol	12	84100	0.041	0.028	64	53.90	0.00
Diethyl ether	15	85260	0.040	0.035	100	34.50	0.50
n-Propanol	30	91200	0.038	0.071	400	8.62	2.53
Dichloromethane	36	93630	0.037	0.085	576	5.99	0.98
2-Butanol	42	96100	0.036	0.099	784	4.40	0.99
n-Hexane	48	24650	0.139	0.113	256	13.40	0.49
Chloroform	66	26570	0.130	0.156	484	7.13	1.52
n-Butanol	78	111600	0.031	0.184	2700	1.28	1.98
Trichloroethylene	81	28220	0.122	0.191	729	4.73	0.25
Benzene	93	29580	0.117	0.219	961	3.59	0.99
Cyclohexane	96	119700	0.029	0.227	4100	0.84	0.56
Carbon Tetrachloride	99	7570	0.456	0.234	272	12.10	0.12
M.I.B.K.	123	33120	0.104	0.291	1680	2.05	2.01
Toluene	243	49280	0.070	0.574	6560	0.53	9.98
Xylene 1	531	16180	0.213	1.255	5010	0.69	9.60
Xylene 2	624	13530	0.255	1.475	4810	0.72	2.59
Key: As for Table I.6.2							

Table I.6.5 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No IV.

Oven Temperature 65°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 48.4 m. Column Volume 12.525 ml. Column i.d. 0.574 mm.				
Solute	t	n	h	k	N	H	R
Air	336						
n-Hexane	3	204300	0.022	0.009	16	302.5	
Diethyl ether	3	204300	0.022	0.009	16	302.5	0.00
Cyclohexane	9	211600	0.023	0.027	144	33.61	2.00
Acetone	18	55600	0.087	0.054	144	33.61	1.50
Methanol	36	61500	0.079	0.107	576	8.40	3.00
Ethanol	39	62500	0.077	0.116	676	7.16	0.50
Dichloromethane	45	64500	0.075	0.134	900	5.38	1.00
2-Propanol	45	64500	0.075	0.134	900	5.38	0.00
Chloroform	45	64500	0.075	0.134	900	5.38	0.00
Benzene	48	65500	0.074	0.143	1024	4.73	0.50
Trichloroethylene	69	72900	0.066	0.205	2116	2.28	3.50
M.I.B.K.	72	73900	0.065	0.214	2304	2.10	0.50
n-Propanol	75	18800	0.257	0.223	625	7.74	0.25
Carbon Tetrachloride	81	77280	0.063	0.241	2916	1.66	1.00
2-Butanol	84	19600	0.247	0.250	784	6.17	0.25
Toluene	93	81800	0.059	0.276	3844	1.26	1.50
Xylene 1	168	28200	0.171	0.500	3136	1.54	6.25
n-Butanol	180	13140	0.368	0.535	1600	3.03	0.67
Xylene 2	186	13450	0.359	0.553	1700	2.83	0.33
Key: As for Table I.6.2							

Table I.6.6 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No V.

Oven Temperature 80°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 46.6 m. Column Volume 10.564 ml. Column i.d. 0.537 mm.				
Solute	t	n	h	k	N	H	R
Air	282						
Diethyl ether	12	38400	0.121	0.043	64	72.80	
Acetone	18	40000	0.116	0.064	144	32.36	1.00
n-Hexane	24	41600	0.111	0.085	256	18.20	1.00
Methanol	30	43200	0.107	0.106	400	11.65	1.00
Dichloromethane	36	44900	0.103	0.128	576	8.09	1.00
Ethanol	45	47500	0.098	0.159	900	5.18	1.50
Cyclohexane	45	47500	0.098	0.159	900	5.18	0.00
2-Propanol	57	22700	0.205	0.202	641	7.26	1.33
Carbon Tetrachloride	60	23100	0.201	0.212	711	6.55	0.33
Benzene	72	24700	0.188	0.255	1024	4.55	1.33
n-Propanol	111	17160	0.271	0.394	1369	3.40	3.25
Chloroform	111	17160	0.271	0.394	1369	3.40	0.00
Trichloroethylene	114	4356	1.069	0.404	361	12.90	0.12
2-Butanol	132	19040	0.244	0.468	1936	2.40	1.50
M.I.B.K.	135	4930	0.964	0.478	506	9.20	0.12
Toluene	165	9867	0.472	0.585	1344	3.46	1.66
Xylene 1	327	10300	0.452	1.159	2970	1.56	6.75
Xylene 2	375	3915	1.190	1.329	1275	3.65	1.14
Key: As for Table I.6.2							

Table I.6.7 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No VI.

Oven Temperature 50°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 49.4 m. Column Volume 12.797 ml. Column i.d. 0.574 mm.				
Solute	t	n	h	k	N	H	R
Air	300						
Diethyl ether	18	179000	0.027	0.060	576	8.57	
n-Hexane	24	186000	0.026	0.080	1024	4.82	2.00
Acetone	57	56640	0.087	0.190	1444	3.42	5.50
Cyclohexane	60	230000	0.021	0.200	6400	0.77	0.99
Methanol	105	18200	0.027	0.350	1225	4.03	3.75
Dichloromethane	132	82900	0.059	0.440	7744	0.64	4.50
Ethanol	147	98800	0.055	0.490	9604	0.51	2.50
Chloroform	150	90000	0.054	0.500	10000	0.49	0.50
2-Propanol	219	119700	0.041	0.730	21310	0.23	11.50
Benzene	237	128100	0.038	0.790	24960	0.19	3.00
Trichloroethylene	282	66900	0.074	0.940	15700	0.31	5.00
Carbon Tetrachloride	300	160000	0.031	1.000	40000	0.12	3.00
M.I.B.K.	339	45300	0.108	1.130	12700	0.38	3.25
2-Butanol	354	47500	0.103	1.180	13900	0.35	1.25
Toluene	417	57120	0.086	1.390	19300	0.25	5.25
n-Butanol	846	64800	0.076	2.820	35300	0.14	23.80
Xylene 1	852	36800	0.134	2.840	20160	0.25	0.25
Xylene 2	960	28200	0.175	3.200	16380	0.30	3.60
Key: As for Table I.6.2							

Table I.6.8 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No VII.

Oven Temperature 65°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 46.6 m. Column Volume 11.305 ml. Column i.d. 0.555 mm.				
Solute	t	n	h	k	N	H	R
Air	372						
Acetone	12	65500	0.070	0.032	64	72.81	
Methanol	21	68600	0.067	0.056	196	23.77	1.50
Ethanol	24	69690	0.066	0.063	256	18.20	0.50
2-Propanol	27	17700	0.263	0.072	81	57.53	0.25
Diethyl ether	30	71800	0.065	0.081	400	11.65	0.50
n-Propanol	45	8587	0.542	0.121	100	46.60	0.83
Dichloromethane	54	8960	0.519	0.145	144	32.36	0.50
2-Butanol	78	5625	0.828	0.209	169	27.57	1.00
n-Hexane	90	3794	1.228	0.241	144	32.36	0.40
Chloroform	108	1264	3.686	0.290	64	72.81	0.33
n-Butanol	138	2359	1.975	0.370	172	26.97	0.71
Carbon Tetrachloride	171	582	8.000	0.459	57	80.67	0.36
Benzene	177	8372	0.556	0.475	870	5.35	0.25
Cyclohexane	180	1119	4.163	0.483	119	39.15	0.04
Trichloroethylene	228	3265	1.427	0.613	471	9.88	1.14
M.I.B.K.	252	534	8.724	0.677	87	53.49	0.22
Toluene	474	881	5.288	1.274	276	16.84	1.95
Xylene 1	1218	1248	3.732	3.274	732	6.36	4.13
Xylene 2	1218	1248	3.732	3.274	732	6.36	0.00
Key: As for Table I.6.2							

Table I.6.9 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No VIII.

Oven Temperature 70°C Detector Temperature 150°C Injector Temperature 200°C			Column Length 34.5 m. Column Volume 8.918 ml. Column i.d. 0.573 mm.				
Solute	t	n	h	k	N	H	R
Air	186						
Methanol	36	5476	0.630	0.193	144	23.95	
Ethanol	60	6724	0.513	0.322	400	8.63	
Carbon Tetrachloride	60	2988	1.154	0.322	177	19.40	0.00
Acetone	66	3136	1.100	0.355	215	16.03	
2-Propanol	78	7744	0.445	0.419	676	5.10	
Diethyl ether	84	8100	0.425	0.452	784	4.40	
Dichloromethane	102	4096	0.842	0.548	513	6.72	
n-Propanol	132	4993	0.691	0.709	860	4.00	
2-Butanol	177	3660	0.942	0.952	870	3.96	
n-Hexane	186	15376	0.224	1.000	3844	0.90	
Chloroform	198	2621	1.316	1.064	696	4.95	
n-Butanol	300	4199	0.821	1.613	1600	2.16	
Cyclohexane	312	1102	3.129	1.677	432	7.97	
Benzene	312	1102	3.129	1.677	432	7.97	0.00
Trichloroethylene	396	1505	2.291	2.129	696	4.95	
M.I.B.K.	462	1866	1.848	2.483	948	3.64	
Toluene	678	1474	2.339	3.645	908	3.80	
Xylene 1	1356	4696	0.734	7.290	3632	0.95	
Xylene 2	1446	2959	1.165	7.774	2323	1.48	
Key: As for Table I.6.2							

Table I.6.10 Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No IX.

Oven Temperature 80°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 49.4 m. Column Volume 12.797 ml. Column i.d. 0.574 mm.				
Solute	t	n	h	k	N	H	R
Air	300						
Diethyl Ether	18	179776	0.027	0.060	576	8.576	
Hexane	24	186624	0.026	0.080	1024	4.824	2.00
Acetone	57	56644	0.087	0.190	1444	3.421	5.50
Cyclohexane	60	230400	0.021	0.200	6400	0.772	1.00
Methanol	105	18225	0.271	0.350	1225	4.033	3.75
Dichloromethane	132	82944	0.060	0.440	7744	0.638	4.50
Ethanol	147	88804	0.056	0.490	9604	0.514	2.50
Chloroform	150	90000	0.055	0.500	10000	0.494	0.50
2-Propanol	219	119716	0.041	0.730	21316	0.232	11.50
Benzene	237	128164	0.039	0.790	24964	0.198	3.00
Trichloroethylene	282	66908	0.074	0.940	15708	0.314	5.00
Carbon Tetrachloride	300	160000	0.031	1.000	40000	0.124	3.00
M.I.B.K.	339	45369	0.109	1.130	12769	0.387	3.25
Propanol	345	46225	0.107	1.150	13225	0.374	0.50
2-Butanol	354	47524	0.104	1.180	13924	0.355	0.75
Toluene	417	57121	0.086	1.390	19321	0.256	5.25
n-Butanol	846	64855	0.076	2.820	35344	0.140	23.83
Xylene 1	852	36864	0.134	2.840	20164	0.245	0.25
Xylene 2	960	28224	0.175	3.200	16384	0.302	3.60
Key: As for Table I.6.2							

Table I.6.11 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No X.

Oven Temperature 65°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 48.4 m. Column Volume 12.525 ml. Column i.d. 0.574 mm.				
Solute	t	n	h	k	N	H	R
Air	336						
Diethyl Ether	2	203581	0.024	0.007	10	472.65	
Hexane	4	205028	0.024	0.011	23	210.06	0.40
Cyclohexane	9	211600	0.023	0.027	144	33.61	1.80
Acetone	18	55696	0.087	0.054	144	33.61	1.50
Methanol	36	15376	0.315	0.107	144	33.61	1.50
Ethanol	39	62500	0.077	0.116	676	7.16	0.50
Dichloromethane	42	63504	0.076	0.125	784	6.17	0.50
Chloroform	43	63908	0.076	0.129	829	5.84	0.20
2-Propanol	45	64516	0.075	0.134	900	5.38	0.30
Benzene	48	65536	0.074	0.143	1024	4.73	0.50
Trichloroethylene	69	32400	0.149	0.205	940	5.15	2.33
M.I.B.K.	72	18496	0.262	0.214	576	8.40	0.25
Propanol	75	18769	0.258	0.223	625	7.74	0.25
Carbon tetrachloride	81	77284	0.063	0.241	2916	1.66	1.00
2-Butanol	84	19600	0.247	0.250	784	6.17	0.25
Toluene	93	20449	0.237	0.277	961	5.04	0.75
Xylene 1	168	7056	0.686	0.500	784	6.17	3.12
n-Butanol	180	13148	0.368	0.536	1600	3.03	0.67
Xylene 2	186	4844	0.999	0.554	615	7.87	0.20
Key: As for Table I.6.2							

Table I.6.12 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No XI.

Oven Temperature 50°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 44.9 m. Column Volume 12.986 ml. Column i.d. 0.606 mm.				
Solute	t	n	h	k	N	H	R
Air	408						
Acetone	23	82484	0.054	0.056	231	19.43	
Methanol	24	82944	0.054	0.059	256	17.54	0.20
Carbon Tetrachloride	25	20851	0.215	0.062	71	63.63	0.10
Diethyl Ether	27	84100	0.053	0.066	324	13.86	0.30
Ethanol	30	85264	0.053	0.074	400	11.23	0.50
Dichloromethane	33	86436	0.052	0.081	484	9.28	0.50
2-Propanol	51	93636	0.048	0.125	1156	3.88	3.00
Cyclohexane	54	94864	0.047	0.132	1296	3.47	0.50
n-Propanol	60	97344	0.046	0.147	1600	2.81	1.00
Chloroform	63	10955	0.410	0.154	196	22.91	0.17
Hexane	84	107584	0.042	0.206	3136	1.43	3.50
Benzene	90	110224	0.041	0.221	3600	1.25	1.00
2-Butanol	108	13148	0.341	0.265	576	7.80	1.00
Trichloroethylene	111	29929	0.150	0.272	1369	3.28	0.25
M.I.B.K.	144	135424	0.033	0.353	9216	0.49	5.50
Toluene	183	155236	0.029	0.449	14884	0.30	6.50
n-Butanol	228	19975	0.225	0.559	2567	1.75	2.50
Xylene 1	369	67081	0.067	0.904	15129	0.30	11.75
Xylene 2	396	71824	0.063	0.971	17424	0.26	2.25
Key: As for Table I.6.2							

Table I.6.13 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No XII.

Oven Temperature 70°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 46.3 m. Column Volume 9.942 ml. Column i.d. 0.523 mm.				
Solute	t	n	h	k	N	H	R
Air	494						
Di thyl Ether	5	110756	0.042	0.010	10	452.15	
Acetone	11	113434	0.041	0.022	52	89.31	1.00
Methanol	12	50655	0.091	0.024	28	162.77	0.13
Dichloromethane	16	115600	0.040	0.032	108	42.81	0.60
Hexane	16	115872	0.040	0.033	117	39.70	0.10
Ethanol	17	116145	0.040	0.034	125	36.91	0.10
Cyclohexane	22	118611	0.039	0.045	219	21.14	0.90
2-Propanol	23	119163	0.039	0.047	243	19.03	0.20
Benzene	28	121104	0.038	0.056	339	13.68	0.70
Carbon Tetrachloride	28	121383	0.038	0.057	353	13.10	0.10
n-Propanol	38	126167	0.037	0.078	655	7.07	1.70
Chloroform	41	127592	0.036	0.084	762	6.08	0.50
Trichloroethylene	46	129600	0.036	0.092	924	5.01	0.70
2-Butanol	49	131334	0.035	0.100	1076	4.30	0.60
M.I.B.K.	52	132787	0.035	0.106	1211	3.82	0.50
Toluene	60	136604	0.034	0.121	1600	2.89	1.30
n-Butanol	92	153037	0.030	0.187	3795	1.22	5.40
Xylene 1	118	166464	0.028	0.238	6147	0.75	4.20
Xylene 2	132	174390	0.027	0.267	7744	0.60	2.40
Key: As for Table I.6.2							

Table I.6.14 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No XIII.

Oven Temperature 65°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 45.9 metres Column Volume 11.051 ml. Column i.d. 0.553 mm.				
Solute	t	n	h	k	N	H	R
Air	432						
Diethyl Ether	4	84565	0.054	0.010	8	585.5	
Acetone	6	85264	0.054	0.014	16	286.9	0.30
Methanol	7	85498	0.054	0.015	19	237.1	0.10
Ethanol	7	85732	0.054	0.017	23	199.2	0.10
2-Propanol	8	85966	0.053	0.018	27	169.7	0.10
Dichloromethane	9	86436	0.053	0.021	36	127.5	0.20
n-Propanol	10	86907	0.053	0.024	46	99.3	0.20
Chloroform	13	87853	0.052	0.029	71	65.1	0.40
2-Butanol	14	88328	0.052	0.032	85	54.2	0.20
Benzene	18	90000	0.051	0.042	144	31.9	0.70
Trichloroethylene	19	90240	0.051	0.043	154	29.9	0.10
Carbon Tetrachloride	20	90721	0.051	0.046	174	26.3	0.20
Hexane	20	90963	0.050	0.047	185	24.8	0.10
Cyclohexane	23	91930	0.050	0.053	231	19.9	0.40
n-Butanol	25	92903	0.049	0.058	282	16.3	0.40
M.I.B.K.	31	95357	0.048	0.072	433	10.6	1.00
Toluene	38	98094	0.047	0.087	635	7.2	1.10
Xylene	79	115872	0.040	0.182	2746	1.7	6.80
Key: As for Table I.6.2							

Table I.6.15 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No XIV.

Oven Temperature 80°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 43.8 metres Column volume 13.314 ml. Column i.d. 0.621 mm.				
Solute	t	n	h	k	N	H	R
Air	398						
Diethyl Ether	2	70969	0.062	0.005	1	3042.0	
Hexane	3	71390	0.061	0.008	4	1095.0	0.20
Acetone	6	72469	0.060	0.015	16	273.8	0.50
Cyclohexane	9	73549	0.060	0.023	36	121.7	0.50
Methanol	12	74638	0.059	0.030	64	68.4	0.50
Dichloromethane	21	19488	0.225	0.053	49	89.4	0.75
Ethanol	24	79073	0.055	0.060	256	17.1	0.50
Carbon Tetrachloride	25	19825	0.221	0.062	67	65.1	0.05
2-Propanol	27	80202	0.055	0.068	324	13.5	0.40
Benzene	30	81339	0.054	0.075	400	11.0	0.50
Trichloroethylene	54	90721	0.048	0.136	1296	3.4	4.00
Chloroform	60	23287	0.188	0.151	400	11.0	0.50
n-Propanol	66	95605	0.046	0.166	1936	2.3	1.00
2-Butanol	67	95852	0.046	0.167	1971	2.2	0.10
M.I.B.K.	67	96100	0.046	0.169	2007	2.2	0.10
Toluene	81	101889	0.043	0.204	2916	1.5	2.30
n-Butanol	165	62567	0.070	0.415	5378	0.8	9.33
Xylene 1	174	64584	0.068	0.437	5980	0.7	1.00
Xylene 2	195	69415	0.063	0.490	7511	0.6	2.33
Key: As for Table I.6.2							

Table I.6.16 - Retention Parameters and Column Operating Conditions for Wall Coated Open Tubular Column No XV.

From these Tables data was extracted in an attempt at demonstrating the effect of the different coating techniques. This extracted data is presented in Table I.6.17 as a comparison of partition ratios for a selected set of solutes on each column after correction for column volume.

Solute\Col	I	V	+ XI	II	VI	XII
Hexane	0.053	0.009	0.055	0.182	0.085	0.033
Benzene	0.456	0.143	0.459	0.497	0.255	0.057
Toluene	0.877	0.277	0.917	1.182	0.585	0.121
Xylene 1	1.579	0.500	1.696	2.387	1.159	0.238
Methanol	0.263	0.107	0.305	0.204	0.106	0.024
Ethanol	0.281	0.116	0.389	0.326	0.159	0.033
n-Propanol	0.719	0.223	0.832	0.785	0.394	0.078
n-Butanol	1.614	0.536	1.735	1.923	0.936	0.187
Solute\Col	III	VII	XIII	IV	+VIII	XIV
Hexane	*	0.023	0.007	0.113	0.154	0.032
Benzene	*	0.225	0.075	0.219	0.303	0.057
Toluene	*	0.395	0.204	0.574	0.812	0.099
Xylene 1	*	0.807	0.437	1.255	2.087	0.189
Methanol	0.213	0.099	0.030	0.028	0.020	0.019
Ethanol	0.298	0.139	0.060	0.028	0.035	0.019
n-Propanol	0.660	0.327	0.166	0.071	0.092	0.032
n-Butanol	1.277	0.802	0.415	0.184	0.236	0.054

Solute\Col	IX	X	XV
Hexane	1.000	0.206	0.046
Benzene	1.677	0.220	0.042
Toluene	3.645	0.448	0.087
Xylene 1	7.290	0.904	0.182
Methanol	0.193	0.059	0.017
Ethanol	0.322	0.074	0.017
n-Propanol	0.709	0.147	0.024
n-Butanol	1.613	0.558	0.058

Table I.6.17 - Partition Ratios for Selected Solutes on All Columns After Correction For Total Volume.

Figure I.6.1 - Correlation Plot of Partition Ratios for Air Plug Dynamic vs. Mercury Plug Dynamic Coating Methods.

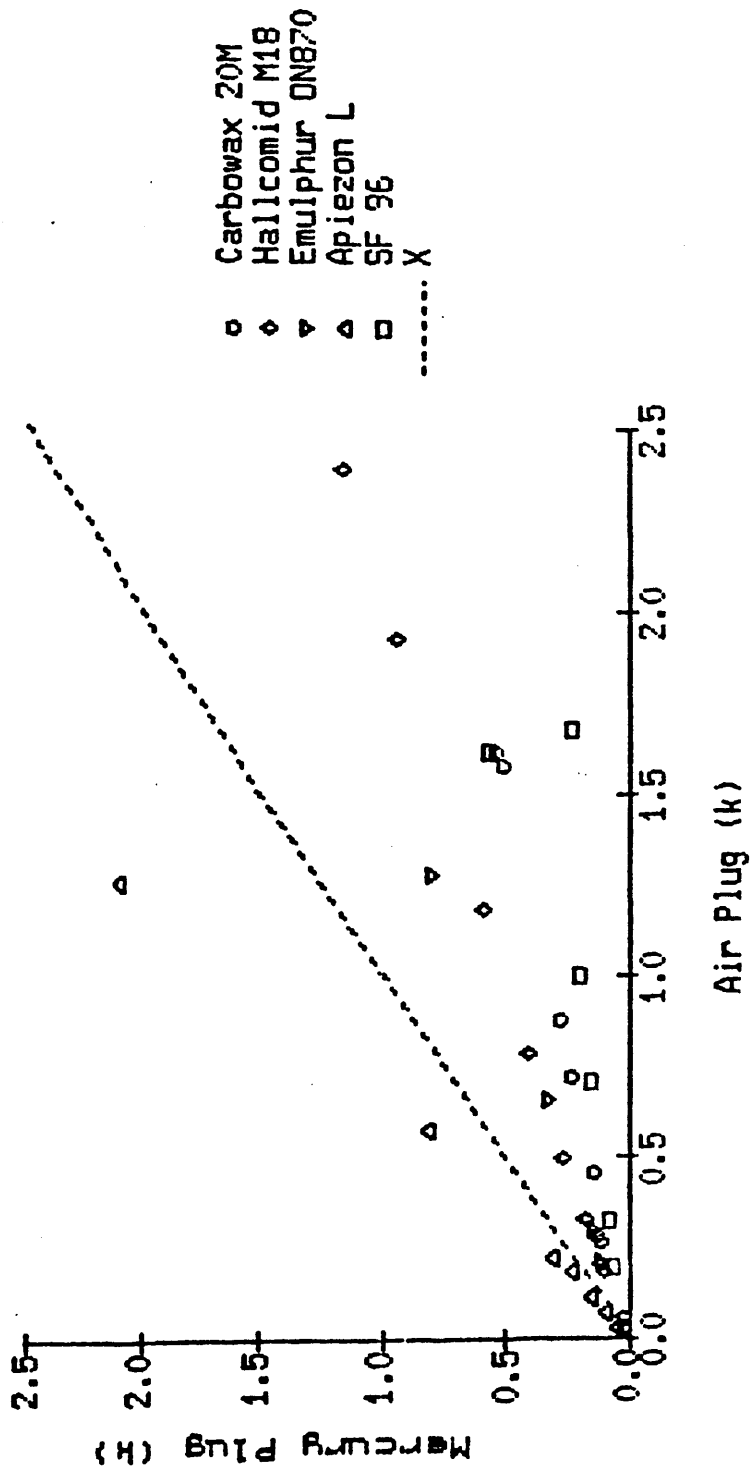
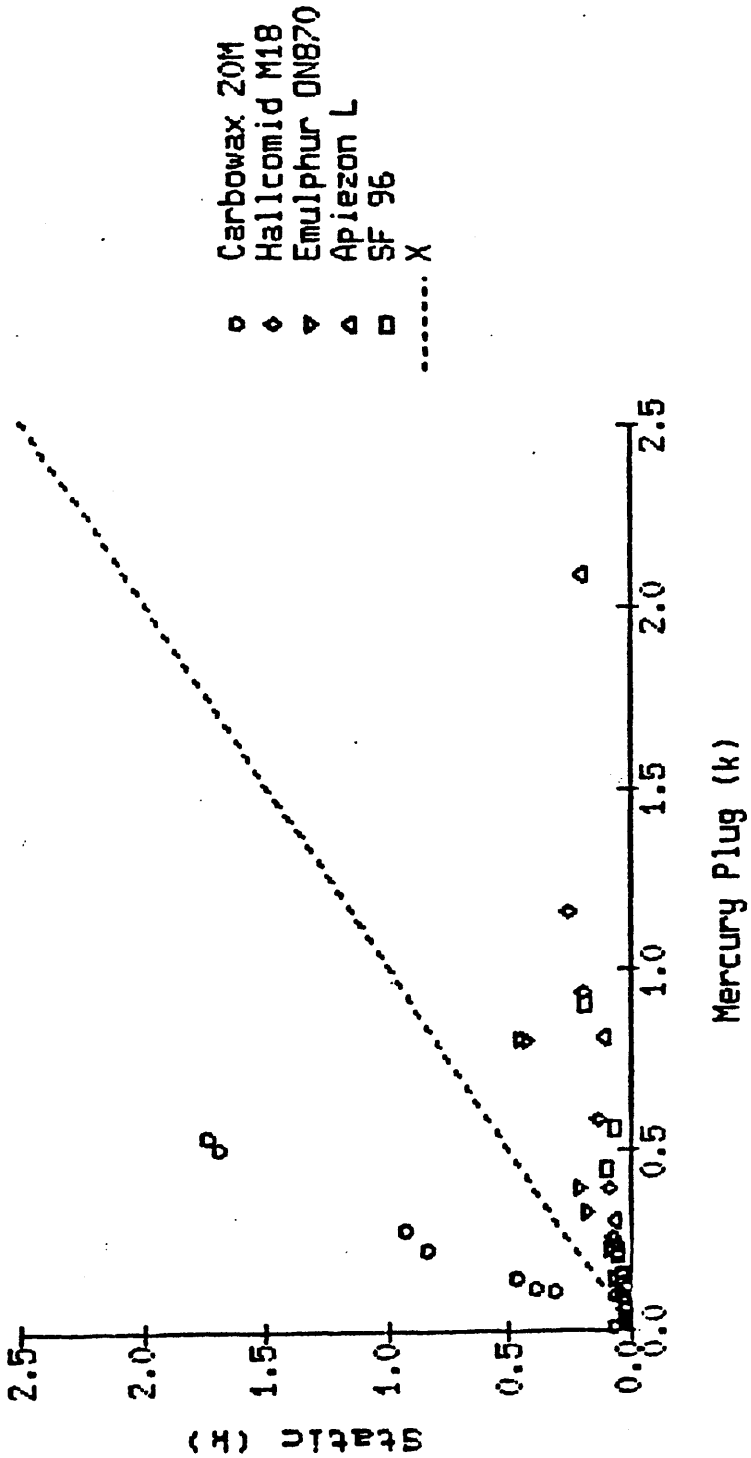


Figure I.6.2 - Correlation Plot of Partition Ratios for Mercury Plug Dynamic vs. Static Coating Methods.



If the partition ratios for a particular series of solutes on one column are plotted against the partition ratios for the same series of solutes on another column the gradient obtained will be equal to the ratio of the respective column phase ratios. This value will be greater than unity if the second column is more efficient than the first column as from theory the higher the value of the column phase ratio the more efficient the column. The data given in Table I.6.17 has therefore been represented graphically and the resulting curves are given in Figure I.6.1, air plug dynamic against mercury plug dynamic coating method, and in Figure I.6.2 , mercury plug dynamic against static coating method, for each of the five stationary phases used in the comparison. All partition ratios were normalised for total volume before plotting.

I.6.4 Discussion.

The phase ratio is dependant on the quantity of stationary phase in a column and the total column volume, therefore assuming constant temperature and the fact that retention times can be measured with reasonable accuracy the partition ratios obtained from these measurements can be relied upon as a method of column comparison. In fact the errors obtained on the plots of partition ratios were all less than 3% (ie. the deviation of any one point from the best line of fit was less than 3% of the x or y value at that point). The correlation graphs produced Figures I.6.1 and I.6.2 illustrate the relative improvement of one coating method over

another.

I.6.5 Conclusion.

Static coated columns were shown to be more efficiently coated than mercury plug dynamic coated columns, which were more efficiently coated than air plug dynamic coated columns. This should result in the static coated columns lasting longer than their counterparts, the more uniform distribution of stationary phase reducing the chances of plugging.

Against this advantage is the fact that static columns are more difficult, and take more time, to manufacture. This balance between efficiency and availability is dependant on the solute separation required. The columns examined here have been compared for their usefulness in the separation of a wide range of solvents and it was found that the number of plates required for a particular separation increased dramatically as the coating technique improved, resulting in the need for longer columns and consequentially longer analysis times. For this reason it was decided to compromise to some extent by using only mercury plug dynamic coated columns for further work.

I.7 Preparation of 2-Chloro-1,1,2-Trifluoroethyl Methyl Ether.

I.7.1 Introduction.

A novel approach to the problem of column conditioning has appeared in the literature (Comins, Pretorius & Schieke 1975) concerning the growth of silica whiskers on the internal wall surface. At first this method appears to be an extension of the etching solution procedure but columns produced in this way were relatively more stable and demonstrated a large increase in the openness of the column. A fluoro-ether was used to produce these whiskers.

To investigate this type of column conditioning a supply of 2-chloro-1,1,2-trifluoroethyl methyl ether, the fluoro-ether used in the above reference, was required. This compound was not available commercially. The excercise in producing sufficient quantity of this ether of suitable purity was considered to be of some benefit.

I.7.2 Method.

A method for the preparation of chloro trifluoroethyl ethers was obtained from a literature search (Fontan, Kirk, Parker & Yee 1962). This general method involved the reaction of 2-chloro-1,1,2-trifluoroethylene with the appropriate alcohol and was illustrated with several alcohols including methanol.

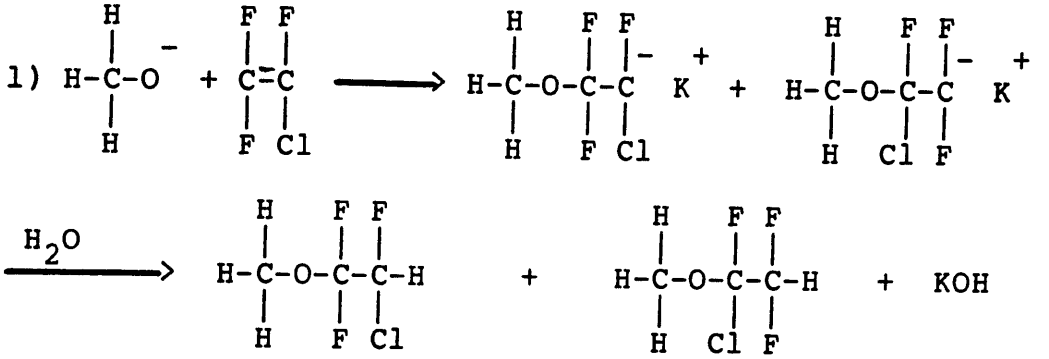
general reaction:

$$ROH + CF_2{=}CFCl + KOH \longrightarrow ROCF_2CHFCl$$

Mechanisms:

$$K^+OH^- + CH_3OH \longrightarrow CH_3O^-K^+ + H_2O$$

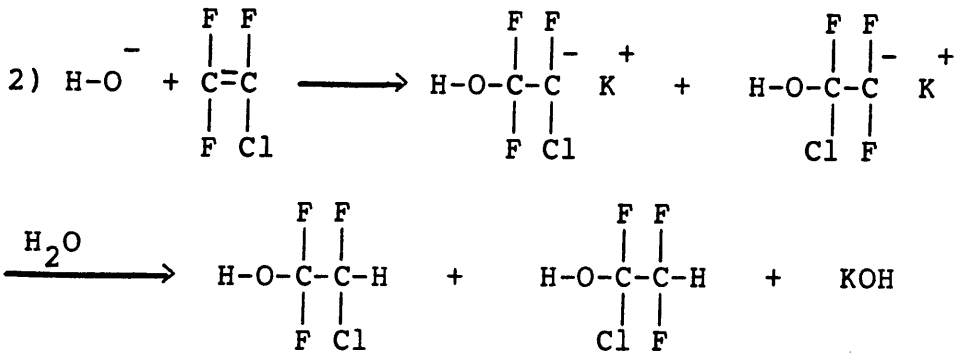
1)



Sterically preferred major product

Major impurity

2)



Minor impurities

As the methoxide ion is a stronger neucleophile than the hydroxide ion reaction mechanism 1 is preferred. These two reactions are also favoured by methanol saturated with potassium hydroxide forcing the initial production of methoxide ions.

The 2-chloro-1,1,2-trifluoroethylene, purchased as a compressed gas, was slowly bubbled through a dispersion disc at the bottom of a one metre vertical tube filled with dry methanol previously saturated with potassium hydroxide. The outlet from this tube was connected to an upright water cooled

condenser to prevent evaporation losses of the ether produced. Any unreacted 2-chloro-1,1,2-trifluoroethylene escaping from the condenser was then passed through a second vertical tube filled with dry methanol saturated with potassium hydroxide. The flow rate of the reactant gas was adjusted so that no gas bubbles left the surface of the liquid in the second reaction tube so that most of the reaction would take place in the first reactor tube. The entire process was performed in a well ventillated fume hood. A cooling bath was provided for the first reactor tube to moderate the reaction rate.

When the reaction had reached equilibrium and the temperature of the second reactor was in excess of that of the first reactor the supply of the 2-chloro-1,1,2-trifluoroethylene was turned off. The reaction was stopped by pouring the contents of both reactors into water. Excess methanol and potassium hydroxide were removed by sucessive washes with distilled water until the washes were of neutral pH. It was not possible to dry the ether as contact with silica gel results in hydrolysis to chlorofluoroacetic acid (Fontan, Kirk, Parker & Yee 1962). The crude ether was purified by glass distillation using a one metre fractionating column containing glass helices.

The purified product was analysed to confirm its identity. Ultra violet and infra-red spectroscopy, gas chromatographic analysis, nuclear magnetic resonance spectroscopy and mass spectroscopy were all performed on the ether.

I.7.3 Results.

The reaction was observed to be slow at first, but as some ether was formed the rate of reaction was increased quite rapidly with a resultant rise in temperature, leading to a further increases in the rate of reaction. A yield of 27.89 g (76.0%) was obtained for the pure product which was found to have a boiling point of 64.4^{o}C $\pm$ 0.2 ^{o}C and a density of 1.3632 at 20^{o}C.

A sample of the crude and a sample of the distilled product were chromatographed on a 2.5% FFAP on Chromasorb W 80/100 mesh packed column using using electron capture detection. The resulting chromatograms given in Figure I.7.1 show five separated impurity peaks in the crude product including some residual methanol, but only a trace of one of the peaks in the purified product.

The ultra violet absorption spectrum of the pure product is given in Figure I.7.2 and shows a maximum absorption at 277 nanometres.

The infra-red spectrum was run as a thin film between CsI plates and is given in Figure I.7.3.

Figure I.7.1 - Chromatograms of Crude and Distilled Product.

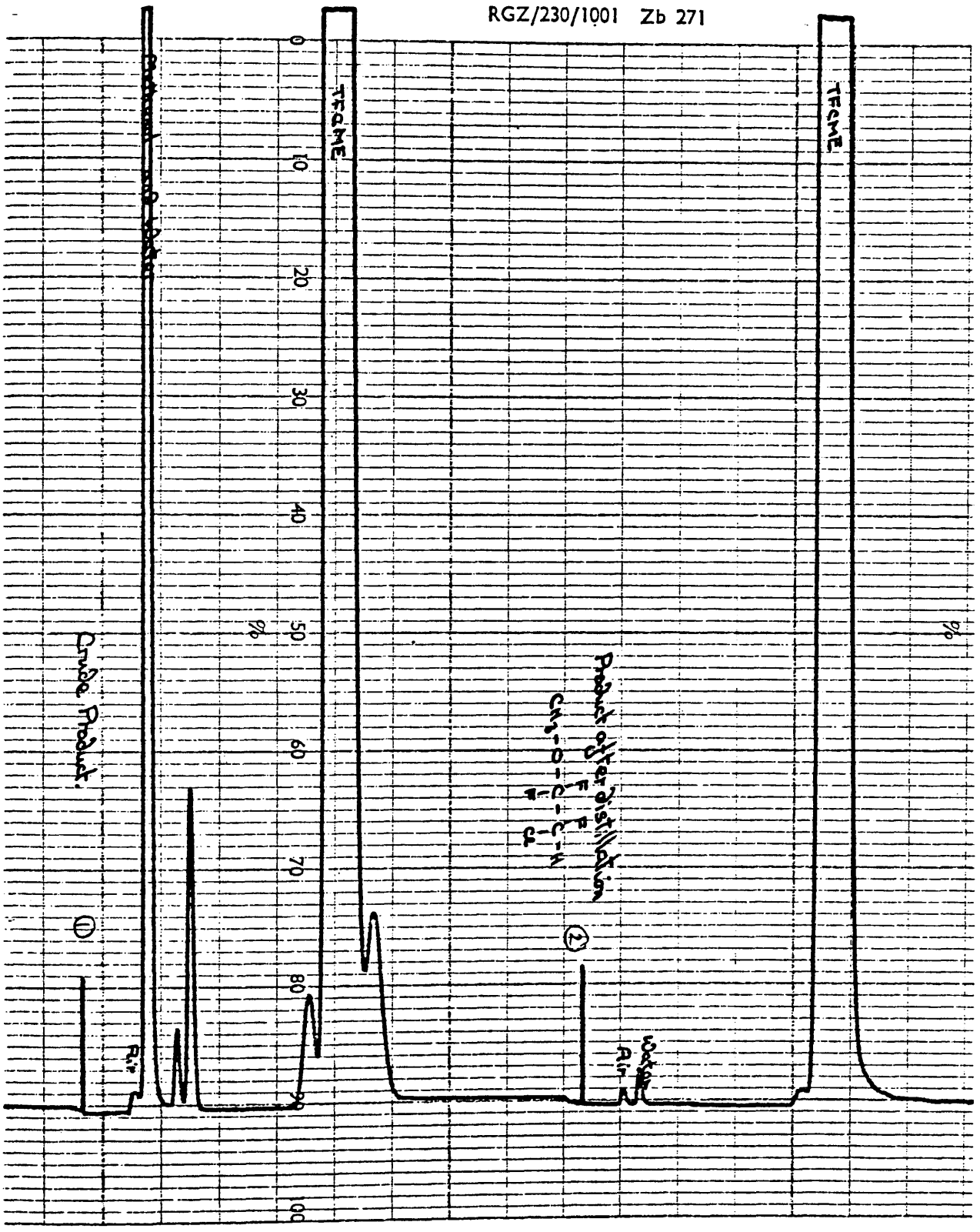


Figure I.7.2 - The Ultra Violet Absorption Spectrum of the Pure Product.

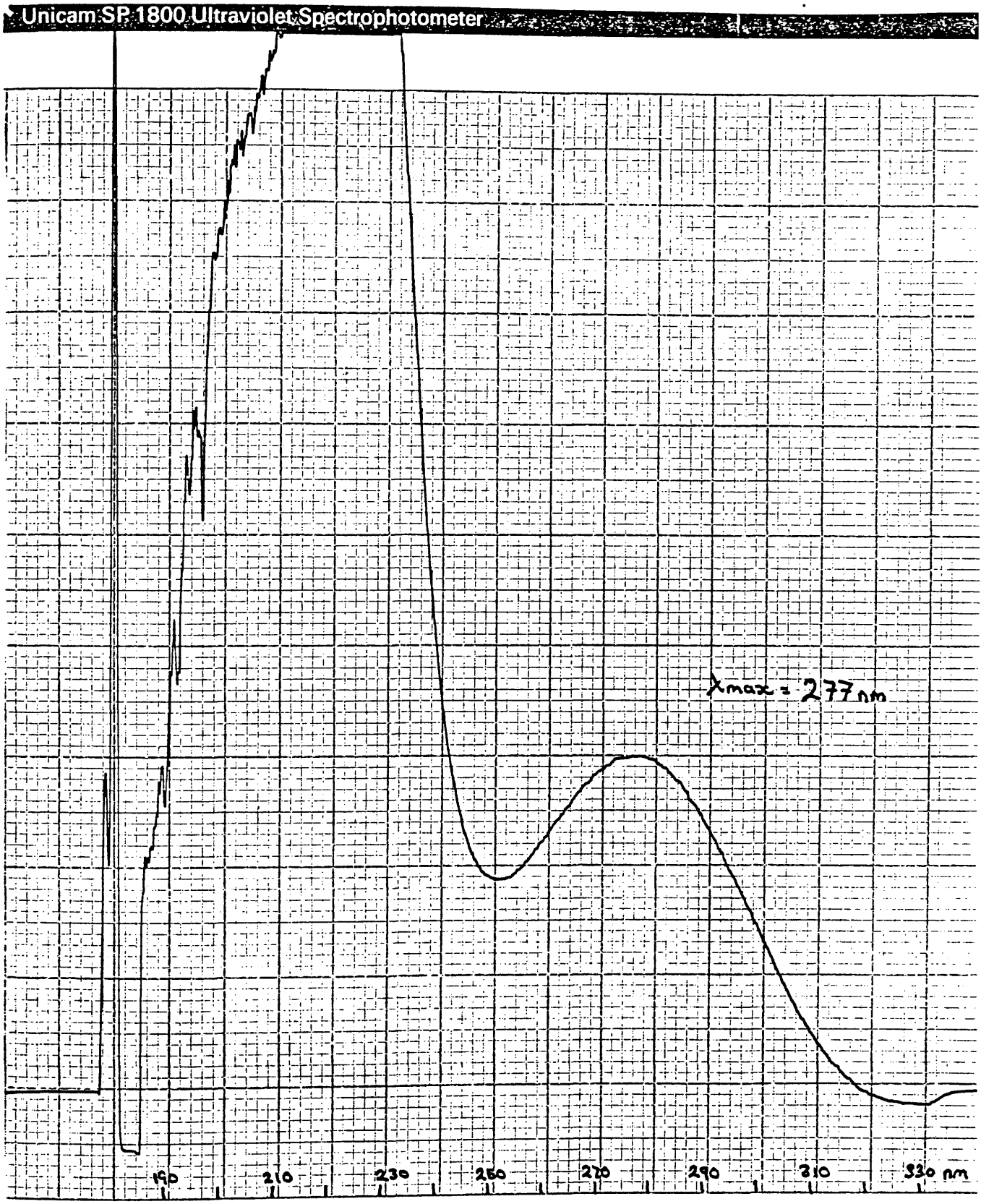


Figure I.7.3 - The Infra-red Spectrum of the Pure Product.

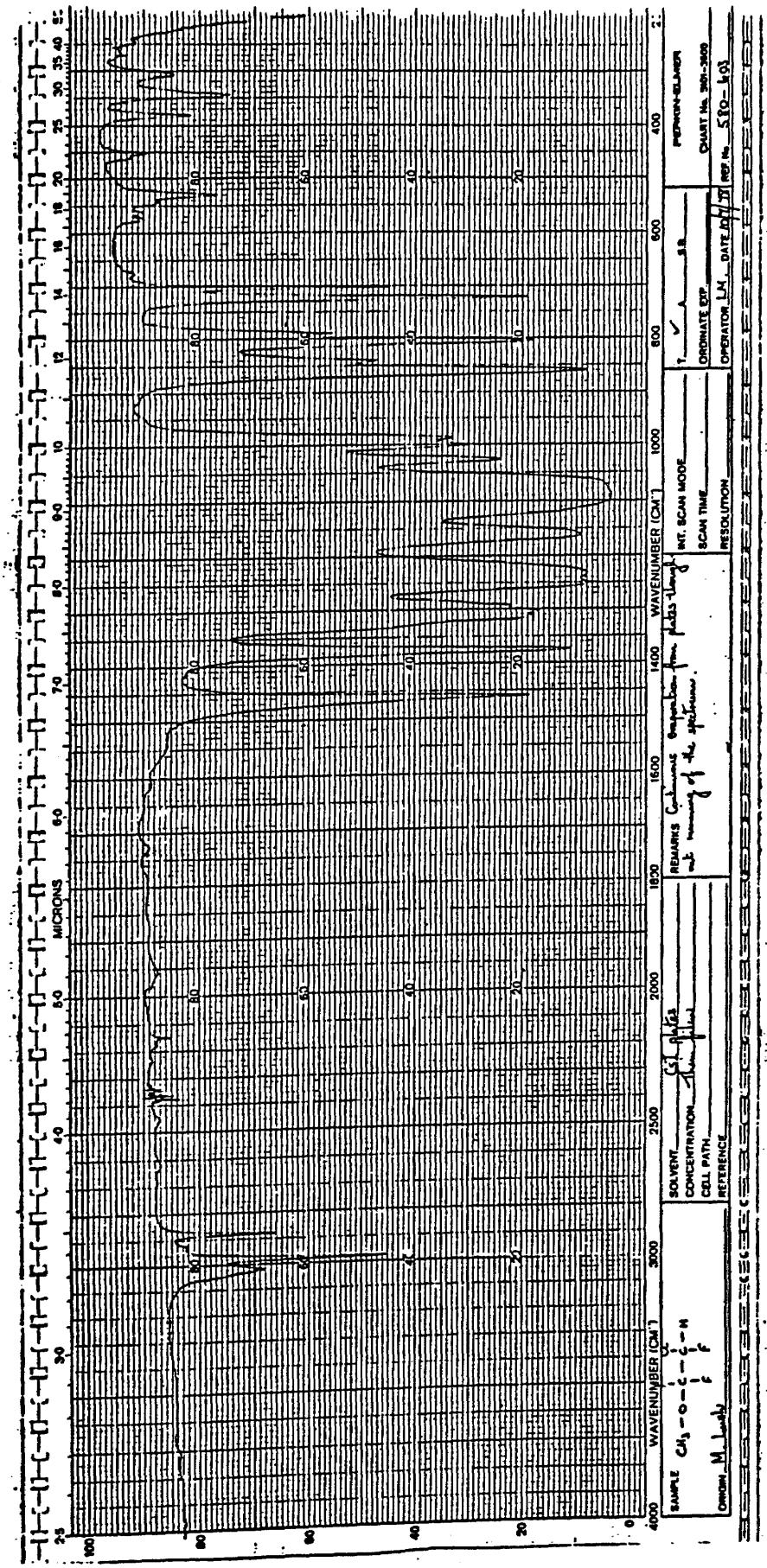


Figure I.7.4 - The High Resolution Mass Spectrum of the Pure Product.

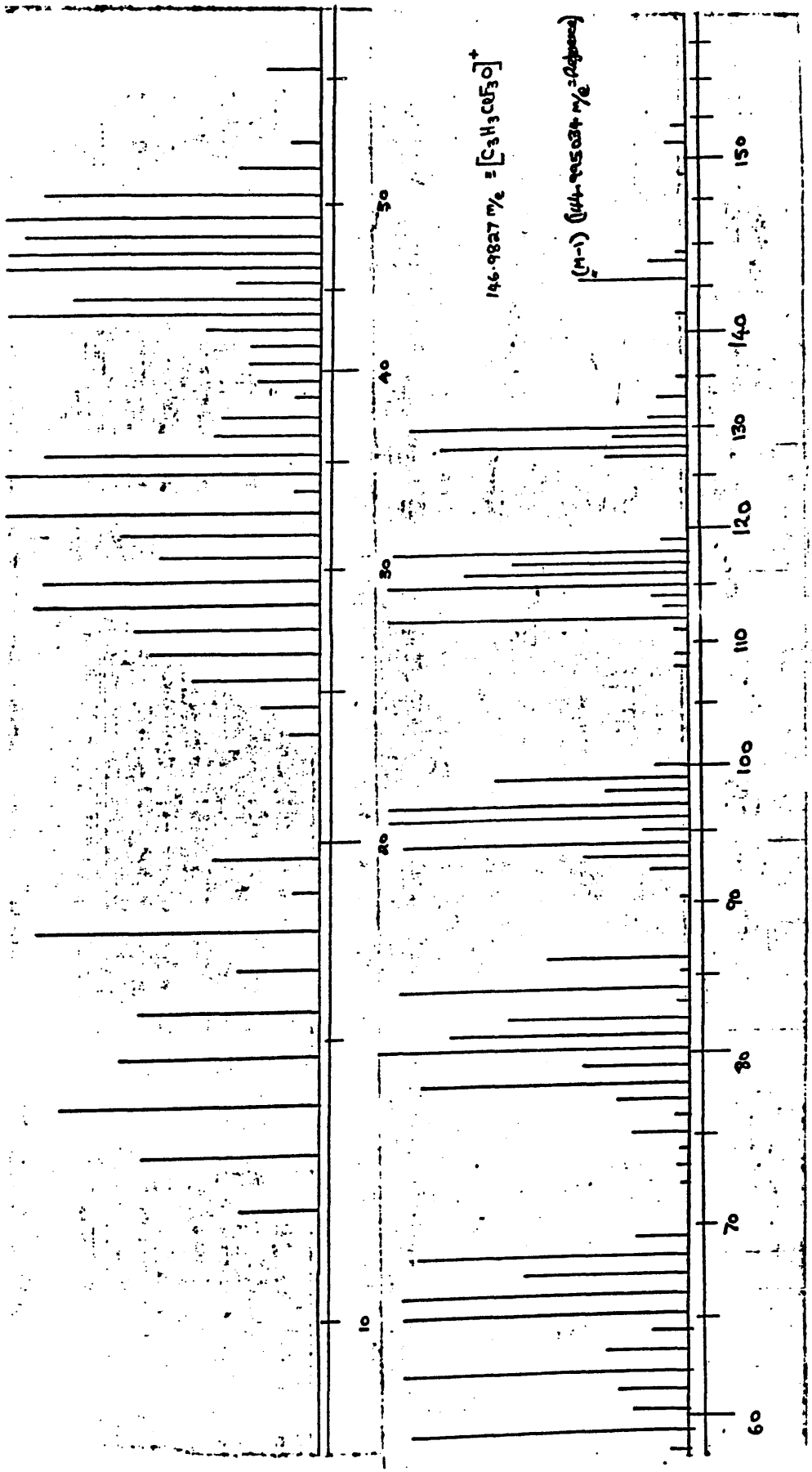


Figure I.7.5a - The Nuclear Magnetic Resonance Spectrum of the Pure Product.

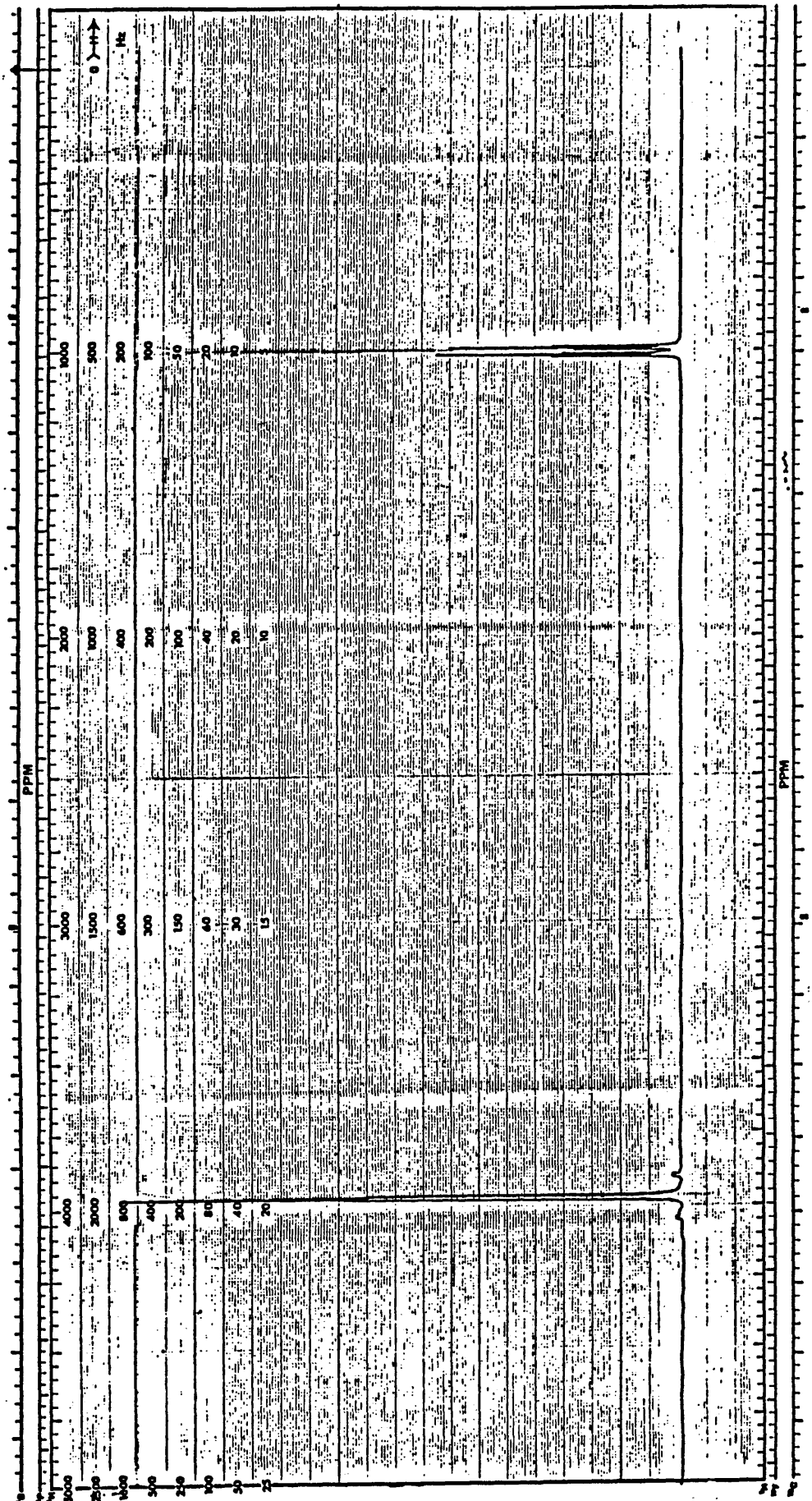


Figure I.7.5b - The Nuclear Magnetic Resonance Spectrum of the Pure Product.

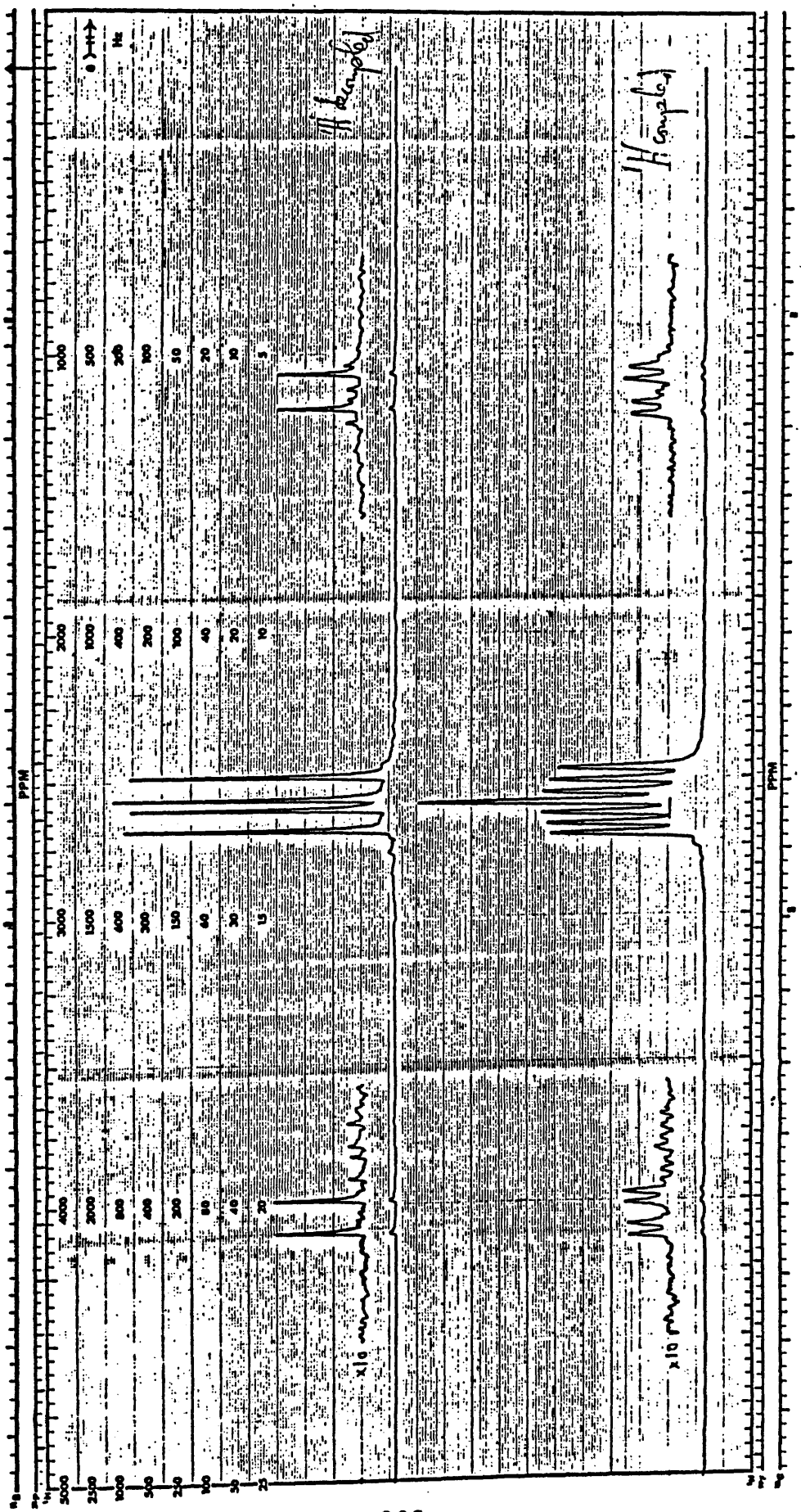


Figure I.7.5c - The Nuclear Magnetic Resonance Spectrum of the Pure Product.

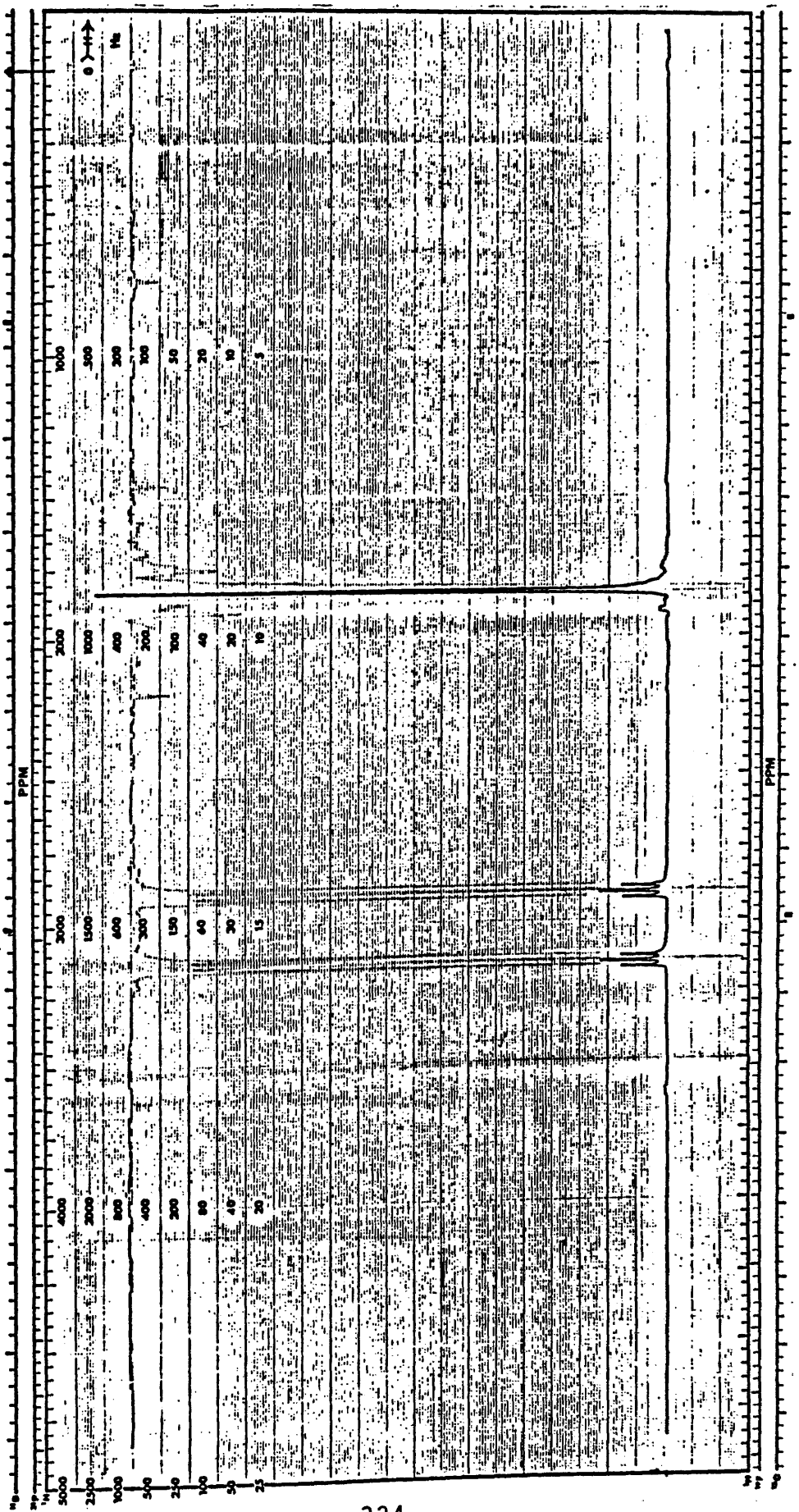
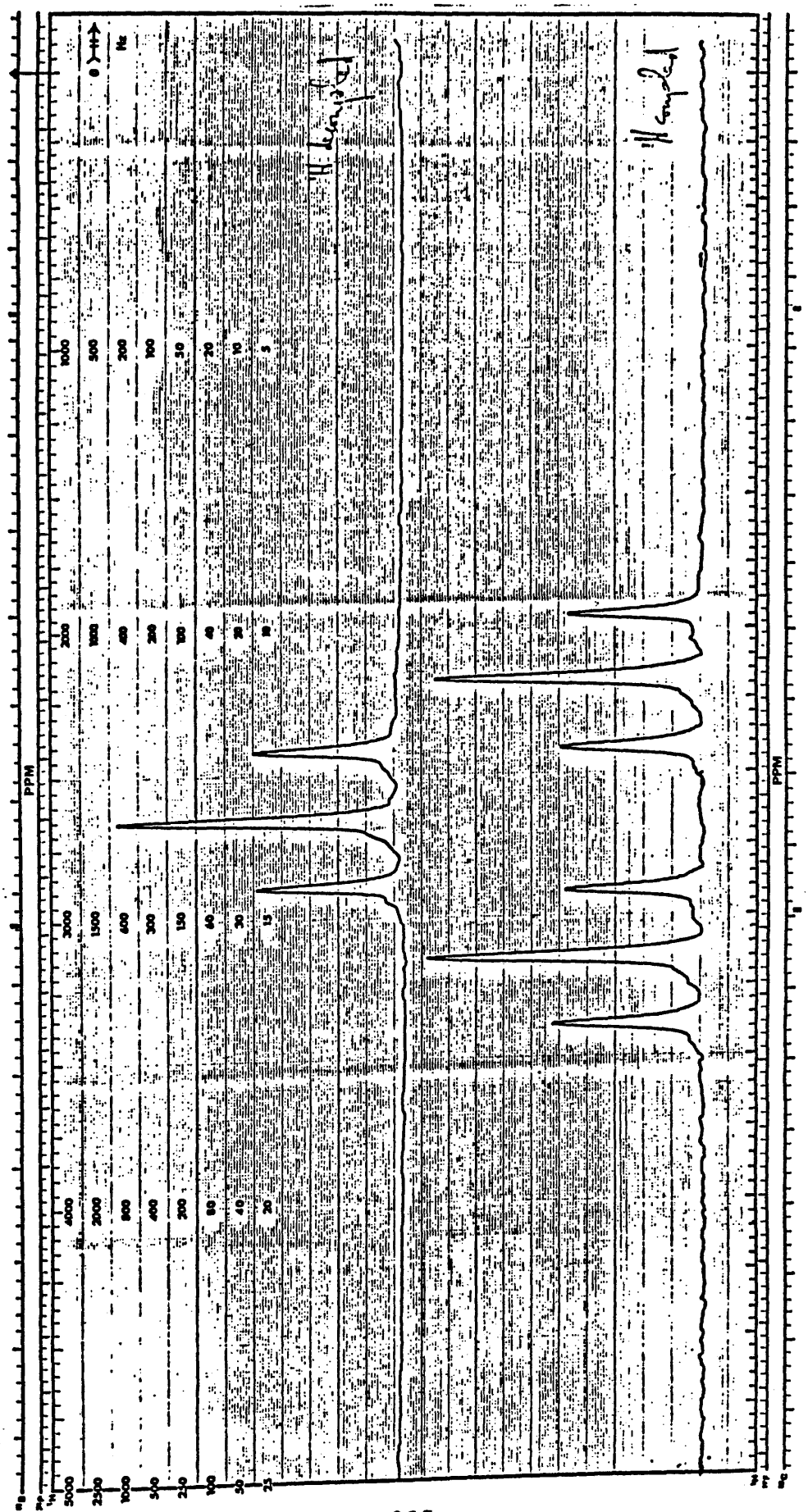


Figure I.7.5d - The Nuclear Magnetic Resonance Spectrum of the Pure Product.



High resolution mass spectroscopy, Figure I.7.4, and nuclear magnetic resonance spectra, Figures I.7.5a to I.7.5b, were obtained by courtesy of Glasgow University Chemistry Department and the results of both techniques confirm the identity of the pure product as that of 2-chloro-1,1,2-trifluoroethyl methyl ether.

I.7.4 Conclusion.

A small quantity of 2-chloro-1,1,2-trifluoroethyl methyl ether was successfully manufactured, purified by fractional distillation and its identity confirmed by spectroscopic techniques. A yield of 76% was obtained.

I.8 Preparation of a Whisker Walled Open Tubular Column.

I.8.1 Introduction.

When the 2-chloro-1,1,2-trifluoroethyl methyl ether, prepared as described in Section I.7, is decomposed in sealed silica tubing a relatively uniform layer of silica whiskers are deposited on the contact surface (Comins, Pretorius & Schieke 1975). The ether acts as a source of hydrogen fluoride. However this alone does not explain the production of whiskers as hydrogen fluoride on its own, like hydrogen chloride, produces a crystaine salt deposit. In the case of the ether decomposition silicon tetrafluoride is produced which is converted into silicon dioxide, and this in turn is then deposited in the form of whiskers, or more correctly as crystaline silica needles (Faktor & Garrett 1974).

I.8.2 Method.

A five centimetre length of glass pasteur pipette containing a teflon septum plug was connected to a glass capilary column blank, Section I.1, using heat shrinkable PTFE tubing. The air in the column was removed using a vacuum pump. After one hour the end of the tubing nearest the pump was sealed using a micro gas burner and five hundred microlitres of 2-chloro 1,1,2-trifluoroethyl methyl ether was introduced into the column through the septum. This end of the column was then also sealed in a micro gas burner.

The sealed column was then placed in an oven and heated slowly to 450°C. After twenty-four hours the ends of the column were opened and immediately flushed with dry nitrogen at 200°C. At this point the column had a brownish appearance due to the deposits of carbon from decomposed 2-chloro-1,1,2-trifluoroethyl methyl ether. This was removed by flushing the column with oxygen at 450°C for twelve hours.

The column produced was then deactivated by deposition of a thin film of Carbowax 20M as described in Section I.2 and coated with a solution of five percent Halcomid M18 in acetone by the mercury plug coating procedure as detailed in Section I.3. The column was tested for efficiency in the same way as its hydrogen chloride etched counterpart, Column No XI, described in Section I.6.

I.8.3 Results.

The results obtained from the chromatography of the series of solvents are given in Table I.8.2.

Oven Temperature 80°C Detector Temperature 200°C Injector Temperature 150°C			Column Length 25.5 m. Column Volume 4.219 ml. Column i.d. 0.459 mm.				
Solute	t	n	h	k	N	H	R
Air	494						
Diethyl Ether	5	81372	.0313	0.010	7	339.0	
Acetone	6	50415	.0506	0.022	23	110.7	0.65
Methanol	7	83736	.0305	0.024	47	54.2	0.14
Dichloromethane	11	65025	.0392	0.032	60	41.9	0.48
Ethanol	12	65178	.0391	0.033	65	38.9	0.06
Hexane	12	65178	.0391	0.033	65	38.9	0.00
Cyclohexane	18	66719	.0382	0.045	123	20.7	0.74
iso-Propanol	19	119163	.0214	0.047	243	10.5	0.17
Carbon Tetrachloride	24	121383	.0210	0.057	353	7.2	0.82
Benzene	24	68278	.0373	0.057	198	12.8	0.00
n-Propanol	34	92694	.0275	0.078	481	5.3	1.48
Chloroform	37	93741	.0272	0.084	559	4.6	0.42
Trichloroethylene	41	95216	.0268	0.092	679	3.8	0.57
2-Butanol	45	73875	.0345	0.100	605	4.2	0.49
M.I.B.K.	48	97558	.0261	0.106	889	2.9	0.42
Toluene	55	76840	.0332	0.121	900	2.8	0.93
n-Butanol	88	86084	.0296	0.187	2134	1.2	4.08
Xylene 1	113	122300	.0209	0.238	4515	0.6	3.60
Xylene 2	127	98094	.0260	0.267	4356	0.6	1.79
Key: As for Table I.6.2							

Table I.8.2 - Retention Parameters and Column Operating Conditions for the Whisker Wall Coated Open Tubular Column.

I.8.4 Discussion.

Whisker walled open tubular columns require an exacting column conditioning stage and are therefore both difficult and time consuming to manufacture. The resulting column was found to be more efficiently coated with stationary phase as predicted but for the purpose of solvent separation this particular column did not perform much better than the comparable hydrogen chloride etched column.

I.8.5 Conclusion.

The 2-chloro-1,1,2-trifluoroethyl methyl ether produced in the previous section was used to manufacture a whisker walled open tubular column for comparison with the columns already examined. The column obtained was shown to have an improved coating efficiency but showed only a small improvement on the wall coated open tubular column in the separation of solvent mixtures.

The assumption was made that the chemical etching and silica deposition process had occurred within the column manufactured and that the projected whisker formation had not been disturbed or destroyed by the deactivation and coating procedures.

I.9 Examination of the Internal Surfaces of Etched Capillaries using Scanning Electron Microscopy.

I.9.1 Introduction.

In Section I.8 a specialised type of column conditioning was performed, which like the more standard method of hydrogen chloride etching, produces a microcrystalline structure on the inner surface of the glass tube that is not evident by direct visual examination. The prime factor in the determination of overall open tubular column performance has been shown to be surface etching and stationary phase coating efficiency. Preparation of two identical columns is impossible. The many variables over which there is no satisfactory control result in extremes of variation occurring even along the length of an individual column.

Some method of quality control is therefore required over the conditioning process. Microscopic examination using scanning electron microscopy has become the technique which is regarded as the most informative (Alexander & Rutten 1974, Comins, Pretorius & Schieke 1975).

I.9.2 Method.

Column Preparation.

Three types of column were prepared and coated for individual examination by scanning electron microscopy representing the

extremes of surface modification which may be applied.

1) Hydrogen chloride etching method involving the removal of alkali metal silicates from the surface of the glass and deposition of a random distribution of fine crystalline chloride salts over the surface (Cronin 1974). As described in Section I.2.

2) Silica whisker growth induced by the decompositiopn of a fluoroether such as 2-chloro-1,1,2-trifluorethyl methyl ether a described in Section I.8.

3) Potassium difluoride etching producing a combination of the whisker growth and crytalline deposits of the above two methods. A column blank was washed with a five percent solution of the potassium difluoride in dry methanol, filled with the same solution and allowing to stand for twelve hours. The column was then flushed and dried in a stream of nitrogen and finally sealed at both ends using a micro flame. The column was heated up to 400^{o}C for one hour, slowly cooled and flushed with dry nitrogen at 100^{o}C for twenty-four hours.

Preparation of Samples for Scanning Electron Microscopy.

It is not possible to examine the surface of glass directly with scanning electron microscopy owing to its lack of conductivity. The surface therefore has to be first coated with layers of carbon and gold to eliminate charging effects and to facilitate scanning.

As the columns were inherently fragile, in particular to mechanical shock, they could not be broken open before conductive coating. An attempt was therefore made to view the column surface through an open end. To accomplish this short lengths of the column, approximately five millimetres, were mounted vertically on a scanning electron microscope stub using aluminium paint. The stub was rotated in a vaccuum chamber while being given a fine coating of gold using an argon arc source. However, the carbon and gold particles emitted during the vaccuum coating process essentially travel in straight lines and are therefore unable to evenly shadow the heavily textured inner surface of the column. This problem was to some extent overcome by subjecting the column fragments to the vapour from a 0.1 percent aqueous solution of osmium tetroxide for twenty-four hours at room temperature prior to conductive coating. The vapour penetrates and coats the surface and a layer was provided for further coating of the carbon-gold film. The stub was then placed in the scanning electron microscope. The operating vaccuum was applied, the electron gun activated at 1200 Kilovolts and scanning commenced. Since this particular type of specimen provides a difficult target, focusing the electron beam became increasingly difficult at higher magnifications due to charging effects.

I.9.3 Results.

The three photomicrographs presented in Figure I.9.1 were taken at low power magnification (x100, x150, x200) of the

inside of a hydrogen chloride etched column. The clearly formed crystals of sodium chloride can be seen randomly dispersed over the surface. The number of crystals appears to be few but it is thought that this was an artifact of the process of breaking open the capillaries.

The photomicrograph in Figure I.9.2 was taken, at a much higher magnification (x100,000), of the surface of the capillary between the crystals on a hydrogen chloride etched column. It clearly shows the surface etching and roughening which contributes to improved coating efficiency.

The photomicrograph in Figure I.9.3 shows the surface (magnification x1000) of silica whiskers grown on the surface of a capillary by decomposition of a fluoro-ether. The whisker growth appears to be relatively uniform. Even under greater magnification (x10,000), Figure I.9.4, the original surface of the glass cannot be observed. The problem of viewing the whiskers from the side is thought to result from the fact that the surface between these whiskers had not been completely cleaned of the carbon residue from the decomposition.

An attempt was made to remove this carbon layer by placing a stub with column fragments mounted on it in an oxygen enriched atmosphere at 300^{o}C. The resulting stub was then coated with gold in the usual way before examination. The photomicrograph (x10,000), Figure I.9.5 shows the branched structure of the whiskers.

The photomicrograph in Figure I.9.6 shows the surface (magnification x1000) of the silica flakes and crystals produced by the potassium difluoride method. The flakes become clearer at increased magnification (x10,000), Figure I.9.7, and when they are viewed at an angle (x10,000) Figure I.9.8.

The photomicrograph in Figure I.9.9 represents a x1000 magnification of the surface of a hydrogen chloride etched column coated with Emulphur 0N870 stationary phase by the mercury plug dynamic method. The sodium chloride crystals have dissappeared and the agrregation of the stationary phase into droplets can be clearly seen.

Figure I.9.1 - S.E.M. Photomicrographs of Crystal Deposits Produced by Hydrogen Chloride Etching (magnification x100, x150, x200).

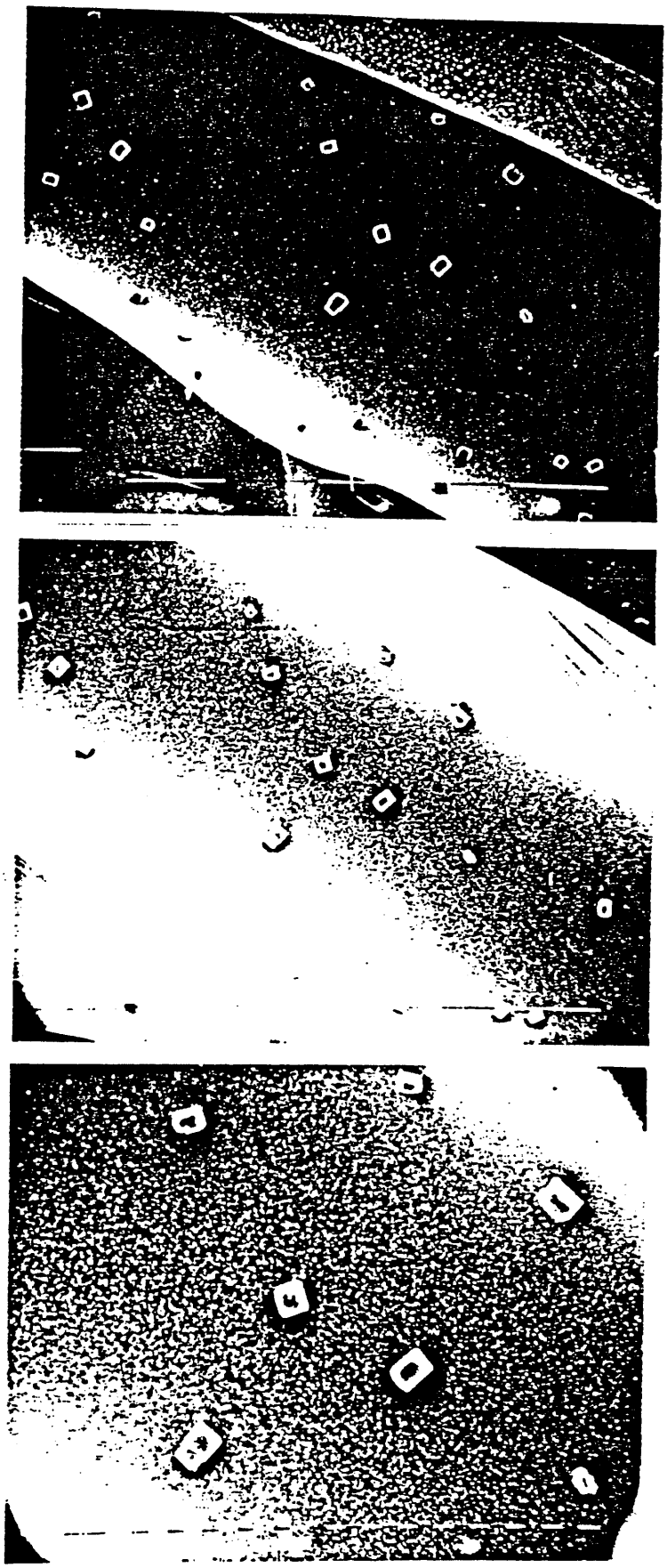


Figure I.9.2 - S.E.M. Photomicrograph of Capillary Surface Etched with Hydrogen Chloride (magnification x100,000).

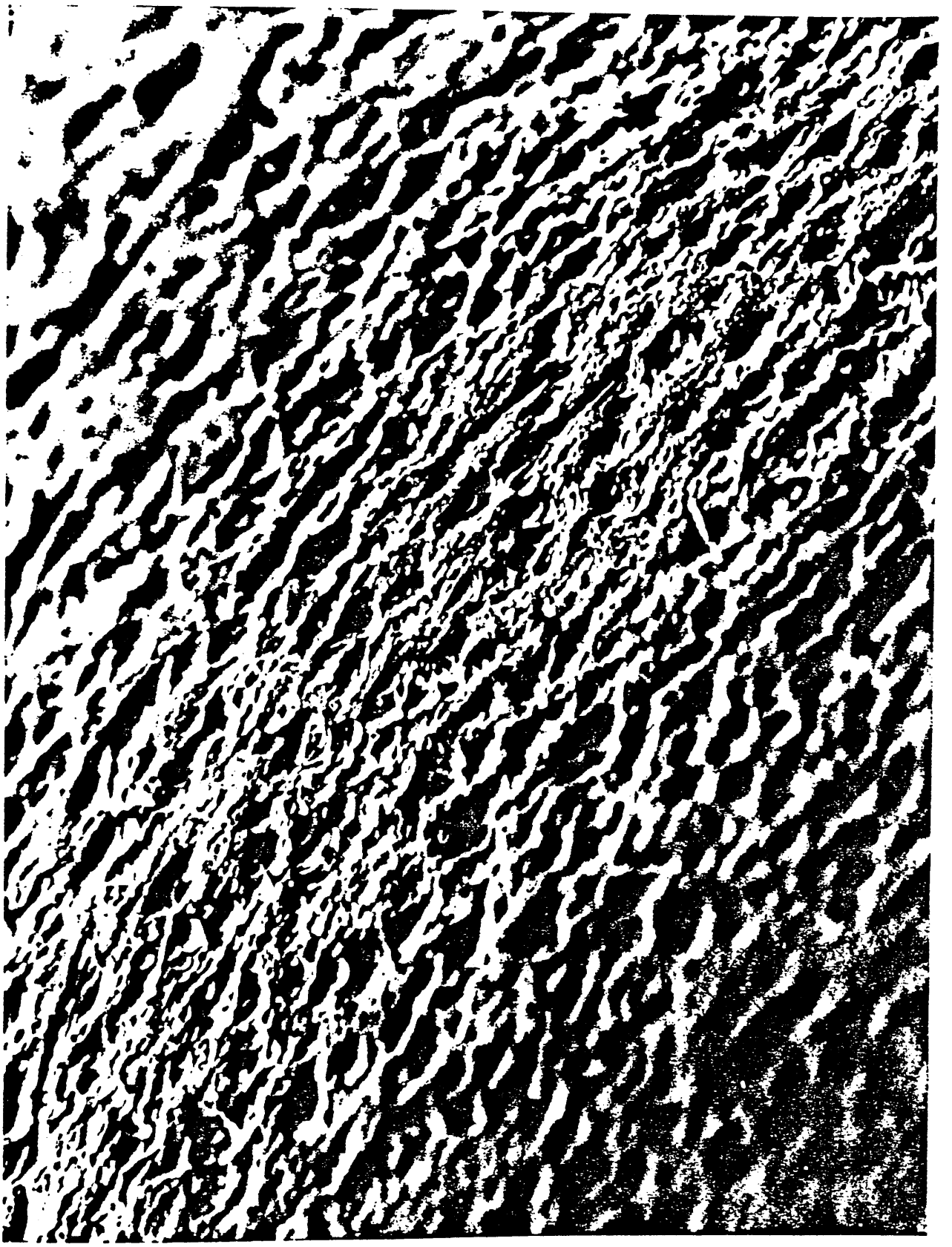


Figure I.9.3 - S.E.M. Photomicrograph of the Surface of Silica Whiskers Produced by Decomposition of Fluoro-ether.(magnification x1000).

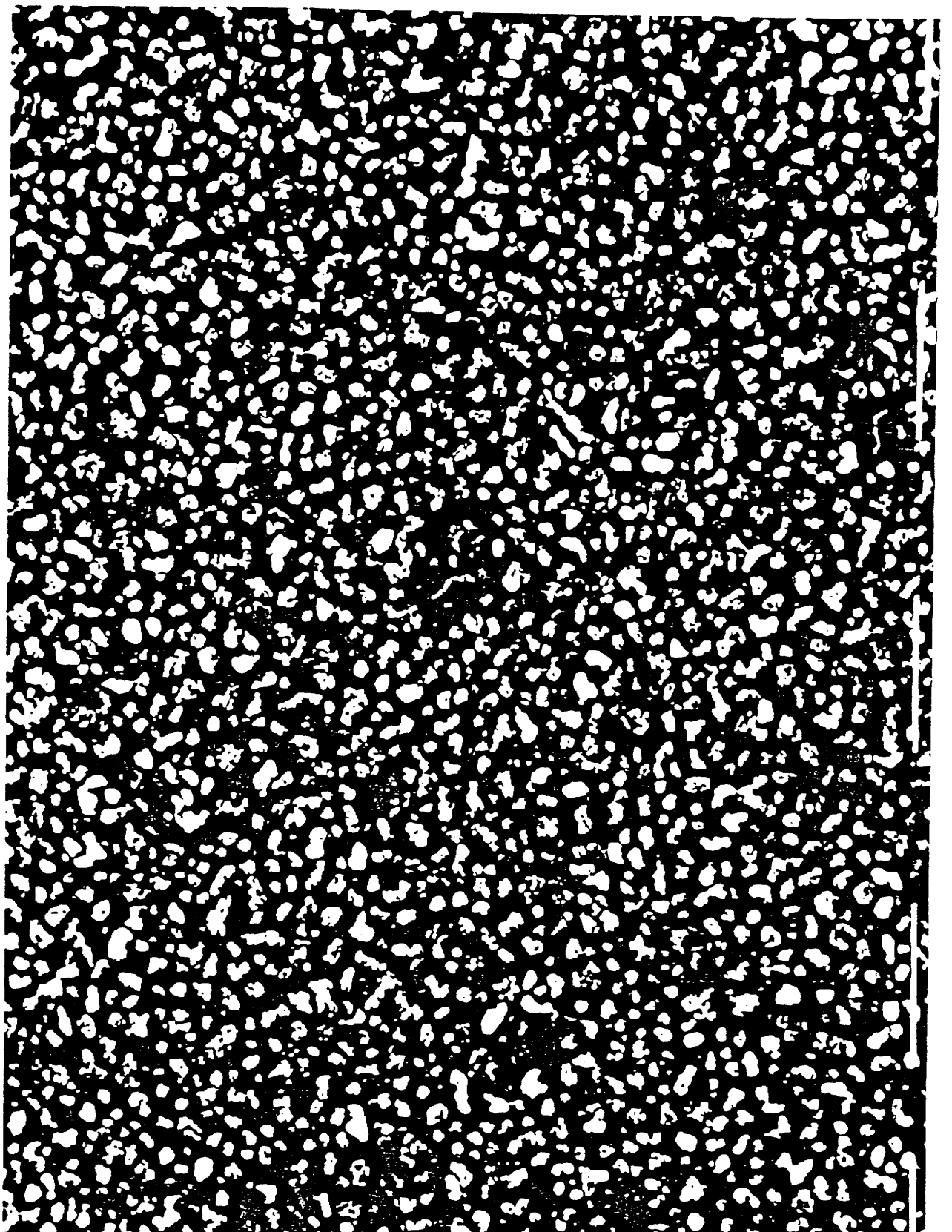


Figure I.9.4 - S.E.M. Photomicrograph of the Surface of Silica Whiskers Produced by Decomposition of Fluoro-ether (magnification x10,000).

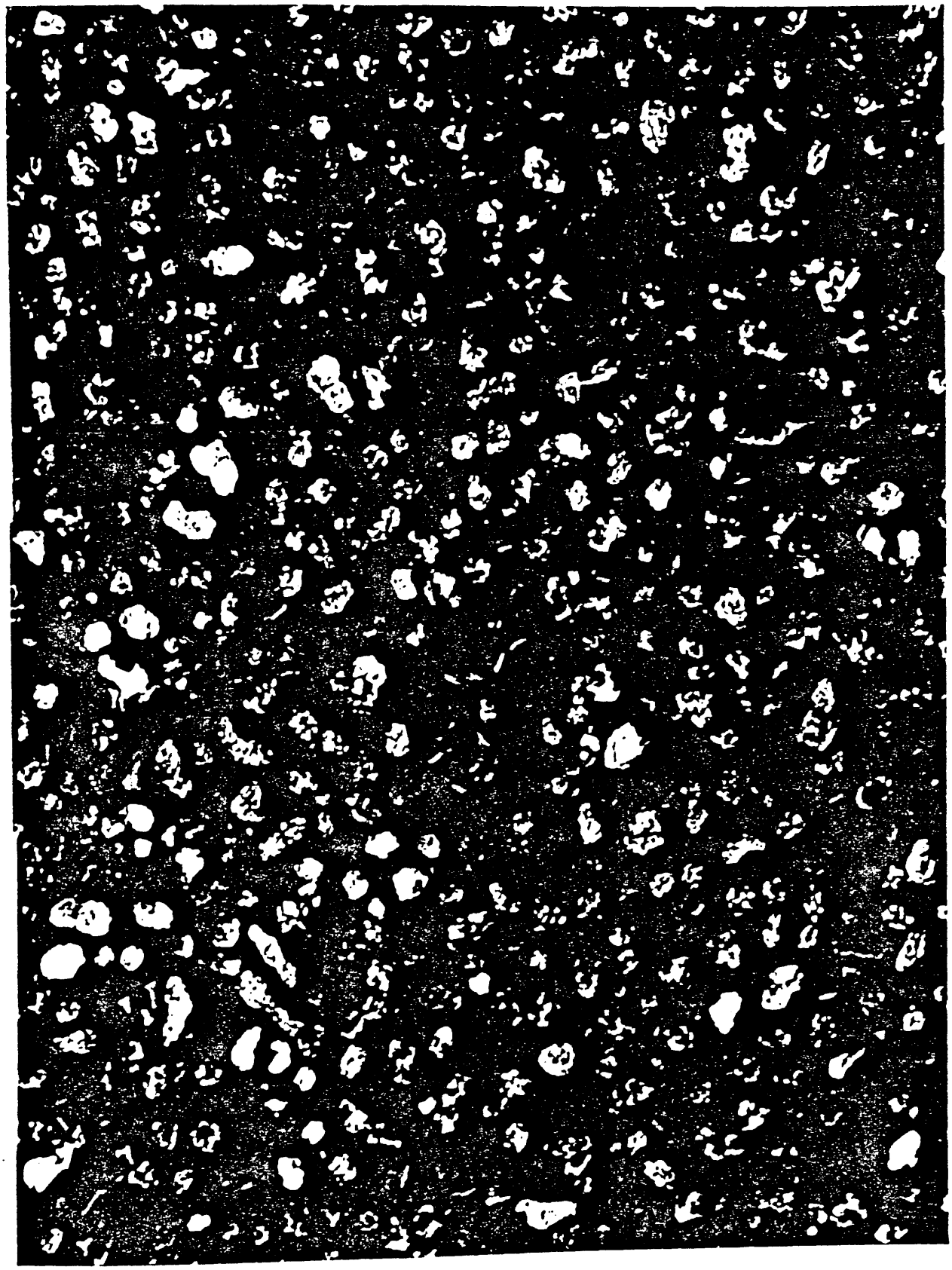


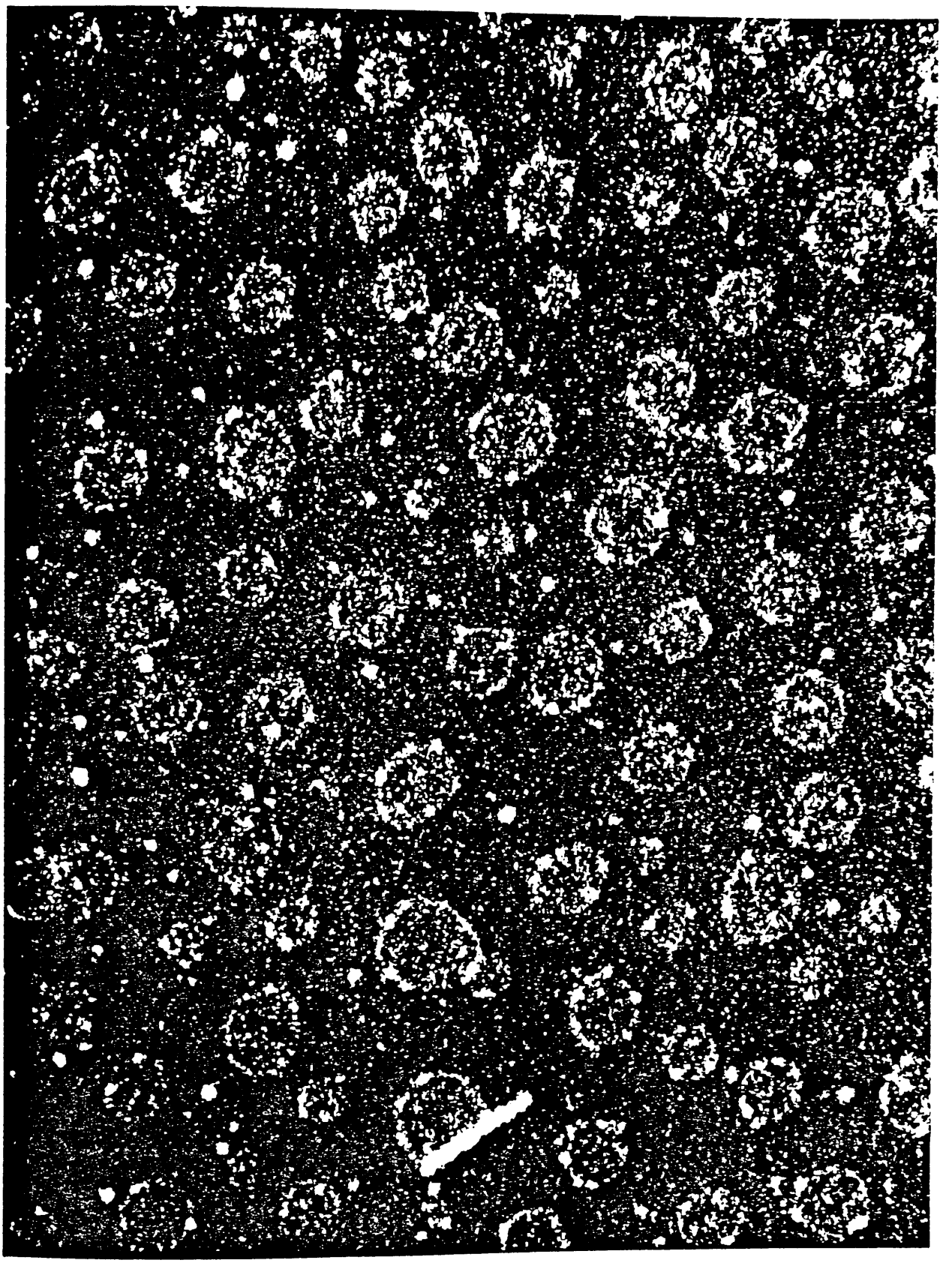
Figure I.9.5 - S.E.M. Photomicrograph of Silica Whiskers after Heating in Oxygen Enriched Atmosphere to Remove Carbon Deposits (magnification x10,000).

Figure I.9.6 - S.E.M. Photomicrograph of Silica Flakes and Crystals Produced by Potassium Difluoride Etching (magnification x1000).

Figure I.9.7 - S.E.M. Photomicrograph of Silica Flakes and Crystals Produced by Potassium Difluoride Etching (magnification x10,000).

Figure I.9.8 - S.E.M. Photomicrographs of Silica Flakes and Crystals Produced by Potassium Difluoride Etching - Angular View (magnification x10,000)

Figure I.9.9 - S.E.M. Photomicrograph of the Surface of a Hydrogen Chloride Etched, Emulphur ON870 Stationary Phase, Mercury Plug Dynamic Coated Column (magnification x1000).



I.9.4 Discussion.

1) The density of the crystalline deposits in hydrogen chloride etched columns is dependant on two factors: the temperature at which etching is performed, and the alkali metal content of the surface of the glass. The first of these factors can be experimentally controlled but the second is dependant on the batch to batch variation of the glass and any cleaning procedures employed. The resulting surface is extremely fragile, the location of the crystals not fixed and is very sensitive to water vapour. Mechanical shock may disturb the position of these crystals even to the extent of permitting them to be blown from the column. Water vapour allows conditions for crytal growth resulting in a few large crystals instead of many smaller ones. Crystal growth should theoretically be perfectly uniform under the conditions used but the presence of even small quantities of water vapour can be assumed from the observation of deformed crystals.

2) The resulting appearance of the column surface in whisker wall columns should be one of an even distribution of whiskers composed of silica. These whiskers are both mechanically stable and insensitve to water vapour. However as the mechanism for their growth is quite complex their formation is very temperature sensitive and even minor fluctuations in temperature during their manufacture can cause the formation of a variety of deformations. The most typical of which is a lattice work appearance produced by axial growth of the normally radially growing silica whisker. As the

formation of these whiskers is dependant on the decomposition of the fluoro-ether it is important that the ends of the whisker remain pure and in contact with the fluoro-ether molecules. This introduces another variable, that of the ether concentration, which can alter whisker growth. Too low a concentration and the whiskers become stunted in appearance being short and often tapering to a fine point rather than the more normal cylindrical shape. Too high a concentration and the whiskers become coated in the carbon produced by the decomposition of the fluoro-ether and assume a spherical appearance. The complex whisker structure acts as a honeycomb for the deposition of stationary phase increasing the true suface area by many magnitudes with only an insignificant loss in the openness of the column. With this also comes the improved stabilisation of the stationary phase and therefore increased column life.

3) The appearance within the column after potassium difluoride etching is one of crystalline sheets or flakes. The mechanism for their production is obscure but it is thought that silica growth occurs on or around crystalline deposits of the difluoride salt resulting in a dispersed lattice of potassium fluoride and silica. These sheet crystals are quite robust and are more resistant to mechanical shock than the chloride crystal deposits described for hydrogen chloride etching. However they remain susceptible to damage by water vapour resulting in a sponge-like appearance. As the surface is layered it does not represent so great an increase in surface area as whiskers and as the surface is

less uniform the efficiency of the column would be less. This surface still represents a significant improvement over the hydrogen chloride etched surface.

I.9.5 Conclusion.

Scanning electron microscopy represents the ultimate tool in the quality control of column manufacture. Its use is both specialised and subject to many artifactual errors. For this reason it can be suggested that the tool is limited to research applications such as in the example given in this section by the comparison of three different column etching techniques.

The photomicrographs obtained in this study clearly demonstrate that the method of choice for increasing surface area must be the method of whisker growth. Though other parameters must be considered; ease of manufacture, coating efficiency, column stability and activity, when judging if this type of etching produces best analytical performance.

I.10 Conclusion.

Manufacture of an efficient wall coated open tubular column is a complex and speciallised process far removed from the simple packing of the short and comparitively wide bore packed column routinely used in gas chromatography. In this appendix an attempt has been made to illustrate experiences relating to the several aspects of their manufacture and to use this to gain some experience in their use.

Columns were manufactured from basic glass tubing using a speciallised glass drawing machine designed specifically for the production of capillary blanks. The type of glass selected was of standard soda glass but it is recognised that there are many different grades of glass available and these too would have to be considered in any extensive research in this field.

Several different treatments of the glass surface in preparation for coating, including a speciallised treatment with a fluoro-ether to produce a whisker growth, and three possible methods of coating were also compared resulting in the conclusion that the best method of surface treatment is that of whisker growth and the best method of coating is the static method.

Methods of determining the efficiency of the manufactured columns were also examined from the simple test of theoretical

plate height to the possibility of using a scanning electron microscope to examine the actual physical dimension of the glass surface.

Several of the columns produced showed good separation of the solvents examined and in fact most of them would produce higher selectivity than the packed columns examined in Chapter Two for the resolution of abused solvent mixtures.

APPENDIX II

SHEEP DRENCH DEATHS - APPLICATION OF QUALITATIVE MEASUREMENT OF SOLVENTS TO A CASE NOT ASSOCIATED WITH THE ABUSE OF VOLATILES.

II.1 Case History.

A sheep farmer of Glendale in Skye purchased a supply of fluke and worm drench during 1976 and in the following April proceeded to drench fifteen rams from his flock. As a direct result of the drenching seven of the rams died. A sample of the drench used was subsequently submitted by the solicitors acting on behalf of the farmer to the Department of Forensic Science and Medicine laboratory at Glasgow University for investigation. This sample was accompanied by a request for the chemical formulation of the drench and comment on any analysis. There was also a suggestion that the drench had been retained for too long or under unsuitable conditions.

One anomaly which later arose out of the drenching was that although seven beasts died as a result, five wedders subsequently slaughtered showed that the same drench used on them had no beneficial effect. In fact on opening up the beasts the livers of all five were unusable because of fluke infestation.

II.2 Product Information.

The data sheet for the product indicated that it was comprised of a clear green oily liquid containing approximately twenty per cent by volume carbon tetrachloride of a standard which complies with the British Vetinary Compendium.

The drench is recommended for use in the control of liver fluke and hookworm (Bunostonum) in sheep, and is also claimed to give some control of the stomach worm (Haemonchus).

The recommended dosage for flukes in both lambs and sheep is five millilitres and for roundworms ten millilitres in lambs of six to twelve months old and fifteen millilitres in adult sheep. These doses are to be given orally as a drench as early in the day as possible, preferably before midday. The first dose for all ewes being given during September and the second dose one month later, about two to four weeks before tupping. A further dose one to two weeks after tupping is recommended with the fourth dose a month later. Instructions are specifically given not to drench while the ram is with the ewes although no reasoning is given as to the exact reasons for this contraindication. In general, according to the date of tupping, the dosage regime consists of four doses at about one month intervals.

Some specific contraindications to this form of drenching do exist. In particular dipping and drenching on the same day should be avoided and sheep in high or fat condition should

not be dosed with this particular product. It is also recommended that sheep should not be dosed when a sudden change of weather to cold and wet seems imminent and if this is unavoidable the sheep should be restricted to a sheltered paddock for a few days following dosing. However the most important warning is that sheep fed on concentrates, oil containing foodstuffs and certain vegetable rations ie. kale, crushed beet tops, clover or mangolds, are liable to be unduly susceptible to the toxic effects of carbon tetrachloride.

It is even suggested that on every occasion before treating a flock with this product that a trial dose be carried out on some of the best and poorest animals. After watching these dosed animals for forty eight hours and it is observed that they appear to tolerate the dose then it should be safe to dose the rest of the flock within one week. The sheep need not be fasted before dosing with this product but as implied above if they are feeding on roots or trough rations, these should be withheld for at least a day, preferably a week, before dosing. Indeed if possible the flock should be fed on pasture or hay only during the dosing period.

Despite these warnings and precautions sheep have been known to show an idiosyncratic susceptibility to normal doses of carbon tetrachloride. Adverse symptoms have included loss of appetite, depression and diarrhoea, with death, occasionally within twelve to twenty-four hours of treatment. However in the majority of cases sheep can normally tolerate large overdoses of carbon tetrachloride, the product remaining an

acceptable formulation for the treatment of these common infestations.

II.3 Carbon Tetrachloride Toxicology.

The main medical use of carbon tetrachloride is in the treatment of Fasciola Hepatica infestations in sheep. Even in this respect it is of no value against immature flukes in normal doses and is not effective at all against Dicrocoelium Dendriticum (Brander & Pugh 1977).

Carbon tetrachloride is absorbed slowly from an oral dose but the presence of vegetable or animal fats and oils will increase the absorption rate. Once absorbed and circulating, the drug is excreted at various points: the lungs; the kidneys; but mainly through the liver. The biliary system returns the active drug metabolites to the intestines, and in so doing the mature and nearly mature adult flukes in the bile ducts are destroyed. The immature flukes in the liver tissues are unaffected and consequently monthly dosing is usually recommended.

Flock susceptibility is known to be responsible for frequent losses in sheep where dosing with carbon tetrachloride is not adequately controlled. However, in Britain, the use of mixtures of carbon tetrachloride with "soft" paraffin containing a mild local anaesthetic has been widely used in sheep and a number of different commercial licensed veterinary products exist.

The toxicity of carbon tetrachloride depends to a considerable extent on the cleavage of the carbon to chlorine bonds, a necessary process in the formation of the active metabolites. It is therefore of no suprise that toxic effects are not uncommon in sheep treated in this way. However under various circumstances this mild toxicity may be aggravated, debilitated animals in particular are more susceptible, though since the fluke or worm infestation causes debility it is difficult to avoid treating debilitated animals. High protein diets or calcium defficiency also accentuate carbon tetrachloride poisoning. The hypocalcaemia which is sometimes observed as a consequence of treatment might be in part due to the accumulation of calcium in the liver as a function of the metabolic process. Undoubtably the early treatment of the problem and the strict maintenance of dietry control is essential in providing best management and highest probability of a sucessful outcome (Alexander 1969).

II.4 Analytical Procedure.

The sample of green fluid supplied for analysis was subjected to an unbiased analysis to ascertain the identity of its constituents. Considering the lack of available information on the product formulation at the time of receipt and the urgency with which a result was required it was decided that the sample should be subjected to qualitative analysis by the methods described in the preceeding chapters of this thesis.

To assist in the dissemination of the results a control sample of the proposed drench was obtained by courtesy of The Wellcome Foundation and a similar comparative analysis was performed.

The samples were subjected to gas chromatography using a Pye 104 instrument equipped with a flame ionisation detector. Oxygen free nitrogen was used as a carrier gas with a flow rate of forty millilitres per minute measured at the detector under ambient temperature. Half microlitre samples were injected and chromatographed on a glass column (1.5m x 6mm) packed with Tenax G.C. and the retention times of each solute were measured using a stopwatch. The samples were chromatographed at one hundred and eighty degrees and repeated in triplicate, the retention times being calculated as the mean of the three. The identities of the component peaks were evaluated from retention index parameters.

The samples were also chromatographed on a similarly equipped instrument connected to a V.G. Micromass 16F mass spectrometer as a detection system. Helium was used as carrier gas at a flow rate of thirty millilitres per minute. The identities of the component peaks were thus confirmed from their mass fragmentograms.

The composition of the control and suspect samples was then determined using the flame ionisation detector and comparison of peak areas with those obtained for standards of known concentration.

II.5 Results.

Both the suspect and control samples appeared to have the same composition and each gave three peaks on both chromatographic systems. From the retention index tables one of these peaks was identified as carbon tetrachloride. This identity was confirmed from the mass fragmentogram of this peak. The other two peaks could not be identified from the retention index data.

These other peaks were tentatively identified by mass spectroscopy as liquid paraffin and an aliphatic ester with a molecular ion of 116 m/e. From additional information supplied by the manufacturers this was subsequently identified as n-butyl acetate.

Semi-quantitation of the samples against standards of known concentration is given in Table II.1.

The low retention time for the liquid paraffin is misleading as the peak obtained was small in proportion to the other peaks and was due to a very volatile fraction of the liquid paraffin. The majority of this constituent was being retained by the column due to its high molecular weight and eventually caused a strong bleed from the column. This had to be removed before the column could be used for other work and which was achieved by conditioning the column at 350^{o}C for sixteen hours.

	Carbon Tetrachloride	n-Butyl Acetate	Liquid Paraffin
Retention Time (sec)	204	456	156
Suspect Sample (v/v)	16.0	0.37	83.63 +
Control Sample (v/v)	18.2	0.40	82.40 +

+ by difference

Table II.1 - Composition of Suspect and Control Sheep Drench Samples.

II.6 Discussion.

The slight decrease in the concentration of carbon tetrachloride in the suspect sample can be accounted for by the loss due to evaporation of this very volatile compound. This loss may in part be due to the storage time of the sample or to the sampling and transportation of the sample. From the formulation of the product there is no reason to expect that adverse changes would occur if stored under normal conditions for long periods of time. The fact that the stored sample composition is effectively the same as the control sample reinforces this statement. In fact there is no reason to suppose that the drench as supplied to the laboratory would be any less effective in treating sheep for flukes and worms than a new batch of the product supplied fresh from the manufacturers.

This type of drench is well known for its sometimes fatal effects on some of the sheep in a flock. There is no reason to suspect that the deaths of the sheep in this case are attributable to contaminating subtances present in the drench.

Obviously to prevent a reoccurence of this misfortune is difficult without further details concerning the events leading up to the deaths of the rams, however some possible explanations are apparent:

a) The product is known to be more toxic to rams than ewes and in fact is specifically not recommended for rams.

b) Dosing should take place as soon as infection is discovered and preferably starting in September, a time of year when the ewes are strongest and more able to cope with the side effects.

c) Diet is important during the early months of the year as it is common to feed sheep on high protein diets and on supplements, these are both contraindications for this type of drench.

d) The product is not effective against all species of flukes and worms, and is only potent during a specific phase of their life cycle. This phase is at its peak during the summer months.

These suggestions as to the conditions which may have brought about the deaths of the rams are supported by the fact that the rams which were slaughtered had not been cured of the infestation.

II.7 Conclusion.

The above case was a typical example of the use of both the retention index method and mass fragmentography in the process of identification of unknown solvents in a product. These

methods, however, were not able to specifically identify the n-butyl acetate fraction without additional information being made available. This failure was mainly due to the fact that the relevant data for this compound was not listed in either the mass spectral library or the retention index library at the time of searching. This example typically demonstrates the major problem when using such library based systems, in that if the compound has not been previously encountered, or suspected, and the relevant data compiled, then the identification process is likely to fail.

This case also illustrates the complexity of analysing commercial products of this type, and although the product contained only two major volatile components the process of identifying these compounds was not as elementary as anticipated.

APPENDIX III

PAPERS PUBLISHED FROM WORK IN THIS THESIS.

III.1

The Analysis of Blood in Cases of Suspected Solvent Abuse, With a Review of Results During the Period October 1987 to July 1979.

M Lush, J S Oliver, J M Watson, In Forensic Toxicology: Ed. J.S.Oliver, Croom Helm Ltd., London, 1980.

Introduction.

Over the past ten years a trend in substance abuse in the U.K. has been drawn to the attention of both the medical profession and the general public, (Watson 1974, Oliver & Watson 1977). This form of substance abuse more familiarly known as solvent abuse or "glue sniffing" appears to be an increasing problem, especially among teenagers. The term "glue sniffing" is in itself misleading as the abuser inhales deeply rather than sniffs the volatile fumes. It is also far from correct to assume that this practice is entirely restricted to the misuse of glues as virtually any solvent based product could be used, and indeed often are experimented with by the abuser to obtain his or her "high".

At present the antisocial problems of this form of abuse seem

to outweigh the possible long-term risks to the health of the abuser. In fact very little evidence has been established regarding these risks, in particular the possiblity of liver, (Comstock & Hayden 1976, O'Brien 1971) or brain damage (Asbury 1975, Allister 1979). It cannot be disputed that the abuser does attain some degree of psychological "high" from the solvent or the combination of solvent and partial anoxia, and it is during this "high" that the abuser may be at most risk to himself or others. It is therefore inevitable that the abuser may attain an overdose level with solvent just as with any other drug.

The problems presented to the forensic toxicologist are in the fact that there are many possible solvents available for potential abuse, and the identification, quantitation and subsequent diagnosis depend on the availability of a suitable method of analysis. Most toxicologists are familiar with the problem of alcohol abuse and considerable effort has been made to establish quick, accurate and reliable analytical techniques to measure this solvent in body fluids (Sedman 1975, Luckey 1971, Jones 1978). However, the products being abused by the solvent abuser contain a far wider selection of solvents and solvent mixtures, ranging from the simple (glues and cleaning fluids) to the more complex (paraffins, petrol and aerosols).

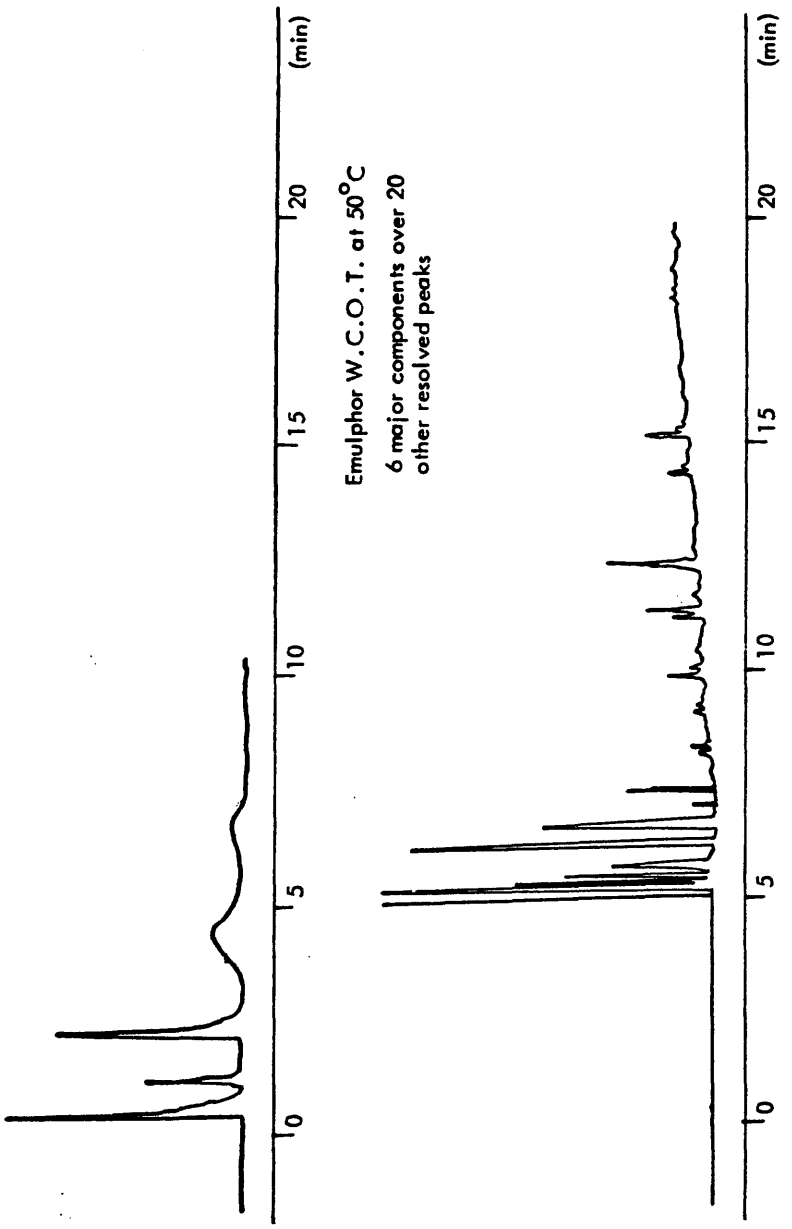
The identification of components in such mixtures requires more efficient separating techniques than the usual gas chromatograph employed for alcohol analysis. The required

degree of resolution can only be met by the use of capillary column chromatography. Figure 1 illustrates the differences in resolution between a good packed column for solvent separation and a capillary column. As can be seen the increase in resolution also increases sensitivity and several components which might otherwise be missed are resolved by the capillary column.

The Problem.

Observation of the current problem of solvent abuse in the Strathclyde region during the last 21 months has indicated that the solvents being abused appear to be restricted to only a few. Of these by far the most popular is toluene. This may be for two reasons: firstly the availability of the solvent. Table 1 gives a list of available abused products and the adhesives form the biggest group. Nearly all of these and the polystyrene cements contain toluene. Dry cleaning fluids are also popular, containing pure solvents, but these are not as easily available to the abuser. Varnishes, paints and lacquers though often easlily available do not appear to be as popular. The other explanation for the greater popularity of certain solvents may be derived from their effectiveness in producing a suitable "high". Solvent abusers themselves seem to progress from acetone based products via chlorinated solvents to toluene based product, and report that the "high" obtained with these products is more satisfactory.

Figure 1. Comparison of blood volatiles from a case of solvent abuse by packed and capillary column.



COMPARISON OF BLOOD VOLATILES FROM A CASE OF SOLVENT ABUSE BY PACKED AND CAPILLARY COLUMN

The pharmacological action of these products is debatable (Browning 1965). The effects may be due to direct chemical action or to the anoxic effect on the tissues. Whatever the action, it is the effect which is of foremost concern and in order to monitor solvent levels in order to attempt to predict this effect suitable methods of analysis are required.

Qualitative Method.

The qualitative method is outlined in Figure 2. A 5 ml venous blood sample is taken from the abuser into a heparinised syringe and stored at $4^{o}C$ until analysis. 500 ul of this blood is then sealed in a 6 ml hypovial. This is heated to $60^{o}C$ in an aluminium block. A 50 ul headspace sample is then taken and injected, via a split injector, into the chromatograph. The column is a wall coated open tubular column with Emulphor as stationary phase. The carrier gas is helium with a flow rate of 2 ml/min. Peaks are identified from their retention index or, in the case of unusual or suspect peaks, by mass spectroscopy. Many peaks are observed which include naturally occurring volatiles, and even a small level (less than 0.1 ng/ml) of toluene is observed even in blank blood samples.

Quantitative Method.

The quantitative method is outlined in Figure 3. An aliquot of blood (500 ul) is taken from the heparinised sample syringe and placed in a 6 ml hypo-vial, to this is added 500 ul of the internal standard solution of 1.5 ug/g redistilled ethyl benzene. The vial is then sealed with a butyl rubber septum.

Figure 2. Qualitative Analysis.

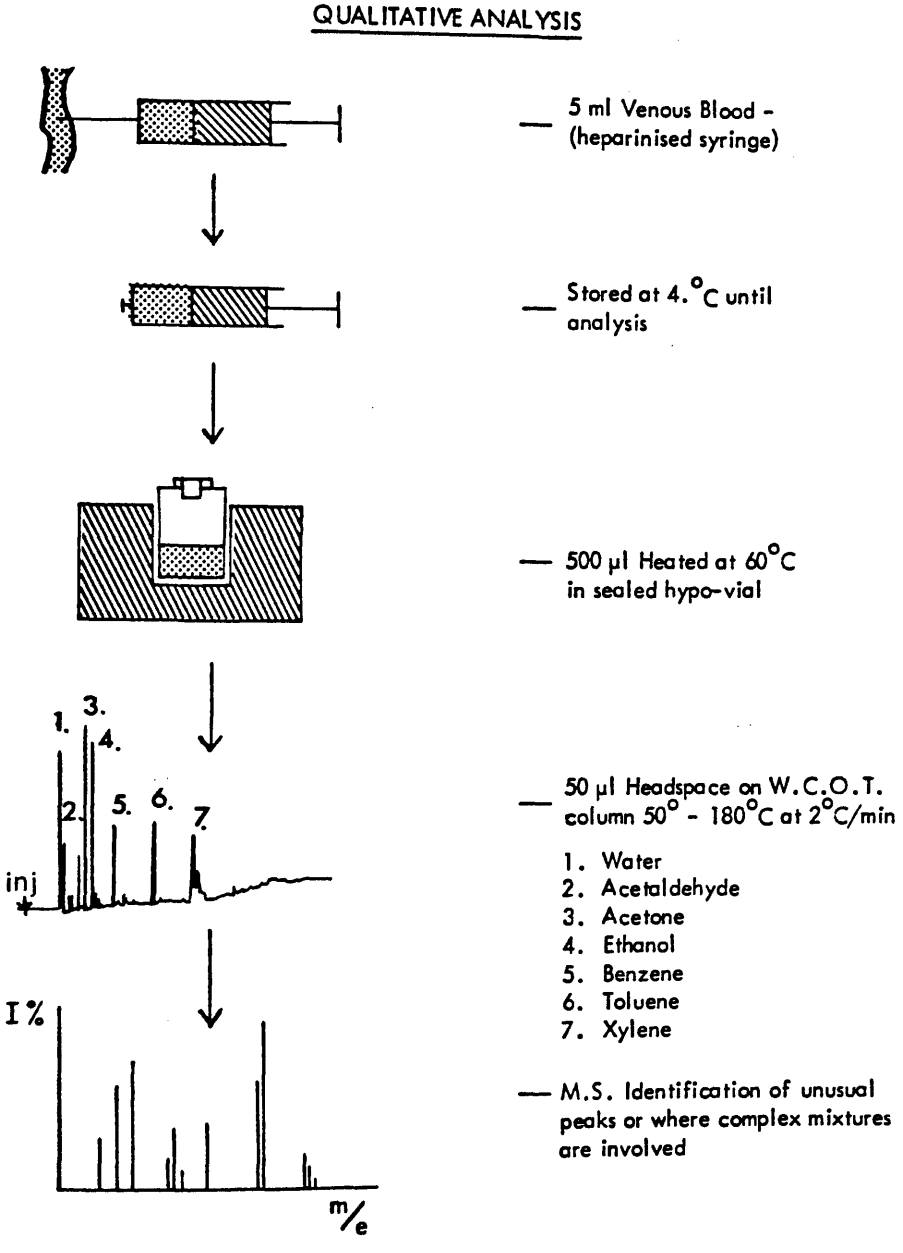
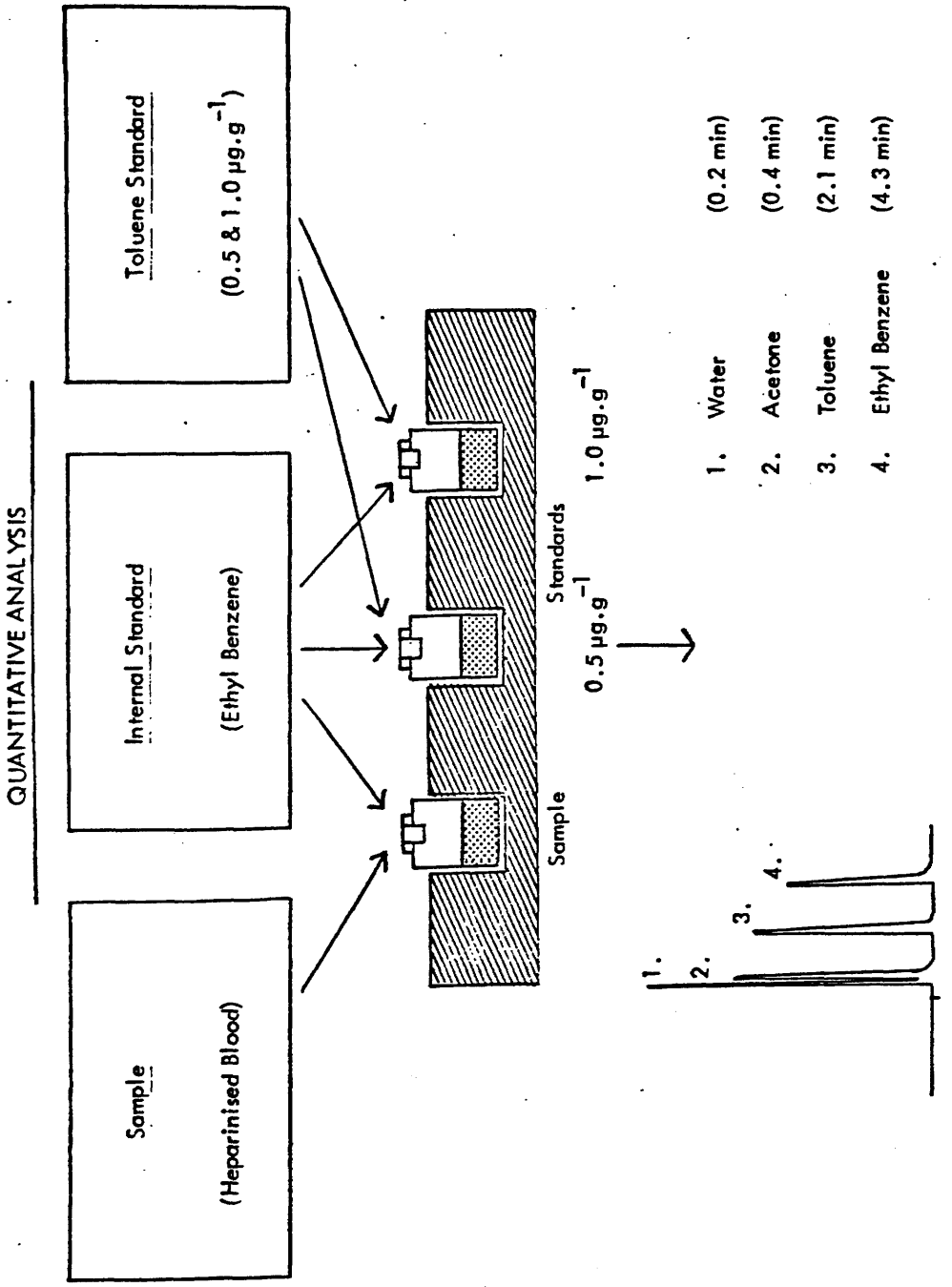


Figure 3. Quantitative Analysis.



Smaller aliquots of blood can be used and as little as 50 ul has been used without significant decrease in the reproducibility of results. Two standard toluene solutions are also run alongside the sample. These are made up to produce blood equivalent concentrations of 0.5 and 1.0 ug/g. The three vials are then placed in an aluminium heating block at 60^{o}C for 45 minutes to allow equilibrium to take place between the blood and the vapour space. A 200 ul headspace sample is then injected into the gas chromatograph using a Hamilton gas-tight syringe.

The column used for the quantitative analysis is a 1.5m x 4mm glass column packed with 30/50 Tenax G.C. polymer. The column is run isothermally at 150^{o}C for the analysis of toluene with a nitrogen carrier gas flow of 40 ml/min. The advantage of this packing material over that generally used for alcohol analysis is the fact that it is nearly ideal for solvent separation and by selecting column temperature to either 100^{o}C, 125^{o}C or 150^{o}C virtually all simple solvent mixtures may be separated. Another advantage is the extremely low bleed characteristics of Tenax G.C. making it an excellent packing material for GC-MS work. Under the above conditions toluene will separate from ethanol, acetone, benzene and xylene enabling direct quantitation to be made by peak area comparisons between samples and standards.

Experiments with this method have shown a detection limit of 1 ng/g with an accuracy of $\pm$ 10 ng/g using the above standards. This limit of detection could easily be improved

but has been found sufficient in cases of solvent abuse and even industrial overexposure.

Results.

During the last 21 months there has been a total of 82 referrals for suspected abuse (Figure 4). These have mostly been referred to us by the police or through hospital casualty departments. Sixty of these referrals were found to have blood solvent levels. Four of these were fatalities one of which showed both ethanol and toluene levels. Of the remaining 56 samples, 52 had toluene levels, 1 had toluene and ethanol, 1 trichloroethylene and 2 ethanol alone.

Of the 60 positives one abuser was referred on three occasions and three were referred twice. Of the remaining 56 different abusers the average age was 14.5 years (Figure 5). It is interesting to note that only two (both of which were fatalities) were over 18 years old. The male to female ratio was 5.7 to 1, lower than other studies where the ratio is given as 9 to 1 or even 20 to 1 (Watson 1974). Though this may be more of a reflection of the sampling and referral procedures than the true state. Figure 6 shows the distribution of the times of abuse. As might well be expected from this age group the most frequent time of abuse is the early evening. Though here again the results are heaviliy dependant on the honesty of the abuser or indeed his ability to give rational answers.

Figure 4. Referrals During Period October 1977 to July 1979.

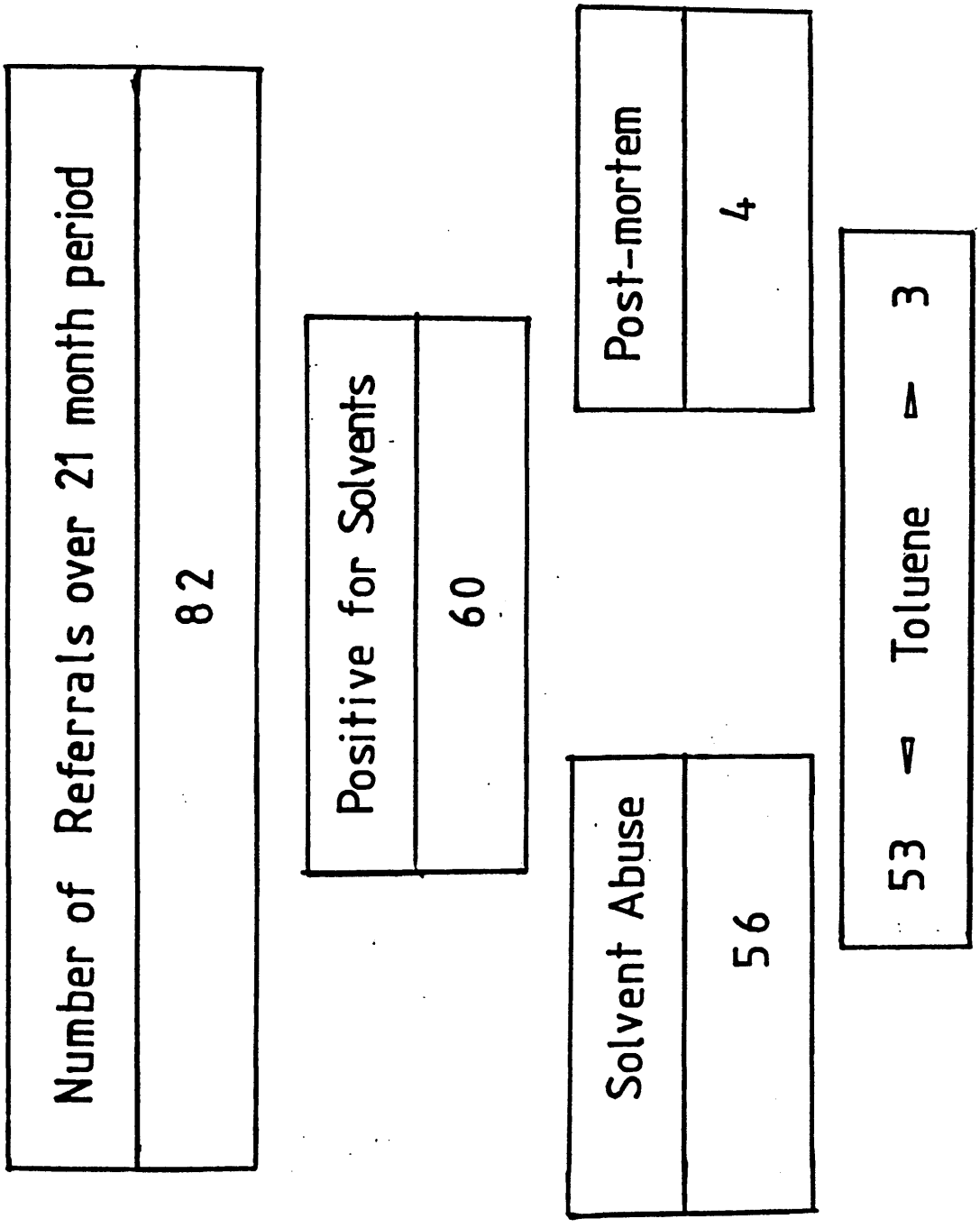


Figure 5. Age Distribution of Positive Abuse Cases.

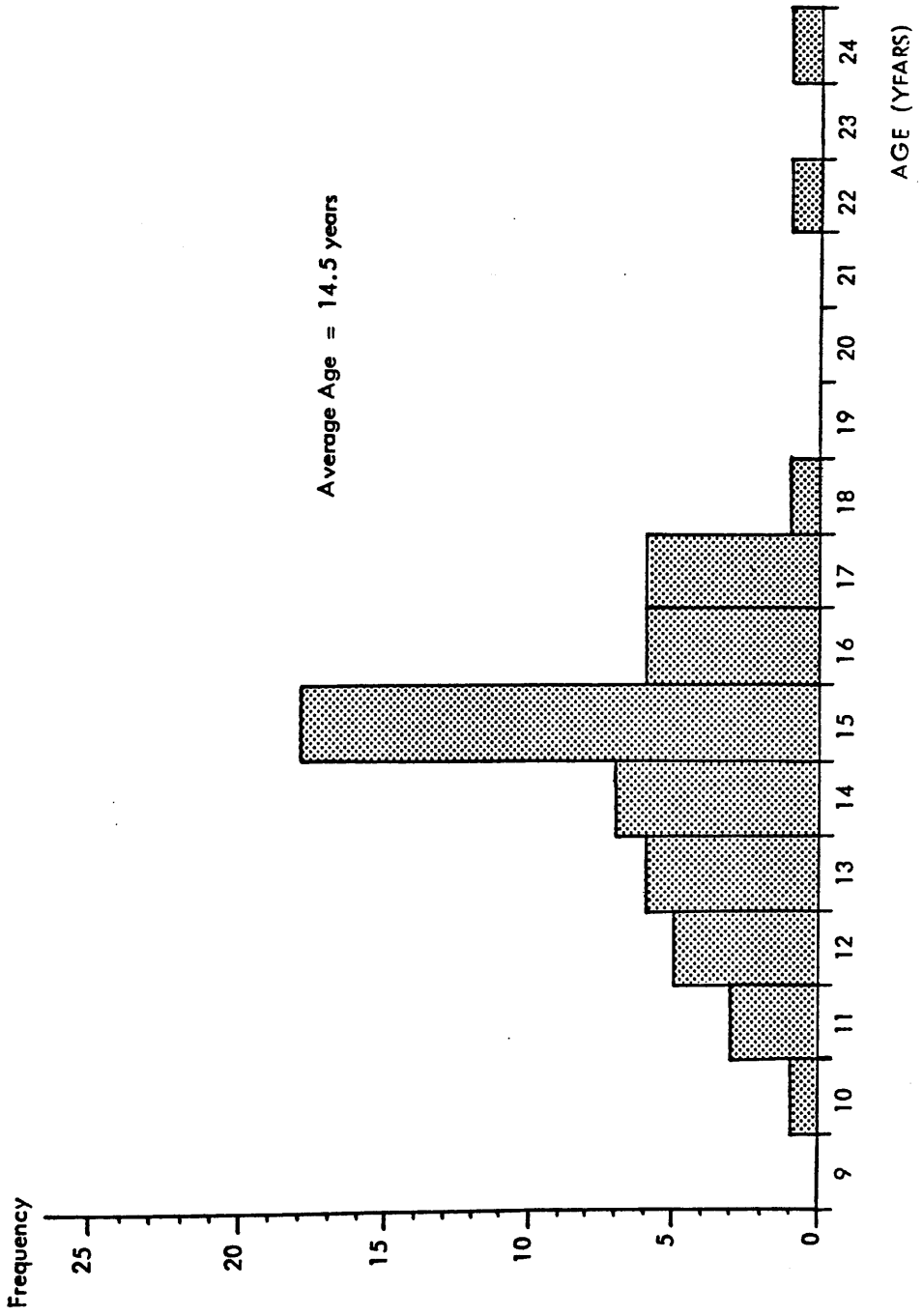
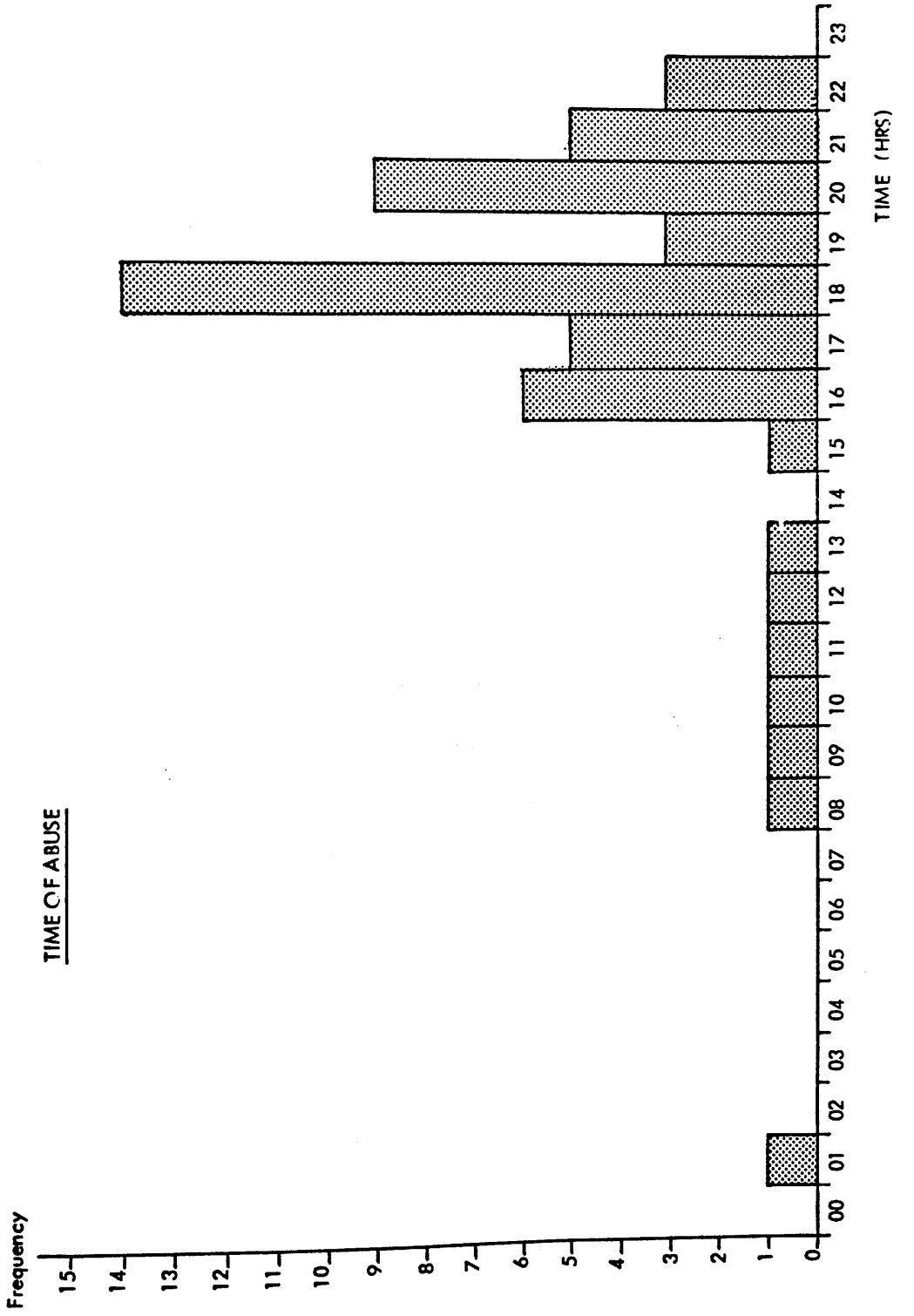


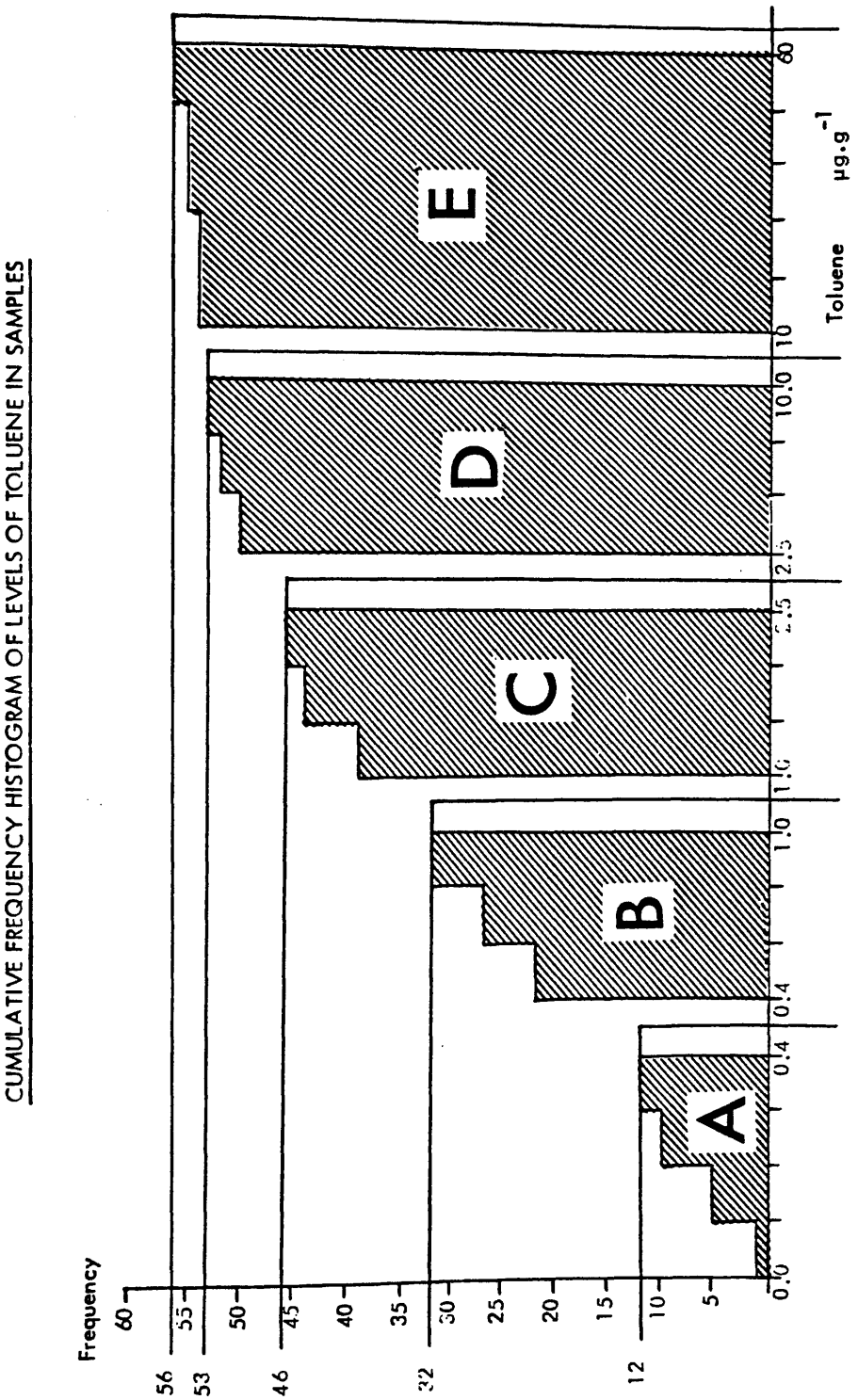
Figure 6. Time of Abuse.



A series of 23 samples from industrial workers exposed to tolene showed blood concentrations up to 0.25 ug/g with two cases where the exposure was known to have exceeded the Threashold Limit Value the blood concentrations were under 0.4 ug/g. In all cases sampling occured within 1 hour of removal from the contaminated area. The cut-off level for the diagnosis of solvent abuse was therefore taken as 0.4 ug/g. This does not assume that levels below this are not due to solvent abuse, but that they might be obtained from normal exposure to toluene at levels considered safe. Abusers with blood levels in the range 0.4 to 1.0 ug/g all showed signs of solvent abuse in particular the smell of solvent on their breath. Those with blood concentrations between 1.0 and 2.5 ug/g showed signs of increasing intoxication. Fifty percent of the abusers with blood concentrations in the range of 2.5 to 10 ug/g were hospitalised and showed signs of marked intoxication with some reporting halucinations. The abusers with blood concentrations above 10 ug/g were either unconscious or deceased at the time of sampling. The one post-mortem sample with a toluene blood concentration of less than 10 ug/g also contained a level of ethanol. These points are illustrated in Figure 7.

The above levels were levels present at the time of sampling and as often a period of 1 to 8 hours elapses between the time of abuse and the time of sampling many of these levels would have been higher at the time of abuse. In fact some of those samples which lie within the ranges C and D might have been over the 10 ug/g level at the time of abuse. The original

Figure 7. Cummulative Frequency Histogram of levels of Toluene in Samples.



dosage is also unknown due to the inconsistent method of inhalation of solvent fumes and the intoxicated state of the abuser.

Conclusions.

From the results obtained in this survey it appears that the problem of solvent abuse is restricted to only a few solvents. Of these there appears to be a destinct preference for toluene and subsequently toluene based products. The use of capillary column chromatography provides a valuable screening technique for the identification of solvents in a blood sample from a suspected abuser. Quantitation can be more easily performed on a packed column. Using a headspace method on a Tenax G.C. packed column, after appropriate selection of column temperature for the solvent mixture involved, levels may be determined with sufficient accuracy.

In the 21 month period of this survey there have been four fatalities involving solvent abuse and 80% of the positive cases showed significant levels of toluene. Of these about half were reported as being intoxicated at the time of sampling. Although it can be argued that solvent abuse is no worse than alcohol abuse it is still of great concern that a person can easily reach an intoxicated state and thereby endanger his or her life or that of others. Possibly of even greater concern is that this form of abuse tends to prevail among adolescents who are perhaps less well equipped emotionally to deal with the problem of intoxication and effectively control the degree of their abuse.

III.2

Solvent Encephalopathy.

R E Day, M D King, M Lush, J S Oliver, J M Watson, British Medical Journal, pp663-665 V283 1981.

Abstract.

Nineteen children aged 8-14 years were admitted over a six-year period with an acute encephalopathy due to toluene intoxication. Seven had a history of euphoria and hallucinations. The remainder presented with coma (4), ataxia (3), convulsions (3), and behaviour disturbance with diplopia (2). A history of glue sniffing was elicited in 14, but in the remainder toluene assay confirmed the diagnosis. Thirteen children recovered completely; five still had psychological impairment and personality change on discharge from hospital but were lost to follow-up, and one had persistent cerebellar ataxia one year after the acute episode, despite absence of further exposure.

Toluene inhalation is an important cause of encephalopathy in children and may lead to permanent neurological damage. Diagnosis is most important if further damage due to continued abuse is to be prevented, and toluene assay is a valuable aid to diagnosis.

Introduction.

Over the past decade glue sniffing has almost reached epidemic proportions among adolescents in some communities where there is extreme social or emotional deprivation. Although some earlier reports (Glasser & Massengale 1962) concluded that the practice was free from serious side effects, evidence now suggests that this is not so. Glue sniffing has been directly responsible for over 60 deaths in the United Kingdom since 1970.

Toluene is the main solvent used in commercially available contact adhesives in Britain, and toxic effects on the central nervous system (Knox & Nelson 1966, Kelly 1975, Boor & Hurtig 1977, Aruffo & Escobar 1980), kidney (Bennett & Forman 1980), liver (Hobby, O´Brien & Yeoman 1971), and heart (Harris & Taylor 1970) have been described. Recently it has become apparent that toluene intoxication is an important cause of encephalopathy in children. We descibe our experience and emphasise the value of a toluene assay as an aid to diagnosis.

Patients and Methods.

During 1974-80 19 patients were admitted to the Royal Hospital for Sick Children, Yorkhill, Glasgow, with neurological symptoms and signs after sniffing glue. Ages ranged from 8 to 14 years and boys predominated with a male-female ratio of 2:1. All patients belonged to socioeconomic class V. Readily available proprietary brands of adhesives containing toluene were used in all cases. Toluene assay was performed by gas

chromatography using a Tenax G.C. packed column (Lush, Oliver & Watson 1980).

Results.

Only seven patients had a history of euphoria or hallucinations. The other 12 presented with coma (4), ataxia (3), convulsions (3), and behaviour disturbance with diplopia (2) (Table I).

The diagnosis was made at presentation in 14 patients. In the remainder a history of sniffing glue was absent, and the diagnosis was established after toluene assay.

Blood concentrations ranged from 0.8 ug/g to 8 ug/g. Haematological and biochemical data were normal in all cases. Electroencephalograms (EEGs) were carried out on ten patients and were abnormal in three, showing diffuse slow wave activity in two and unilateral slowing in the other. Repeat EEGs three weeks later in these patients were normal. Recovery was complete in 13 cases. Five still showed psychological and personality changes on discharge from hospital, but the ultimate outcome is unknown as they were lost to follow-up. The remaining child (case 17) showed persistent signs, which are descibed in detail.

Case No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Age (years)	12	13	9	11	11	11	8	8	14	9	12	13	10	12	13	9	11	13	12
Euphoria (6)		*	*	*								*				*	*		
Hallucination (3)												*				*			*
Suicidal (3)					*							*						*	
Headache (3)							*						*				*		
Vomiting (2)	*																*		
Drowsiness (6)		*					*						*			*		*	*
Diplopia (2)										*	*								
Convulsions (3)								*					*		*				
Coma (5)	*		*			*			*					*					
Dysarthria (5)				*	*		*											*	*
Ataxia (5)				*	*		*											*	*
Behaviour (2)									*	*									
Ulceration (3)						*										*	*		
Outcome	L	N	L	N	L	L	N	L	L	N	N	N	L	N	L	L	P	N	N

N = Normal.
L = Lost to follow-up
P = Persistent signs.

Case No.	Toluene assay (ug/g)
13	0.8
14	0.9
15	1.46
16	0.8
17	1.16
18	7.7
19	3.53

Table I - Presenting features, toluene concentrations, and outcome in 19 children after glue sniffing.

Case Report.

An 11-year old boy presented with a one-week history of headache, vomiting, abnormal behaviour, slurred speech, and unsteady gait. The medical history, family history, and review of the systems were unremarkable. There was no recent contact with infectious disease, and exposure to drugs and toxins was denied. He was a thin apyrexial boy with superficial ulceration of the lips and nares. His mood was euphoric, speech was slurred, and he had coarse rotatory nystagmus in all directions of gaze. There was severe ataxia of gait with moderate limb inco-ordination and intention tremor. Cranial nerves, deep tendon reflexes, and sensory examination results were normal, and there was no muscle wasting or weakness.

Investigations.

Examination of full blood count, urea and electrolyte concentrations, cerebrospinal fluid, skull radiograph, and computerised brain scan gave normal results. Initial EEG showed diffuse slow wave activity with left-sided predominance.

There was no change in the clinical signs over 72 hours, and at this stage the possibility of a toxic encephalopathy due to sniffing glue was considered. Blood toluene concentration was 1.14 ug/g, and it was later confirmed that the patient had been sniffing glue for several months.

He improved slightly over the following three weeks. EEG at

this stage was normal. On review one year later, however, despite confirmatory evidence of abstinence from glue sniffing, cerebellar signs persisted.

Discussion.

Toluene is a hydrocarbon solvent that is insoluble in water. On inhalation it is absorbed by the lungs and bound to lipo-proteins. Some 70-80% of toluene is metabolised in hepatic microsomes by oxidation to benzoic acid, which is conjugated with glycine to form hippuric acid, and is eliminated in this form through the kidneys.

The mechanism of toluene toxicity is unknown, although it may be related to the lipid dissolving property, often used in vitro for extracting membrane lipids. The main toxic impact of toluene is on the central nervous system, probably explained by high cerebral perfusion and the affinity of toluene for lipid rich tissues, from which it is slowly released. Experiments in animals after toluene inhalation have shown toluene concentrations in fatty tisues 80 times higher than in blood (Carlsson & Lindquist 1977). We have assayed toluene concentrations in brain and blood in six fatal cases after glue sniffing (Table II). All showed higher concentrations in brain tissue than in blood, supporting the experimental findings.

Case	Age (years)	Clinical features	Brain	Blood
1	15	Status epilepticus	14.5	0.035
2	24	Coma	22.5	5.9
3	16	Hallucinations:suicide (hanging)	98.6	39.6
4	18	Hallucinations:suicide (drowning)	15.3	13.0
5	16	Hallucinations:suicide (jumped off roof)	55.9	54.0
6	18	Hallucinations:suicide (jumped from window)	1.1	0.13

Table II - Toluene concentrations (ug/g) and clinical features in six fatal cases after glue sniffing.

The effect on the central nervous system may be depressant or excitatory, with euphoria in the induction phase followed by disorientation, tremulousness, mood lability, tinnitus, diplopia, hallucinations, dysarthria, ataxia, convulsions (Brozovsky & Winkler 1965), and coma. The early features of euphoria hallucinations, and behaviour change were absent in seven patients and should not therefore preclude consideration of the diagnosis. Irreversible neurological sequelae, such as encephalopathy (Grabski 1961, Knox & Nelson 1966, Kelly 1975, Boor & Hurtig 1977, Aruffo & Escobar 1980, Malm & Tunnell 1980), optic atrophy (Keane 1978), and equilibrium disorders (Sasa 1978), have been descibed in adult chronic abusers but not in children. One patient in this study had a persistent cerebellar ataxia, and another five patients, who were lost to follow-up, had neurological impairemnt when discharged from hospital. Permanent neurological damage may therefore be more common than recognised (Glasser & Massengale 1962, Wyse 1973).

In patients intoxicated by toluene, investigations may show

impaired renal and hepatic function, cerebrospinal fluid pleocytosis (Kelly 1975), or widening of cortical sulci on pneumoencephalogram or compurised brain scan (Knox & Nelson 1966, Boor & Hurtig 1977, Aruffo & Escobar 1980). EEG may show diffuse or focal slow or sharp wave complexes (Knox & Nelson 1966, Contreras, Fernandez-Guardiola, Gonzalez-Estrada & Zarabozo 1979) and unilateral features may be misleading (see case report). Toluene assay, however, is the most sensitive indicator of exposure to toluene, and the correlation between blood concentrations and clinical features (based on 110 positive samples including 25 from industrial workers exposed to toluene) is shown in Figure I.

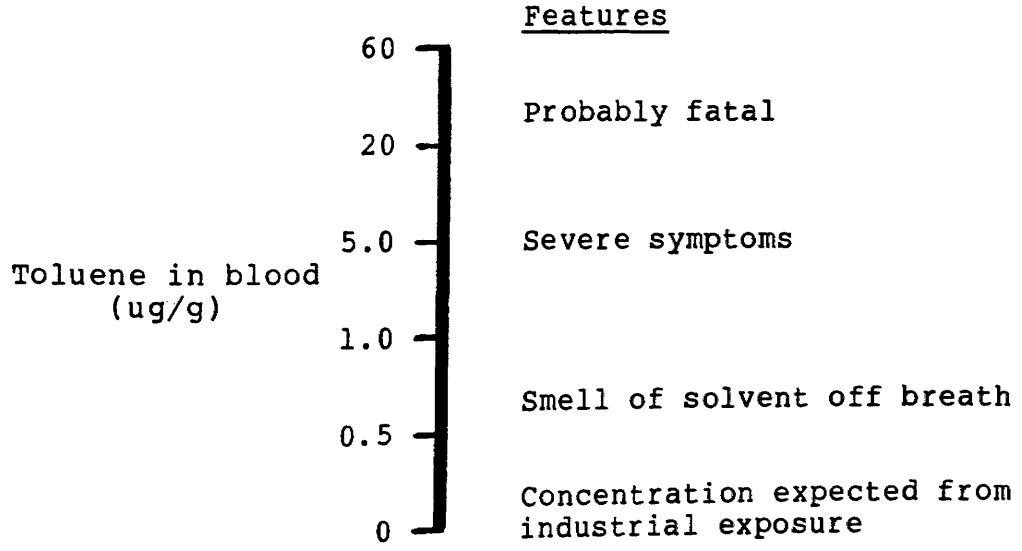


Figure I - Clinical features encountered at a range of blood toluene concentrations; based on analysis of 110 samples, including 25 from industrial workers exposed to toluene.

The interval between exposure to toluene and sampling is important when interpreting results. Blood concentrations of toluene may be biphasic; an initial peak followed by a trough reflecting lipid binding by the central nervous system, and a subsequent peak may appear as toluene is slowly released into

the blood. The assay may be positive up to several days after withdrawl from toluene (see case report). Measurement of urinary hippuric acid concentrations after exposure to toluene is not of diagnostic value, as hippuric acid is a normal urinary constituent that is influenced by diet. Reports of post-mortem findings are few: diffuse cerebral and cerebellar cortical atrophy with giant axonopathy have recently been descibed (Aruffo & Escobar 1980).

Toluene inhalation is an important cause of encephalopathy in children and in some patients may lead to a permanent neurological damage. The wider social implications of glue sniffing are beyond the scope of this paper. Diagnosis is most important if further damage due to continued abuse is to be prevented. This demands an awareness of the possibility of glue sniffing in any child who presents with otherwise unexplained coma, convulsions, ataxia, or behaviour disturbance. Toluene assay is a valuable aid to diagnosis.

III.3

Status Epilepticus Caused by Solvent Abuse.

C Allister, M Lush, J S Oliver, J M Watson, British Medical Journal, p1156 V283 1981.

Glue sniffing may present in various ways (Lubash, New, Rubin & Scherr 1962, Done 1967), and the condition may be fatal (Bass 1970, Cohen 1973). We describe an adolescent with severe status epilepticus due to solvent abuse.

Case Report.

A 15-year-old previously healthy boy presented after a grand mal seizure in the school gymnasium followed by severe uncontrolled status epilepticus. He had had two febrile convulsions in early childhood. On admission he was in coma but was localising painful stimuli; there were no focal signs, and general examination showed no abnormality. Results of routine biochemical, haematological, and bacteriological examinations were normal, as were chest and skull radiographs and cerebrospinal fluid. Computed tomography showed small ventricles and no focal abnormality. On the third day the frontal lobe was sampled for biopsy. Histologically there was no evidence of encephalitis. A few neurones showed features of recent necrosis, but whether this was a focal infarct or part of a diffuse disorder could not be defined. Viral serological and immunofluorescent antibody studies excluded

herpes simplex infection. An anonymous telephone call then suggested that the boy might have been sniffing glue. An aliquot of the brain biopsy specimen was therefore sent for analysis for solvents by gas chromatography (Lush, Oliver & Watson 1980) and showed a toluene concentration of 14.5 ug/g tissue.

Despite therapeutic serum concentrations of various anticonvulsant combinations the patient continued to have frequent seizures. He was therefore also treated with intermittant positive-pressure ventilation, muscle relaxants, and sedation and required a tracheostomy. Continuous electroencephalographic monitoring indicated seizure activity at around six/hour. After two weeks he began to improve. The frequency of seizures decreased without any major change in treatment. Mechanical ventilation was discontinued. He began to obey commands, and after removal of the tracheostomy tube he began speaking at 10 weeks, at which time computed tomography showed dilatation of the lateral and third ventricles and several areas of low density resembling multiple infarcts. He began to walk 15 weeks after onset and was dischaged home after five months. Follow-up two years later showed no focal neurological deficit, and psychometry indicated no definite impairment. He continued to have one or two seizures a month, however, and exhibited major behavioural problems.

Comment.

This case presented major difficulties in diagnosis, and many aetiological factors were investigated. Though hypoxia may have influenced the neurological disturbance, solvent intoxication probably initiated the illness and contributed to its severity. A latent predisposition to convulsions may also have been significant in view of the patient's reaction to fever in childhood. Blood concentrations of toluene usually encountered in cases of solvent abuse range from 0.4 to 1.0 ug/g in samples taken within 24 hours after abuse and from 1.0 to 10.0 ug/g in samples taken when the abuser was obviously intoxicated. Two subjects who hanged themselves when under the influence of toluene had blood concentrations of 52 and 39 ug/g (Lush, Oliver & Watson 1980). The concentration found in the brain tissue was above that found in sniffers who are acutely intoxicated, presumably because the solvent was concentrated in lipids. The true concentration was probably higher, as there would be loss of solvent owing to the small size of the sample, transport in a container of relatively large volume (32 ml), and the delay of seven days between biopsy and analysis.

Without laboratory confirmation it would have been difficult to establish that glue sniffing had been an important aetiological factor in this boy's life-threatening illness. The diagnosis of solvent intoxication should be considered in cases of epilepsy or encephalopathy of undetermined aetiology arising for the first time in the adolescent age group.

We thank Professor Bryan Jennett for permitting us to report this case and for the advice in presentation.

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ADDENDUM

Other notable references on the subject of inhalation abuse published since the work detailed in the preceeding pages of this thesis.

Methods of Analysis of Solvents

A temperature programmed gas chromatographic method has been described (FLANAGAN & RAMSEY, 1982) with similar incubation and headspace sampling conditions as presented in this thesis but using a 0.3% Carbowax 20M on Carbopak C packed column. The method illustrated good separation of several important solvents of abuse.

Details of methods similar to the ones reported in this thesis have also been published for analysis of urinary trichloroacetic acid and urinary hippuric acid (FLANAGAN & RAMSEY, 1982). Hippuric acid levels were reported from several cases and the hippuric acid:creatinine ratio was proposed as a measure of toluene exposure.

Of significance is a novel method described for the analysis of solvents in products suspected of having been used for inhalation abuse (FLANAGAN & RAMSEY, 1982). This technique, vapour-phase infra-red spectrophotometry, has been used to provide "fingerprint" information on the volatile components of abused products.

Epidemiology and the Effects of Abuse

A summary review of solvent abuse effects and clinical findings from several cases which have appeared after the work reported in this thesis have been described in the literature (OLIVER, 1984). Included in this paper were details of several cases involving halogenated solvents and inhalation abuse of fuel-gas. The abuse of these products becomming as apparent as the abuse of toluene based products.

Another very extensive epidemiological study of deaths from volatile substance abuse in the United Kingdom for the period 1971 to 1983 has also been published (ANDERSON, MACNAIR & RAMSEY, 1985). This was subsequently updated with further data for the period 1981 to 1985 (ANDERSON, BLOOR, MACNAIR & RAMSEY, 1986). These papers also describe an apparent trend away from the abuse of toluene to fuel-gases and halogenated solvents.

Finally a case of profound motor neuropathy attributed to deliberate inhalation of petrol has been exhibited in a 4 year old boy. Although the neuropathy was attributed to N-hexane there were no blood levels measured (DOOKUN, HALL, RAMSEY, & SCHWARTZ, 1986).

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