RISK ASSESSMENT OF OCCUPATIONAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

A thesis submitted in accordance with the conditions governing candidates for the degree of

DOCTOR OF PHILOSOPHY

To the University of Newcastle-upon-Tyne

by

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CERTIFICATE

I hereby certify that the work embodied in this thesis is the result of my own investigations, except where reference has been made to published literature.

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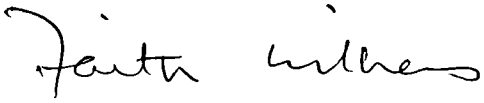
Signed  **Director of Studies**

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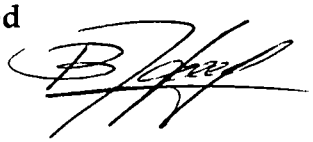
Signed  **Candidate**

TO MY DEAREST PARENTS

BARON & FIDA

ACKNOWLEDGEMENT

No words can describe my gratitude to my dear parents. To them I owe my life.

Dr. Iman Nuwayhid was my inspiration to pursue a career in occupational health. Dr. John Steel refined my knowledge, talents and made me believe in my abilities. Last but not least, is Dr. John W. Edwards who gave me the opportunity to go far. To them I owe my career.

The smile and continuous assurance I received from Professor Peter G. Blain were my drive. The guidance and finest concern of Dr. Faith M. Williams I depended on. To them I owe my PhD. and more.

Dr. Peter Kelly's patience and assistance during my "statistics crisis" will always be remembered. The least I can do to repay him is to be as helpful to my colleagues as he was to me.

Everyone at the Department of Environmental and Occupational Medicine contributed, in their own way, to the completion of this thesis. I thank you all.

I would also like to express my thankfulness to The Arab-British Chamber Charitable Foundation, The Benlian Trust, The Mihran and Azniv Essefian Trust, The Gulbenkian Foundation, The Colt Foundation and The British Occupational Health Research Foundation for their priceless financial contribution to my maintenance and finance of the project.

To all my dearest friends. You were, are and will be always on my mind.

Mary Evans/Harry Price Coll Univ of London

Biological monitoring often resembles a witches brew

"The Other Side Of Animal Studies"

ABSTRACT

A novel strategy was used to assess the risk to health from exposure to polycyclic aromatic hydrocarbons (PAHs), an important class of chemical carcinogens, in workers in the paving and roofing industries using bitumen.

The aim was to evaluate the risk of exposure to PAHs at three different stages: these were external exposure "external dose", internal exposure "internal dose" and biological effect "biologically effective dose". The approaches used for quantifying the risk at these stages were ambient monitoring, biological monitoring and biological effect monitoring, respectively.

Ambient monitoring was carried out by personal air sampling and measurement of PAH-exposure. Excretion of thioethers (a non-selective biomarker) and 1-hydroxypyrene (a selective biomarker of exposure to pyrene) in urine were determined. Urinary d-glucaric acid, sister chromatid exchange in peripheral blood lymphocytes and micronuclei in exfoliated cells were use for biological effect monitoring. Seven groups of workers were studied. These were office workers, departmental staff and manual workers who were not occupationally exposed to PAHs and who were included as controls, and two groups of pavers and roofers who were exposed to bitumen fumes during their work.

The ambient monitoring program revealed that concentrations of PAHs in environmental air could be as high as those found in the pavers' and roofers' occupational environments. The contribution of these background levels to the external dose of the pavers and roofers was considerable. Pavers and roofers were occupationally exposed to low levels of PAHs. The PAH profile in the personal air sample of the workers was different between the two industries. This was attributed to the presence of PAH-emission sources other than hot bitumen, such as vehicle exhaust. The concentration of naphthalene measured in air samples was a good indicator for identifying the existence of these sources. The quantitative evaluation of the carcinogenic risk inherent in the external dose using the 8 hour TWA concentration as a measuring stick did not seem to be effective due to the low "noise" levels of carcinogenic PAHs present. A semi-quantitative measure of the external exposure was suggested which appeared to be a better reflection of the

carcinogenic risk.

Urinary thioethers are not sensitive enough to be used as a non-selective biomarker of exposure to low levels of PAHs; however, urinary 1-hydroxypyrene was a good indicator of internal exposure to pyrene and therefore PAHs in bitumen fume. The relationship between 1-hydroxypyrene in urine and specific airborne PAH-exposure indices in the results of the pavers, but not the roofers, suggested that the route of exposure in the latter is mainly dermal while that in the pavers is more related to respiratory uptake. This observation, however, did not agree with the subjective occupational hygiene assessment where it was observed that the roofers were closer to the source of PAH-emissions due to the nature of their job and both groups did not use any respiratory or personal protective equipment.

The measurement of urinary d-glucaric acid excretion suggested, but not strongly, that hepatic enzyme activity was induced in the occupationally PAH-exposed groups in comparison to controls and reflected the internal dose of pyrene or total PAHs. These observation; however, need further investigation. Micronuclei in exfoliated epithelial cells could not be measured in these populations as insufficient bladder epithelial cells were obtained for analysis. Sister chromatid exchange in peripheral blood lymphocytes of pavers and roofers was significantly elevated in comparison to control and manual worker group. The group mean frequencies of SCE were significantly correlated with the external exposure to carcinogenic PAHs. This observation suggested that sister chromatid exchange is a very sensitive cytogenetic endpoint and is useful for evaluating the risk of exposure to low levels of carcinogenic PAHs.

Risk assessment, such as the one conducted in this study, allows a quantitative estimation of risk associated with long-term exposure to moderate and low levels of airborne exposure to PAHs in bitumen fume. Knowledge of dose response relationship may allow a review of current Occupational Exposure Standards (OES) and Biological Exposure Limits, which are at present either unavailable or based on poor background scientific knowledge for most PAH compounds.

In this study we have suggested an Occupational Exposure Limit for PAHs based on the sum of fourteen PAH-species.

PUBLICATIONS

Ambient, Biological, and Biological Effect Monitoring of Exposure to Polycyclic Aromatic Hydrocarbons (PAHs), B.A. Hatjian, J.W. Edwards, J. Harrison, F.M. Williams, P.G. Blain, Proceedings of the International Symposium on Human Health and Environment, September 1994, p. 78.

Ambient, Biological, and Biological Effect Monitoring of Exposure to Polycyclic Aromatic Hydrocarbons (PAHs), B.A. Hatjian, J.W. Edwards, J. Harrison, F.M. Williams, P.G. Blain, Toxicology Letters, (Full article in press)

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Abbreviations

1-HP	1-hydroxypyrene
2-FB	2-fluoro-biphenyl
6ß-OHF	6ß-hydroxycortisol
ACGIH	American Conference of Governmental Industrial Hygienists
AS	Static air sampling
BaPDE	Benz(a)pyrene diol-epoxide
B(a)P	Benz(a)pyrene
B(a)A	Benz(a)anthracene
B(b)F	Benz(b)fluoranthene
BDH	British Drug House
B(k)F	Benz(k)fluoranthene
BEIs	Biological Exposure Indices
BM	Biological monitoring
BEM	Biological effect monitoring
BOC	British Oxygen Company
BrdU	5-bromo-2'-deoxyuridine
BSM	Benzene soluble material
CSM	Cyclohexane soluble material
CV	Coefficient of Variation
CTPV	Coal-tar pitch volatiles
DCM	Dichloromethane
dia.	Diameter
DiB(a,h)A	Dibenz(a,h)anthracene
DMBA	7,12 dimethylbenz(a)anthracene
DMSO	Di-methylsulphoxide
DNA	Deoxyribonucleic acid
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
EDTA	Ethylenediamine tetraacetic acid
EH	Environmental hygiene
EI^+	Electron ionisation (positive)
EIA	Enzyme-linked immuno-sorbent assay
EM	Environmental monitoring
EPA	Environmental Protection Agency

ETS	Environmental tobacco smoke
FDA	Food and Drug Administration
FF	Free cortisol
GC	Gas chromatography
GC-EC	Gas chromatography/Electron Capture
GC-FID	Gas chromatography/Flame Ionisation Detector
GC/MS	Gas chromatography/Mass spectrometry
GM	Geometric mean
GLP	Good laboratory practice
GSH	Reduced glutathione
GST	Glutathione-S-transferase
Hb	Haemoglobin
HMEA	Hepatic microsomal enzyme activity
HPLC	High performance liquid chromatography
HSE	Health and Safety Executive
IARC	International Agency For Research On Cancer
IR	Infra-red (spectrometry)
MeOH	Methanol
MFO	Mixed function oxygenase
MN	Micronucleus (micronuclei)
MWt	Molecular weight
NAC	N-acetyl-cysteine
n.d.	Not detectable
NIOSH	National Institute for Occupational Safety and Health
OELs	Occupational Exposure Limits
OES	Occupational Exposure Standard
OSHA	Occupational Safety and Health Administration
P-448	Cytochrome P-448
P-450	Cytochrome P-450
PAH(s)	Polycyclic aromatic hydrocarbon(s)
PBLs	Peripheral blood lymphocytes
PBS	Phosphate buffer saline
PM	Personal air monitoring
PPE	Personal protective equipment
PTFE	Polytetrafluoroethylene
RI	Replicative Index
RIA	Radioimmunoassay
RNA	Ribonucleic acid

RPE	Respiratory protective equipment
S9	9000 x g supernatant fraction (of liver homogenate)
SCE	Sister chromatid exchange
SD	Standard deviation
SFS	Synchronous fluorescence spectrometry
SIR	Selective ion recording
SG	Specific gravity
SM	Susceptibility monitoring
SMR(s)	Standard mortality rate(s)
SSC	Standard saline citrate
SSM	Solvent-soluble matter
STEL	Short-term exposure limit
$t_{1/2}$	Half-life
TLV	Threshold limit value
TPM	Total particulate matter
TRIS	Tris(hydroxymethyl)aminomethane
TWA	Time weighted average
U1-HP	Urinary 1-hydroxypyrene
UCr	Urinary creatinine
UCot	Urinary cotinine
UDGA	Urinary d-glucaric acid
UDS	Unscheduled DNA synthesis
USERIA	Ultrasensitive enzymatic radioimmunoassay
UTh	Urinary thioethers
UV	Ultra-violet
WHO	World Health Organisation

SECTION I

INTRODUCTION

CHAPTER 1

RISK ASSESSMENT

1.1 RISK ASSESSMENT

The origin of risk assessment, in its most basic form, can be traced back to that of early humans (Paustenbach, 1989). Risk assessment is a thinking process which is done by everybody on daily basis. For the most part these assessment are performed almost subconsciously.

Books have been written on the topic of risk assessment. It's virtually impossible to cover this subject in great detail in the boundaries of this thesis; however it is essential to understand the definition, the main components or parameters of risk, and the steps involved in the process of risk assessment in order to appreciate the role of occupational monitoring in the different stages of risk assessment.

Risk assessment can be defined as "the quantitative evaluation of the likelihood of an undesired event and the likelihood of harm or damage to be caused, together with value judgement concerning the significance of the results" (Edmondson, 1994).

Risk evaluation, a major step in risk assessment, is dependant on two parameters; the frequency, the likelihood of occurrence of the unwanted event, and the consequences resulting from its occurrence. The following mathematical equation defines the existing relationship:

R = F (frequency, consequence)

The risk assessment process involves four major steps which answer essential questions raised by environmental/occupational health research programs. The steps are as follows:

1) **Hazard identification**: answers the question if the agent of interest is capable of causing an adverse effect in humans (Fowle and Sexton, 1992). Quantitatively speaking it determines whether human exposure to the agent could cause an increase in the incidence of a health condition (cancer, birth defects, etc.). It involves the process of characterising the nature and strength of the evidence of causation between exposure and toxic outcome (Paustenbach, 1989).

2) **Exposure assessment**: determines what exposure occurs or is anticipated to occur for human populations (Fowle and Sexton, 1992). In terms of quantitative assessment, the process involves measuring or estimating the intensity, frequency, and duration of human exposure to an agent contaminating the environment, or of a hypothetical exposure that might occur upon the release of new chemicals or a change in processes. A fully comprehensive exposure assessment should identify the magnitude, duration, schedule, and route of exposure; the size, nature and types of populations exposed and most importantly define the uncertainties in all estimates (when interpreting the outcomes of such investigations). From the control or prevention point of view exposure assessment can often be of help in suggesting prospective hazard control options and attempting to predict efficacy of currently accessible control technologies for restraining exposure (Paustenbach, 1989).

3) **Dose-response assessment**: examines what is the quantitative relationship between dose and effect in humans (Fowle and Sexton, 1992). It is the process by which the relations between the dose of an agent absorbed and the incidence of the an adverse health effect in exposed populations is characterised. It estimates the incidence of effect as a function of exposure to the agent. It studies the effect of factors as intensity of exposure defined by dose and duration of exposure and confounding variables that might effect response such as sex, lifestyle etc.. Classical dose-response assessments require extrapolations from high to low doses and in many instances from animals to humans. These methods of extrapolations introduce a number of biological and statistical uncertainties into the prediction of the incidence of the undesired effect. The methods used should be properly identified and justified and where possible the uncertainties quantitatively rather than qualitatively defined (Paustenbach, 1989).

4) **Risk characterisation**: (based on the synthesis of dose-response assessments) attempts at estimating human health risk from the anticipated exposure to the agent (Fowle and Sexton, 1992). Basically, it is performed by combining the exposure and dose-response assessments as well as summarising the effects of uncertainties described in the preceding steps (Paustenbach, 1989).

1.2 RISK ASSESSMENT IN OCCUPATIONAL HEALTH

Historically, exposure assessment was almost exclusively based on environmental monitoring or prediction of pollution concentrations in different media; while dose-response assessments were founded primarily on experimental toxicology studies. Only recently, studies have focused on actual environmental/occupational human exposures and the understanding of the causal relationships between exposure, dose and effect by carrying out risk assessments in human populations.

The risk assessments conducted are mainly quantitative, however in some instances, as in cancer assessment, where the answer could be "yes or no" (i.e. hazard does or does not cause cancer), the risk assessment might be qualitative. In some other instances, where there is no information on possible potential hazard (i.e. new industrial process), risk assessment starts qualitatively in the hazard recognition step (Paustenbach, 1989).

The goal of regulatory and legislative bodies like the Health and Safety Executive (HSE) in UK and Environmental Protection Agency (EPA) in USA is to eliminate or, where not possible, reduce critical uncertainties associated with health risk assessment for environmental/occupational exposure to pollutants (Fowle and Sexton, 1992). Unfortunately, currently for most pollutants little is known about the causational relationships existing between exposure and toxic outcome. The quantification of these causal relationships is the basis of health risk assessment.

Biological monitoring provides the possibility of clearing these uncertainties that usually encumber health risk assessment. To understand the role of biomarkers in human health risk assessment with environmental / occupational exposure we must define the train of events between external exposure to xenobiotics and the resulting health effects. For a health effect (biological response) to take place a xenobiotic must be absorbed into the body, reach the target organ and result in a biological change. This cascade of events is illustrated in the schematic diagram (fig. 1.1).

Biomarkers provide the possibility of understanding, qualifying and quantifying the relationship existing between the different stages in the train of events. Biomarkers of exposure, effect, and susceptibility in humans provide information on the extent of absorption of pollutants and how much reaches the target organ, how the target dose ("biologically effective dose") is related to effect and who in the population at risk has increased or decreased resistance to absorption of and/or effect from xenobiotics, respectively. The selective use of biomarkers that are related to a biological response or toxic outcome will improve the reliability of predicting risk to human health.

Good examples of biomarkers are blood lead in lead exposure, pulmonary function in response to respiratory irritants such as formaldehyde and nitrogen oxides, acetyl cholinesterase inhibition predicting exposure to an organophosphate compound and measurement of DNA-adducts for identifying exposure to chemical carcinogens.

For biomarkers to aid in a quantitative risk assessment they should be properly selected for the purpose. They should prove to be linked to a pathological change which by turn influences the course of the disease development. They must be analytically sensitive, reliable, reproducible and validated. Baseline values in the normal populations and confounding factors need to be known. All this information is crucial for the validation of the biomarker. Proper quality control measures should be utilised to ensure the precision, accuracy, and reliability of the results which will ultimately improve the accuracy of the risk assessment process.

Current challenges in the field of environmental/occupational toxicology are to improve the process for assessment of risk to human health from exposure to xenobiotics by using biological monitors that are able to predict all potential pathological alterations and toxic outcomes. The objective of this research program will provide the opportunity for better effective preventive control measures to alleviate exposure deemed risky to human health. These programs are adopted by agencies like EPA. The agencies rely heavily on quantitative assessment of environmental/occupational health risks as the scientific basis for deciding on how best to safeguard the public health. Adopted programs are

of two types, the long range basic research and short-term type of research. The study described in this thesis falls under the latter type. Both types answer questions at the interface between the two ends of the risk assessment cascade of incidents.

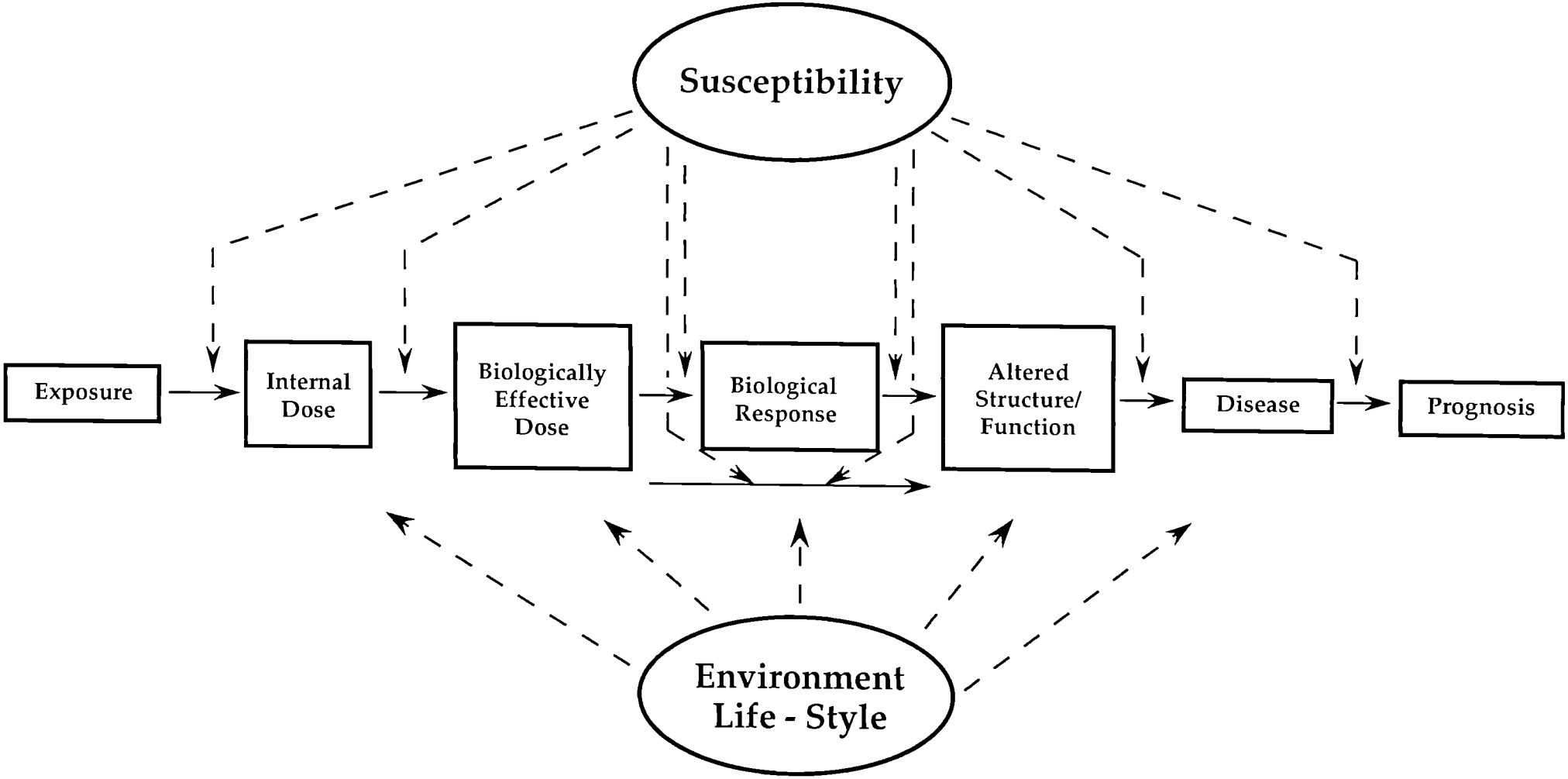

Susceptibility
Exposure
Internal Dose
Biologically Effective Dose
Biological Response
Altered Structure/ Function
Disease
Prognosis
Environment Life - Style

Figure: 1.1

CHAPTER 2

OCCUPATIONAL MONITORING

2.1 MONITORING EXPOSURE TO OCCUPATIONAL HAZARDS

The main function of occupational hygiene is to control exposure to hazards in the occupational environment which is part of a multi-disciplinary approach to the prevention of occupational disease or unacceptable discomfort (King, 1990). This could be achieved only if hazards are recognised, quantified, controlled and finally re-evaluated.

There are three main ways of measuring an exposure to hazards which are usually based upon routine sampling protocols or monitoring unless it is used in research.

The first type of measurement used for hazard quantification is ***ambient monitoring,*** which is utilised to assess external dose. Another way of assessing hazards is by ***biological monitoring*** which is the tool for quantifying internal dose. Last but not least is ***biological effect monitoring*** which quantifies a biological response to hazard exposure.

The three types are discussed below in more details highlighting the advantages and disadvantages of each.

2.2 AMBIENT MONITORING (fig. 2.1A)

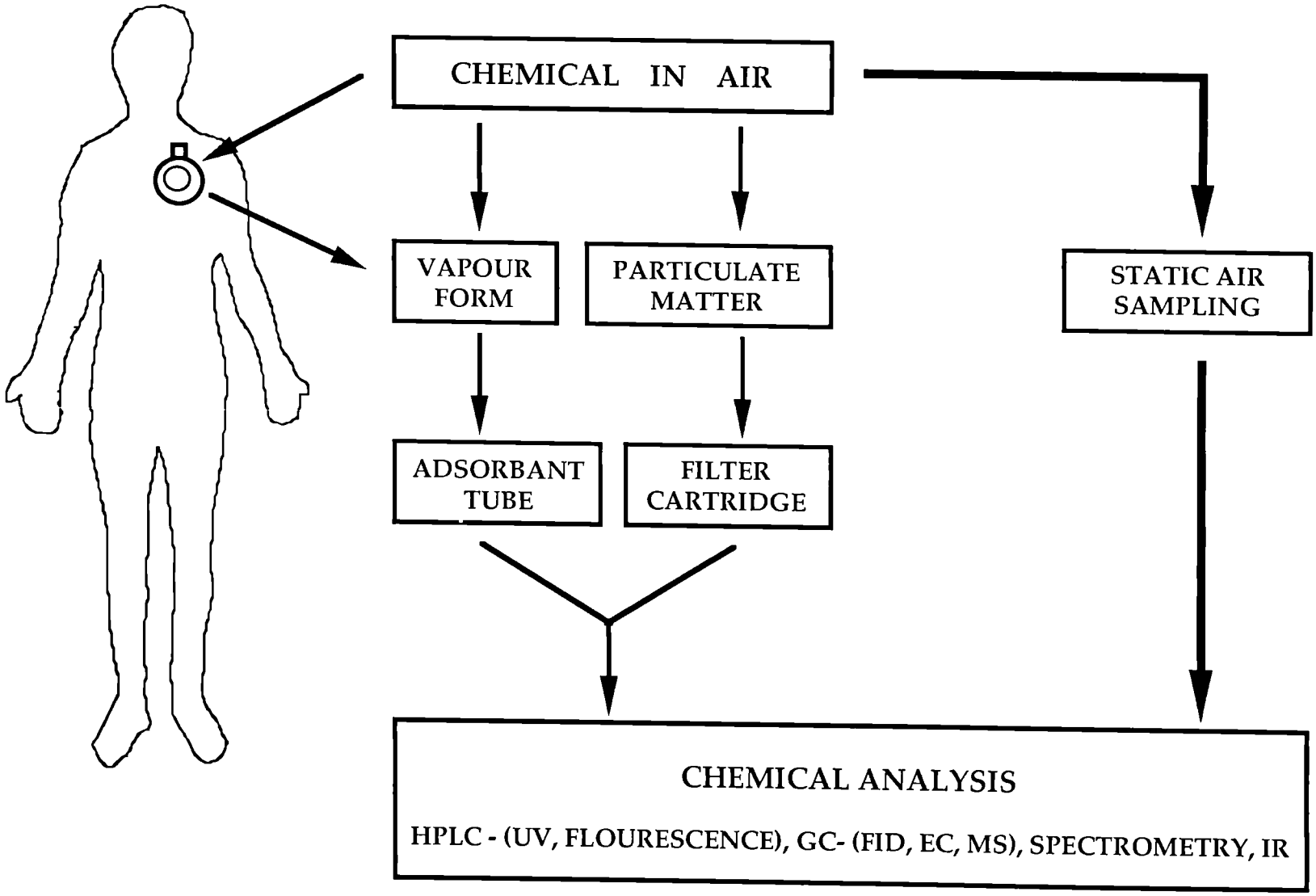
2.2.1 Definition

Ambient monitoring (environmental monitoring, EM) is "the measurement and assessment of agents at the workplace to evaluate ambient exposure and health risk compared to an appropriate reference" (Zielhuis and Henderson, 1986).

In other words, ambient monitoring is the measurement and assessment of potential exposure to agents in the occupational environment. It is the evaluation of external exposure "external dose" by its quantification and comparison to appropriate set standards (i.e.

Figure : 2.1a

AMBIENT MONITORING



Threshold Limit Values (TLVs), Occupational Exposure Limits (OELs), etc.).

2.2.2 Types

There are two types of ambient monitoring. These are area sampling and personal air sampling. Each type has got its uses, advantages and disadvantages.

2.2.2.1 Area Sampling

Area sampling (static sampling) (AS) is primarily performed to monitor the performance and effectiveness of hazard control systems (e.g. local exhaust ventilation) or changes in the industrial process. Its main advantages is that it can be used to monitor and check on efficiency of hazard control. It could be interchanged with personal air monitoring if the workers are not mobile. The equipment used can sample at a wide range of flow rates since the pumps can be main power operated. They are easy to handle and cause minimal trouble to the workers as well as the occupational hygienist.

2.2.2.2 Personal Air Monitoring

Personal air monitoring (PM) is mainly used to assess and estimate the health risk to the occupants of the workplace. They are used to check for compliance with occupational exposure standards (OELs). They can also be used in monitoring for a change in the industrial process. They are useful as part of a strategy design to investigate a complaint.

The main advantages of PM are its usefulness in assessing individual exposure by sampling air as close to the breathing zone as possible. Personal monitoring is important in risk assessment of exposure to hazards, defining dose-response relationships and setting occupational exposure standards.

The disadvantages of PM techniques is that they suffer from non-response from the workers. They are not easy to handle and equipment

used can not sample large volumes of air which is necessary in circumstances where the pollutant's air concentration is quite low. They are of limited use in assessing the performance of hazard control systems especially when the workers are not static.

2.2.3 Practical Considerations

There are a number of methodological limitations which should be taken into consideration when choosing and applying EM techniques, interpreting results, and designing the sampling strategy. The type and concentration of the pollutant of interest are the major factors affecting the choice of the EM method. The purpose of the investigation or risk assessment is also of primary importance. For example in some occupational environments direct reading instruments are of more use when compared to indirect reading methods. Choosing from available qualitative, semi-quantitative and quantitative EM methods should be considered carefully in the light of the investigation under study. Knowledge of methodological issues like characteristics of the EM method (i.e. specificity, sensitivity, reproducibility etc.), instrument design and use, errors in calibration, sampling rate, sample position, duration of sampling, sample preparation and analysis and variability due to operator errors is crucial for results interpretation and reliability of the risk assessment. Other factors such as the industrial process, the effects of external confounding factors (e.g. weather conditions) should be understood prior to designing the sampling strategy.

The advantages and disadvantages of ambient monitoring are listed in table 2.1.

2.3 BIOLOGICAL MONITORING (BM)

2.3.1 Background

Biological monitoring of exposure to a xenobiotic in the work

Table : 2.1 Advantages and Disadvantages of Ambient, Biological, and Biological Effect Monitoring

Ambient Monitoring

Advantages	Disadvantage
* Can be used for the evaluation of engineering controls * Cost-effective for preliminary identification of risk * Easy to collect and analyse samples * Less confounding factors to take care of * More welcomed by workers with respect to other types of monitoring * No need for an ethical permission * Relatively less time consuming	* Can not evaluate individual's exposure and ultimately true internal dose * Can not pick susceptible individuals * Can not account except for occupational exposure * Gives information about external exposure through inhalation only

Biological Monitoring

* Can give an estimate of true internal dose * Accounts for all routes of exposure.(Inhalation, ingestion, and skin absorption) * Can pick up susceptible individuals * Important for setting OES, TLVs etc. * Accounts for exposure in all environments * They could be selective and non-selective * More related to adverse health effects	* Sufficient knowledge about toxicokinetics of agent/s of interest is needed * Confounding factors affecting biologic output. * Sometimes costly * Results might be misleading. (Especially when site of action is important and not the metabolite. * Needs ethical approval * Not always welcomed by workers (especially blood sampling)

Biological Effect Monitoring

* Could be utilised to predict mutagens and carcinogens * Best indicator of an adverse health effect * Not much information is needed about toxikinetics of the xenobiotic/s of interest * Can pick up susceptible individuals * Accounts for all routes of exposure * Accounts for exposure in all environments	* Sometimes analysis is cumbersome * Sometimes costly * Needs ethical approval * Relevance to disease is not always known * Not always welcomed by workers * Very specialised techniques are needed sometimes * Specificity must be high

environment means evaluation of the internal exposure of the workers to the toxic agent(s) (i.e. the internal dose) by a biological method (Lauwreys, 1984).

Several papers in the early 1980's have broadly used the term biological monitoring for more than one type of biomonitoring activity (Friberg, 1982; Lauwreys, 1983 and Aitio *et al.*, 1983). This loose usage of the term "biological monitoring" is misleading. To avoid misusage, it is necessary to elaborate on the topic of biological monitoring since the rapid expansion of monitoring activities in occupational health has increased the confusion among the different programs (Zielhuis and Henderson, 1986). Understanding the meaning of the different activities, their uses, characteristics and applications is important to avoid misuses of terminology, erroneous result interpretation and decision making. It is worth noting that there is an unclear overlap between the different activities which results from our limited knowledge for understanding the implications of certain biological indicators.

It is useful to classify biological monitoring activities into three types: biological monitoring (BM), biological effect monitoring (BEM) and biological susceptibility monitoring (BSM). For each type of monitoring a different class of biomarkers (biological indicators) is used. "Biomarkers of exposure", "biomarkers of effect" and "biomarkers of susceptibility" are applied in BM, BEM, and BSM programs, respectively.

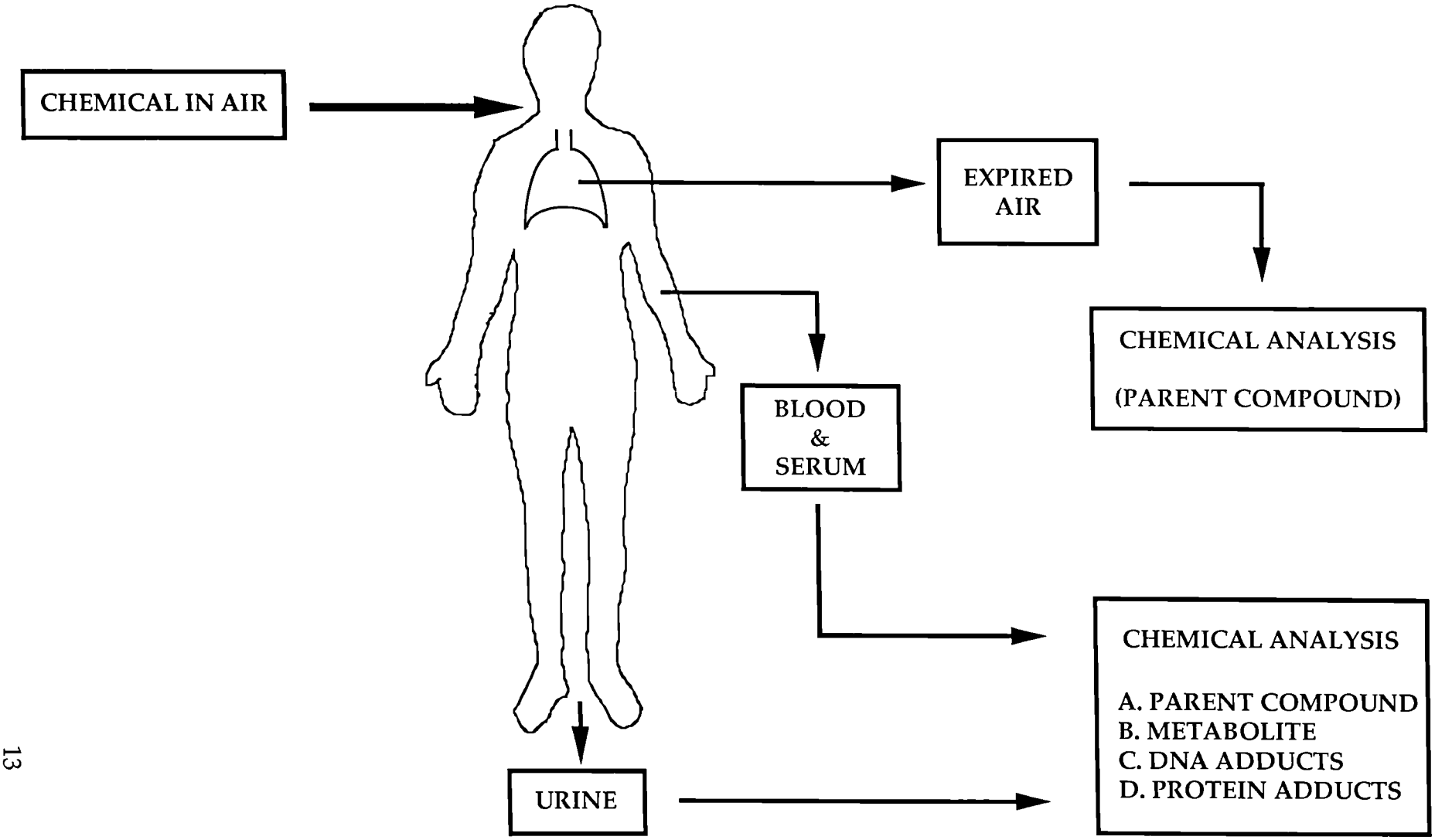
2.3.2 Types of Biological Monitoring Activities

2.3.2.1 Biological Monitoring of Exposure (Fig. 2.1b)

Biological monitoring of exposure is "the measurement and assessment of agents or their metabolites either in tissue, secreta, excreta or any combination of these to evaluate exposure and health risk compared to an appropriate reference" (Zielhuis and Henderson, 1986). In other words, BM of an industrial hazards is the evaluation of the internal exposure "internal dose" of a worker or a group of workers to the hazardous agent/s by a biological method. It is important to define the term "internal dose" before its usage since it may mean the amount of

Figure : 2.1b

BIOLOGICAL MONITORING



agent absorbed in a specific period of time or the amount already stored in the body (body burden).

Biological monitoring is related to "uptake" (intake x fractional absorption) through several pathways simultaneously. It is based upon the knowledge of the *impact of man on the agent*. An understanding of biological monitoring indices depends on knowledge of the xenobiotic's toxicokinetics including uptake, distribution, absorption, biotransformation, accumulation and elimination.

The main advantages and disadvantages of biological monitoring of exposure are listed in table 2.1.

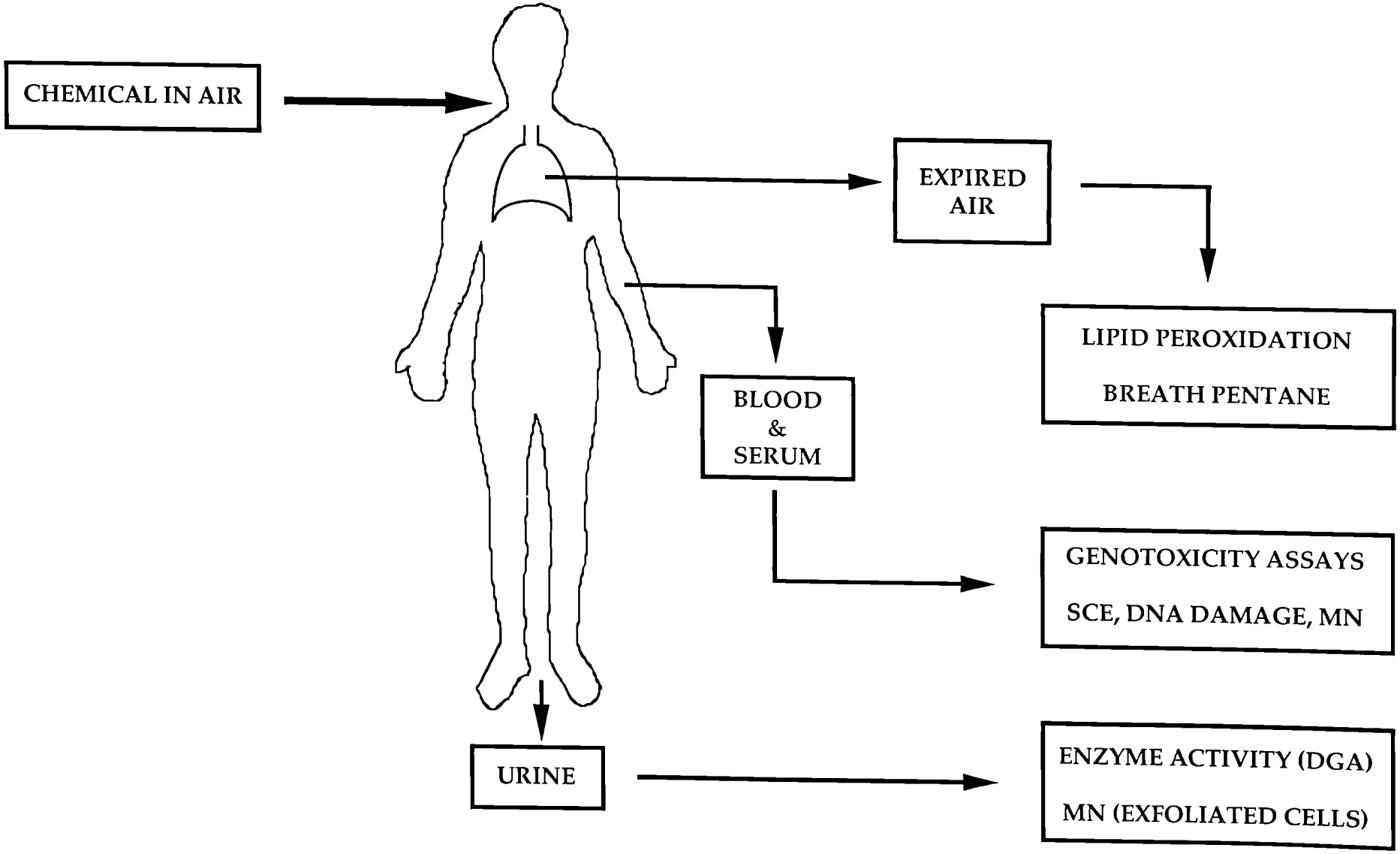
2.3.2.2 Biological Monitoring of Effect (Fig. 2.1c)

Biological effect monitoring is "the measurement and assessment of early biological effects, of which the relationship to health impairment has not yet been established, in exposed workers to evaluate exposure and/or health risk compared to an appropriate reference" (Zielhuis and Henderson, 1986). Biological effect monitoring is related to the biologically effective dose (i.e. the dose at the target organ/tissue where the toxic effect is manifested). It is the *impact of the agent on man*.

BEM screening methods are utilised to identify or detect adverse and non-adverse effects preferably when they are preventable and still reversible. It is of importance to make a distinction between adverse and non-adverse effects. In some cases the distinction is somewhat ambiguous since the health significance of an effect is not known. The key question is, of course, which of those different methods used for monitoring exposure to hazardous agents are reasonably reliable for prediction of disease. Currently, research in the field of occupational toxicology and other disciplines is trying to answer this question. The major problem lies in extrapolating from observed biological effect levels to estimates of risk for exposed populations (Saffiotti, 1980). When the health pertinence of a biological effect marker frequency is not regarded as "adverse" then it cannot assess health impairment but will point to the existence of a health risk. Solely when the effect biomarker level is considered as "adverse" can it be used to appraise the impairment of health.

Figure : 2.1c

BIOLOGICAL EFFECT MONITORING



The main advantages and disadvantages of biological effect monitoring are listed in table 2.1.

2.3.2.3 Biological Monitoring of Susceptibility

Biological susceptibility monitoring could be defined as "the measurement and assessment of an internal or acquired frailty of an individual in responding to the challenge of exposure to a specific xenobiotic substance, used for subjects' classification with regard to metabolic susceptibility and in relation to health risk". Biological susceptibility monitoring is related to the capability of the subjects to respond to the challenge of exposure to a particular physical, chemical or any type of hazard(s) (Committee on Biologic Markers, 1989).

2.3.3 Uses of Biological Monitoring

Scientists have used occupational monitoring for many years to assess the health risks of workers. In early years, air monitoring techniques were largely confined to area sampling. This meant that biological monitoring was the only means of assessing personal exposure to hazards. With the advent and development of personal air monitoring, the use of BM as the only means for monitoring personal exposure progressively disappeared.

Direct comparison of external dose to internal dose became possible with the advances of personal air monitoring techniques. It was observed that for apparently identical ambient exposure the inter-individual variability of the biomarkers' results was large which discouraged industrial toxicologist and other scientists to use BM for exposure assessment. More recently, scientists have been thoroughly addressing issues of BM such as uses, selection, validation, quality control, variability, practical and ethical considerations which all assisted the BM approach to regain its importance in risk assessment of exposure to hazards. Another positive outcome of that intensive research program was highlighting the usefulness of BM in the field of environmental/ occupational health and research in molecular epidemiology.

2.3.3.1 In Occupational Health

A major objective in environmental and occupational health is the prevention of health impairment from exposure to hazards in the general and work environment. To achieve such an objective permissible estimated exposure levels for hazards must be set. These levels must cause no adverse health effects if individuals are exposed to the hazard(s) over a life time period. To ensure that such an objective is met the industrial hygienist must be able to compare past and current exposure data of workers with recommended occupational exposure levels. For that purpose biological monitoring techniques were introduced and results obtained needed to be compared to permissible biological levels (Lauwreys, 1984). Once a sampling strategy has been conducted the health professional will need reference levels against which to evaluate the results obtained. Various countries use different permissible biological levels and their way of interpreting the results is fundamentally different. Industrial toxicologists in U.S.A. use Biological Exposure Indices (BEIs) which are set by the American Conference of Governmental Industrial Hygienists (ACGIH). BEIs are the biological equivalent of exposure at current TLVs. They have two disadvantages, first, they do not account for dermal route and secondly the values relates to average uptake at the recommended TLVs which suggest that 50% of the exposed working population at these levels will exceed the BEIs (Pryde and Gompertz, 1994). When analysing their data and comparing them to BEIs, US. scientists do not base their decision and recommendations on individual results however they consider the mean levels followed over a period of time or across different control and exposed working populations (Droz and Wu, 1991). In Germany, health professionals use the Deutsche Forchungsgemeinschaft (DFG) or a what is known as Biological Tolerance Values (BAT). They are the maximum permissible quantity of a chemical or its metabolite or any deviation from the norm of biological parameters induced by these substances that do not impair the health of employees even if exposure is repeated and for a long duration. German health professionals seems to take an opposite approach. They do consider that individual results are interpretable. They do base their judgement on the results either individually and/or collectively. Up to date, there is no formal strategy followed in UK for setting permissible biological limits. The only two biomarkers which have upper permissible biological limit and advisory

limit are blood lead and urinary mercury, respectively.

The advent and use of biomarkers and biological exposure limits had an impact on several aspects of the field and practice of occupational health and medicine. Occupational physicians need validated markers and are sometimes pressurised to use markers that are not entirely validated in the process of diagnosis of diseases arising from occupational exposure to hazards (Schulte, 1991). The fact that biomarkers can indicate occupational and non-occupational exposures, implied that their use will eventually introduce difficulties over the distinction between occupational and other medical specialities. Their detection forces the occupational physician to consider non-occupational factors such as genetic characteristics, diet, smoking and drinking habits, use of medication, domestic exposure to hazards in addition to occupational exposures.

The use of BM might prompt the health professionals to efficiently use exposure, risk assessment, and pre-clinical registries. This will have a major contribution to epidemiological research in the field of occupational health. This information however might be misused in a discriminatory mode by the employers, while from the workers' point of view the presence of any exposure documentation might help as a catalyst to raise a lawsuit. As for courts and regulatory bodies BM would satisfy their need for evidence that accounts for the magnitude of occupational risk in the light of confounding factors (Hulka and Margolin, 1992). Workers' compensation ultimately depends on the causation relationship between occupational exposure and disease. The use of biomarkers in qualifying and quantifying exposure will assist in understanding of that relationship. This will lead to their increased application in the compensation procedures.

2.3.3.2 In Epidemiological Research

Biological markers are becoming prominent features of many classical epidemiological studies. Their existence has also modified the character of some epidemiological research. Collaboration between laboratory and epidemiological investigations became indispensable to research on public health.

Current epidemiological research on occupational diseases is carried out at cellular and molecular levels. This is a recent advancement which offers new avenues for diseases prevention and control. These avenues are what the current available biomarkers can pave.

Their high sensitivity gives them the potential of detecting interactions of xenobiotics with macromolecules and determining alterations occurring in the early stages of the continuum between exposure and disease, at a time when the response is still a reversible homeostatic adaptation. This characteristic is essential since today's type of exposures in developed countries are usually low and chronic and the effects are less prominent in many occupational environments.

Different types of biomarkers have different uses in epidemiological research. While biomarkers of effect determines changes taking place in the continuum between exposure and health impairment others like biomarkers of susceptibility allow the separation of people at risk from those not at risk thus leaving less room for misclassification. This eventually increases the power of the epidemiological study in identifying risks to health (Hayes, 1992).

The main contribution of the molecular epidemiological approach to research on the prevention of environmental/occupational diseases is that scientists no more have to study the morbidity or mortality of an unfortunate cohort so that coming working generation will harvest the results and benefit from the outcome. In other words we do not have to "count the bodies" instead we can "count the molecules" (Schulte, 1991).

The promise that the biological monitoring approach holds towards the contribution of transitional epidemiological research to disease prevention is great.

In contrast to the multiple uses of biomarkers in the field of molecular epidemiology there are several misuses that are almost overlooked by scientists. Scientists main concern is focused on the ways biomarkers could aid in risk assessment and contribute to the field of molecular epidemiology.

Hammond (1991) stated clearly that in a short opening sentence "markers can be misused". Hammond (1991) through three examples, stressed the misuses of environmental markers in the risk assessment of exposure to complex mixtures. Similar misuses can happen when using biological indicators.

A good example is the replacement of biomarkers of effect in target organs by surrogate non-target organ biomarkers. In some cases the non-target organ effect biomarker becomes of great interest to the scientific community, due to a series of evidences which eventually side-track the investigators from the fact that a target-organ effect biomarker is what is needed for an accurate health effect characterisation.

Side-tracking can take place in molecular epidemiology studies. For instance, scientists are currently concentrating on trying to clarify the potential relationships existing between the mechanisms of DNA adduct formation and their levels in lymphocytes with the incidence of bladder and lung cancer (Gaylor, 1992 and Jacobson-Kram, 1993). Instead they should try to develop and validate DNA adduct in exfoliated cells of the urinary tract and respiratory tract as a target-organ effect biomarker. This means that "effect biomarker-tumour incidence correlation" will be drawn at the same site in the same organ.

Likhachev *et al.* (1993) in the introduction to their article supported the fact that certain biomarkers can be misused. They wrote "many endpoints, discovered by currently used markers, such as haemoglobin adducts and non-identified DNA adducts, are non-specific and not related to carcinogenesis and cannot be used as reliable biomarkers of individual susceptibility to carcinogens".

2.3.4 Current and Future Research

Current research in the field of environmental/occupational health and molecular epidemiology biological monitoring activities have received increased attention.

Literature review, on biomarkers of exposure, shows that in 69% of the published research studies the parent compound is the measured endpoint; while only 19% looked at metabolite levels. Twelve percent of the literature assessed agent-specific, non-adverse effects biomarkers. Cross-sectional design was used in about 73% of all studies; 50% and 28% used blood and urine as source of specimen, respectively. The xenobiotic compounds most frequently studied were lead (23%), cadmium (5.2%), toluene (3.8%), polychlorinated bi-phenyls (3.8%), asbestos (3.6%), mercury (3.6%) and styrene (3.1%). The publications studying the listed compounds total up to 46.1% (Yager, 1991).

These figures give us a profile of the current research on biological markers up to 1991 only. The picture conveyed by this data is neither comprehensive nor clear. For instance, 53.9% of the studies on biological monitoring of xenobiotic compounds were not identified. It is also worthwhile noting that this data might not be true at this point in time when a great deal of work is being done on biological monitoring of exposure to carcinogens.

Most progress has been made in the area of biomarker development and validation and ensuing refinement of innovative techniques. The available methods are remarkably sensitive for detecting marginal increases in biomarkers levels over the observed background levels (Yager, 1991).

It is quite obvious that, after these advancements have been made, there is an urge for **future research** to aim at understanding the characteristics (i.e. toxico-kinetics, toxico-dynamics etc.) and implications of all the types of biomarkers. This will aid in unveiling temporal relations thereby allowing rational biological monitoring programs to be conducted. The relationships of these characteristics to the critical site of toxic action of chemical need to be known. These in turn allow us to assess the relation of those characteristics to a biologically significant effect.

Future studies should further the validation of biomarkers in humans and increase our knowledge about baseline frequencies of biomarkers in the "control" general population are needed. After knowing the exposure-response relations, the influence of exposure patterns (dose

rate) on induction and persistence of these endpoints need to be determined. This will allow better effective remedial control measures to be recommended and practised after the occurrence of exposure. Research into the possible relationships existing between endpoints and disease should be carried out through experimental animal studies and classical and molecular epidemiological studies. Addressing the following issues is necessary for tightening our understanding on the usefulness of these assays for risk assessment.

Some governmental agencies, such as EPA, have set their priorities regarding biologic markers research in environmental health. Fowle and Sexton from the Office of Health Research at U.S. EPA published an article in 1992 describing current research interests on exposure biomarkers and effect biomarkers of carcinogenesis, pulmonary toxicity, neurotoxicity, reproductive/developmental toxicity, hepatotoxicity and other toxicity effects. They stressed future research needs with respect to EPA priorities in meeting regulatory requirements. In general, current research is at developmental and validation stages; while future research needs are to find more appropriate, non-invasive biomarkers for large population monitoring as much as establishing possible relationships between exposure, dose, non-adverse effects and health impairment.

2.4 BIOLOGICAL MARKERS

2.4.1 Definition

Biological markers in the context of environmental and occupational health are indicators signalling events in biological systems or samples.

2.4.2 Types

There are three types of biomarkers used in biological monitoring programs. These are biomarkers of exposure, effect and susceptibility. They

are tools to recognise a sequence of biologic events taking place upon the occurrence of exposure to toxic agents. These happenings may serve for identification of initial exposure, acquired dose, biologically effective dose (i.e. dose at site of toxic action, dose at the receptor site or target macromolecule) and altered function/structure with no subsequent pathological development or possible or factual health detriment (Committee on Biological Markers, 1989). Regardless of the exposure, biological variability in organisms can bring about some to be hyper- or hypo-susceptible to environmental/occupational induced diseases. In essence, biomarkers are indicators that can be utilised to elucidate the connections existing between exposure to a xenobiotic substance(s) and health impairment.

2.4.2.1 Biomarkers of Exposure

A biologic marker of exposure is "an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism". These biomarkers measure the levels of the parent compound or its metabolite(s) in different body fluids. If the parent material is responsible for the toxic outcome then the amount of metabolite reaching the target site or being excreted in body fluids might be of limited importance. On the other hand, if the metabolites are causing the toxic insult then the metabolism occurring in different compartments in the organisms is an important determinant of absorbed dose and biological effective dose (Committee on Biological Markers, 1989).

Basically, exposure biomarkers are used to evaluate internal exposure (internal dose). If the exposure biomarker levels are quantified in the target organ, tissue or cells they will reflect the extent of the biologically effective dose.

2.4.2.2 Biomarkers of Effect

A biologic marker of effect is "a measurable biochemical, physiological, or other alteration within an organism that, depending on magnitude, can be recognised as an established or potential health impairment or disease". In other words a biologic marker of effect or

response can be referred to as a qualitative or quantitative change capable of predicting potential or actual health impairment arising from exposure. They could also be indicative of normal health (e.g. blood glucose) (Committee on Biological Markers, 1989).

For better comprehension of the meaning of the biological effect marker, the term effect must be put in the right context. An effect is defined as a real health detriment or recognised disease, an early precursor of a disease process that indicates a potential for health impairment, or an event peripheral to any disease process, but correlated with one and thus predictive of development of an impaired health status.

2.4.2.3 Biomarkers of Susceptibility

A biological marker of susceptibility is "an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance(s)". For instance, a phenotypic or genotypic characteristic or a pre-existing disease that results in an increase in the absorbed dose, the biologically effective dose, or biological response can be a potential for a marker of susceptibility. This type of biomarker can serve as a biomarker of exposure or effect (Committee on Biological Markers, 1989).

Up to date their application have been limited due to the lack of availability of validated markers. However scientists efforts have been directed towards recognising potential markers and assessing the extent of their usefulness and their impact on the fields of occupational health and molecular epidemiology.

2.4.3 Selection

There are several criteria that must be considered when selecting a biological marker. Ideally, the biomarker should respond in a consistent and quantitative fashion to exposure (especially at low doses) and should be selective for the environmental exposure of interest. In some instances non-selective biomarkers maybe useful if they can be related to external

exposure to the xenobiotic of concern (Committee on Biological Markers, 1987).

Basically, the biomarker's method of analysis must be cheap, non-invasive and easy to measure in a large population. It should be able to indicate qualitatively and/or quantitatively reactions between the agent and the exposed organism and be associated with toxic outcome. Of course, its specificity to the reaction is crucial. Reproducibility with defined precision and accuracy is an important attribute of a good biomarker. It must be prevalent in the sampled population with known variability in the non-exposed population. Being common between species would be of great assistance for result interpretation and proper development (Mercier and Robinson, 1993).

When the exposure is of a complex nature and the chemical composition of the mixture is known; the ratio of the marker to the active agent(s) should be established (Hammond, 1991). A good biological indicator of choice is one which the complex mixture is the only possible source. It should not be affected by other contaminants. The biomarker or its precursors should undergo chemical transformation or be removed from the environment at the same rate as the agent of interest. The national research council recommends that the toxicity of the marker should represent the toxicity of the mixture. Hammond (1991) disagrees with that recommendation stating that the relevant toxicity of the mixture in most instances is unknown. So as long as the marker is properly quantified and proportional to exposure it can be useful at a later stage when the toxicity of the mixture's active ingredient is identified.

In summary, all the above criteria and recommendations should be considered when selecting the biomarker. Ultimately, selection regardless of the characteristics of the marker depends upon the aspect of exposure which is to be predicted. The question that has to be answered is whether external exposure, total uptake or target dose of the active intermediate is what is being assessed. For example, a marker with a high residual variability when correlated with external exposure nevertheless can be very good predictor of certain aspects of internal exposure. The reverse is also true (Droz and Wu, 1991).

2.4.4 Validation

For biomarkers to contribute to occupational health, epidemiological research and to the risk assessment process they must be validated. The biomarkers' validations usually require time-consuming appraisals and are achieved by establishing that a relationship exists between an exposure and the biological change of interest.

When addressing validity the topic must be put in the right context. Validity has got several definitions. In the interest of this study only two will be considered: laboratory and population validity.

In the context of epidemiology, population validity refers to how well the markers depict an event in a population such as disease occurrence. In the epidemiological approach evaluating validity implies assessing sensitivity and specificity and more importantly prevalence. These factors, and in particular prevalence of the marker in the sampled population, affect the predictive value of the biomarkers. There are two epidemiological approaches for evaluating validity. Those that focus on outcome (disease) and those that focus on exposure (environment). The first is the clinicians' approach whereby sensitivity and specificity are studied in relation to diseased or healthy. On the other hand the health scientists' approach refers to sensitivity and specificity of a biomarker relative to exposed and non-exposed individuals.

Laboratory validation of a biomarker means the minimum level of an analyte that an assay can detect (Hulka and Margolin, 1992). In technical terminology sensitivity means the smallest single value that can be distinguished from zero with 95 % confidence interval. Specificity in the laboratory indicates the ability to detect a unique analyte among a group of closely related structures. In short laboratory validity depends on the characteristics of the test (reliability, accuracy, precision etc.) and on biological characteristics of the marker.

One way of validating biomarkers is to define the correlation between the biomarkers and the external exposure quantified by ambient monitoring. This approach, however has been criticised by Hulka and Margolin (1992) as well as Schulte (1991). They both did not recommend

this approach because it appears to lack conceptual coherence. This incoherence is due to the fact that biomarkers are better and more accurate indicators of exposure to xenobiotics. This does not mean that information obtained from an observed correlation is not useful. They can improve the relevance of external measurements which is needed in cases where the biomarker is too costly to obtain or unavailable.

An alternative way for validating a biomarker is to develop a matrix of information from experimental toxicology studies in animals and clinical studies in humans, that enables one to make estimate for environmentally/ occupationally exposed humans. Information collated from studies on markers of acute effects for short-term high level exposure to pollutants can be compared to markers for chronic effect resulting from long-term low level exposure of animals to the same pollutants. This can lead to the development of markers that are predictive of health effects in chronically exposed humans (Committee on Biological Markers of the National Research Council, 1987). The use of animal studies that restrict various host and environmental confounding factors are necessary for assay development, to generate dose-response curves, to assess sources of assay variability and to understand whether the biomarker has a role in the pathogenic pathway or merely presents an adaptive response on the part of the organism. Preliminary studies in human tissue must be conducted to evaluate the usefulness of the marker for biologically monitoring an exposed population. This sometimes requires persons who are or were heavily exposed to the xenobiotic of interest before moving onto individuals with low to moderate levels of exposure to pollutant.

The current situation is that very few biomarkers have been able to satisfy these standards. Preliminary studies, such as the one described in this thesis, are necessary before studies of predictive validity can be proposed.

2.4.5 Variability

Variability is an inherent component of all our data and data sources. There are three main categories of variability in molecular

epidemiology studies utilising biomarkers for risk assessment of exposure. The first category is environmental variability which is mainly related to type and patterns of exposures (e.g. duration of exposure, concentration of pollutant, form of pollutant etc.). The second category is laboratory variability. This could be of two types random and systematic. The third category is biological variability. That is also of two types intra-individual and inter-individual variation. The following section explains what is meant by the different types of variability and their effect on different aspects of biological monitoring such as choice, sampling strategy, data analysis and interpretation.

i) Environmental Variability: It is important to understand the characteristics of the biological biomarker in order to understand how environmental variability (i.e. variability in external exposure) might affect its results. Droz and Wu (1991) in their article have demonstrated how exposure profiles might affect different biomarkers. They studied the effect of continuous exposure (constant), constant industrial exposure (cyclic) and fluctuating industrial exposure (erratic) on two hypothetical biomarkers one with a short half-life ($t_{1/2}$) of 5 hours and the other with a long $t_{1/2}$ of 50 hours behaving according to a one-compartment kinetic model. In the first case they observed that the excretion of the biological indicator reaches a steady state after certain period of time. So sampling after that point in time would give an accurate measure for exposure and dose-response assessment. This situation leads to a conclusion that biomarkers with a long $t_{1/2}$ (>40 hours) should not be utilised to evaluate transient situations arising from the implementation of new engineering control measures or use of personal protective equipment. A sufficient period of time must elapse before these indicators can provide accurate information on internal exposure levels.

In the second type of exposure (cyclic) the biological monitoring strategy becomes more complex and sampling time more critical. The biomarker levels fluctuate considerably within a day. This makes it more difficult to control for the variation introduced by sampling time.

In the third type of exposure (erratic) the levels of the biological indicator are even more variable than those observed in the case of cyclic exposure especially for the biomarker with a $t_{1/2}$ of 5 hours. For defining

long-term mean exposure levels in order to relate it to chronic health-effects; indicators with a long biological $t_{1/2}$ are of more use than the one with a short biological $t_{1/2}$. They concluded that with the use of biomarkers with long $t_{1/2}$ (> 40 hours) less than 50% of exposure variability will be transmitted to the biomarker's measurements thus damping the effect (Droz and Wu, 1991).

Other factors can contribute to environmental variability which in turn will introduce variability in the biomarker's findings. The form (i.e. gas, vapour, particulate matter etc.) and particle size the pollutant is present in are types of environmental variability that potentially can affect the internal dose of a xenobiotic thus affecting levels of the biomarker in target organs or body tissues.

The composition of the mixture usually varies with variation in particle size. With a decrease in particle size the surface-to-volume ratio increases thus increasing the proportion of chemical adsorbed to the surface in relation to its mass. This also alters the site of toxic action and the area of absorption. A good example is the difference between respirable (< 10 μm in dia.) and inhalable (> 10 μm in dia.) particulate matter. The former is related to alveolar effects while the latter to tracobroncheal effects.

The form the contaminants are present in will contribute to environmental variability. If the pollutant is in the gaseous or vapour form rather than particulate matter it will be more readily available to be absorbed through the respiratory system. On the other hand, if the reverse is the case the dermal route might play a considerable role in the absorption of the xenobiotic. So the form the pollutant is present in alters the route of absorption which in turn will introduce variability in the biomarker measurements as well as affecting the health risk associated with the exposure. It is well known that gas solubility of a certain pollutant can influence the part of the respiratory tract that is affected which might not be true for other forms of the same pollutant (Hammond, 1991). Therefore, the above mentioned characteristics of the contaminants introduce variability in the biological measurements and are important factors to account for in the selection process of the biomarker.

Particle size, form, and complexity of the mixture individually and collectively affect the bioavailability of the pollutant for absorption and eventually the dose reaching the target organ. For example it is well known that vehicles "solvents" have an effect on the absorption of chemicals through skin. In 1969, Allenby *et al.* demonstrated that the ability of a number of liquids "solvents" to accelerate the percutaneous penetration of tri-n-propylphosphate (TPP) through human full thickness skin could vary significantly. They found that di-methylsulphoxide (DMSO) is capable of accelerating the permeability of full-thickness human skin to TPP up to 173 times. They anticipated that its effectiveness may be due to its ability to decrease the diffusional resistance of the stratum corneum. The importance of the pollutant's form is also demonstrated in the example of gas solubility presented above.

ii) Laboratory Variability: This type of variability is of two kinds random and systematic (bias). In the laboratory, random error is an issue of error in the sampling. Random error is reflected quantitatively in the coefficient of variation (CV). It is usually determined from the CV of the data acquired from analysing aliquots of the same sample under identical conditions. With the increase in the number of aliquots the precision can be improved. Systematic error is reduced by blind analysis of the samples. There are several sources of potential error in the laboratory that could bias the results of assays. Day to day variation in the equipment's performance and batch to batch variation are common sources. Operator's errors are quite common especially if they are new and lack the experience required. All these types of errors could be controlled for by the use of internal and external standards and positive and negative controls. Subjectivity in the individual analysing the sample (e.g. microscope slides scoring, reading autoradiograms) introduce intra- and inter-reader variability which requires standardisation procedures. They could be corrected for by blind and where possible double blind analysis of samples. Another way for better standardisation of procedures is through automation. This will provide better reproducibility and quantification. Results interpretation is biased when inter-laboratory data is compared. This is due to inter-laboratory variability in the methodology applied and other factors (Hulka and Margolin, 1992)

iii) Biological Variability: Biological variability is of two types intra- and inter-individual variability. Intra-individual variability has two components random (errors in sampling) and real variability that is due to true biologic differences in the individual. Real intra-individual variability contains two conceptual variants. The first is time dependant. Several events (e.g. ageing, exposure to xenobiotics, administration of medication etc.) can occur in the time interval between two sampling sessions that will affect the levels of the biomarker measured by introducing intra-person biologic variability. The second type is time independent. This is variability between different compartments in the body. The biomarker and the active chemical levels vary across various organs and fluids in the body. A good example is what Cuzick *et al.* (1990) were able to show in their study. They observed that toxicants from tobacco smoke are three times as likely to form adducts in the lung tissue as in the liver. Another factor introducing variability could be due to none homogeneous distribution of the marker in the organs due to heterogeneousity of the organ itself. Another good example of intra-individual variation is diurnal variations in urine formation. This will alter the concentration of the urinary biomarker. This is classically corrected for by the use of creatinine which is believed to be independent of diurnal variations. The use of creatinine as the means to correct for this type of variability has been questioned by several scientists (Greenberg and Levine, 1989).

Inter-individual variation in a biological indicator refers to the classical concept and measurement of variability in epidemiological studies. The susceptibility factor is one of the main contributors to this type of variability. Predisposing factors, such as malfunctioning of organs (e.g. kidney damage, emphysema, etc.), can also endow to inter-person variability. This type of variability is controlled for in the data interpretation stage of the study. For instance extreme outliers (unusually high or low values) compared to the distribution of the marker's value can be excluded, with caution, from the statistical analysis of the results.

2.4.6 Quality Assurance

It is quite clear that some degree of precision is required for the

measurement of biomarkers in the practice of biological monitoring. This is true since recommended BEIs generally have a very narrow margin of protection. Hence, scientists must be aware of the probability of making a false decision due to variability and errors in measurements. This variability and errors must be controlled for. One way of achieving that is through quality assurance (Sakurai, 1993).

Quality assurance is a process by which the reliability of laboratory results is ensured. It involves sound scientific inquest (i.e. appropriate selection of method and maintenance of good analytical standards) and technical laboratory investigations. An appropriately quality controlled investigation includes proper sample selection, collection, transport, analysis and result recording, interpretation, and documentation. An efficient quality control scheme includes prior to the investigation sufficient training and management of personnel to ensure their ability to conduct the investigation and ultimately secure the reliability of the results (Aitio, 1984).

Quality assurance involves quality assessment which is carried out to assess the quality of the results. Quality assessment has two components internal quality control and external quality control. An **internal quality control** program is a procedure executed by the laboratory staff for continuos evaluation of the reliability of the results with the aid of control specimens. In more practical terms in internal quality control programs a control specimen is included within every analytical series and analysed identically as the samples. The results obtained from the control specimen are compared to the known values. If the result is acceptable only then the results of the samples are reported. An **external quality control** program is a procedure performed by external bodies for continuos objective checking of laboratory performance. Its aim is to control for inter-laboratory variability, by improving the accuracy of results. Technically, it assess both accuracy and precision of results.

Quality assurance programs are part of the U.S.A.'s Food and Drug Administration (FDA) guidelines for good laboratory practice (GLP) as well as other operational units within the Centers for Disease Control, the National Bureau of Standards and various private laboratories (Committee on Biological Markers, 1987). It is worthwhile noting that the

mandatory use these programs does not necessarily mean that the biological monitoring results are precise and accurate unless all the steps of quality assurance are applied. Aitio (1984) stated that some laboratories take notice of their bad performance and try to improve on it; while others tend to ignore them and keep on reporting inaccurate and deviating results. This obviously does not mean that quality assurance programs are ineffective and should be abandoned.

2.4.7 Ethical and Legal Issues

The use of biological monitoring raises a number of ethical and legal issues that should be considered at all stages of the biological monitoring program. The issues can be dealt with at either the stage of development and validation of the marker or the use of the validated marker (Schulte, 1991).

i) Development and Validation Stage: Protocols for developing and validating markers in field testing must pass ethical committee approval before the scientists approach the target population. Volunteer recruiting or soliciting worker's participation is a very sensitive process. The individuals conducting the study must clearly state the objectives of the study and explain the procedures involved during its execution. They must agree upon how should the state of knowledge about biomarkers be reported accurately to subjects on their recruital. They must respect the dignity, rights, cultural practices and freedom of choice of individuals (Mercier and Robinson, 1993). A written consent form must be signed by each of the subjects and witnessed by another individual. The volunteers must be assured that collection and handling of data are dealt with in strict privacy and confidentiality and that they will be informed about any subsequent research findings regarding the potential of the biomarkers in predicting risk. A main requirement in the use of biological monitoring studies is to be open, friendly and communicative with subjects before, during and after the study. This will help the subjects to be at ease and co-operative.

The major part in the validation process is to direct the efforts towards distinguishing between adaptive markers from those representing

maladaptive changes and to determine whether a change is truly a critical effect. It is also essential to define whether the marker is totally predictive of an adverse response, reasonably highly predictive, or only minimally predictive (Committee on Biological Markers, 1987). Strategies using this approach and sticking to the above procedures, should lead to continued productive research using biomarkers in molecular epidemiology and toxicology studies (Schulte, 1992). They lessens the potential for data indicating risk, susceptibility or potential early changes from being used inappropriately in relation to occupational opportunities or insurability.

ii) <u>While Using the Validated Biomarkers</u>: When selecting a validated biomarker the medico-toxicologist expert should choose the easiest however the most appropriate biomarker for better insight on the workers health risk irrespective of its inconvenience and matters like technological development and economical consideration (i.e. cost-benefit evaluation). The application of the biological monitoring method recommended should be evaluated through (occupational health team - workers) discussions and method evaluation. If workers do not trust the occupational health team, whose job is to safeguard their health at all expense, then there is something wrong in the occupational health service (Zielhuis, 1984). Health surveillance of the workers should not be internalised in the costs of production otherwise biological monitoring as a primary strategy will not seem as an economically attractive program (Ashford *et al.*, 1990).

Although biological monitors provides tremendous opportunities for disease prevention their use might have a non-affirmative societal impact. The impact could either be of direct implication or just ethical questions to answer. Practically, issues like the workers' ability to maintain their jobs or obtain health insurance can originate. Findings of workers with excess frequencies of various markers may impose official obligations on the employers for follow-up health surveillance. This is however difficult in countries with no national health service. As for the employee's responsibilities, the question is regarding whether or not they have to disclose the results of marker evaluation performed on them to insurers or potential employers. If the answer is yes, then insurers might unfairly utilise this information by not insuring the susceptible individuals or insuring them at higher premiums. As for the employers

they also might misuse these results for discrimination. They might not employ the hyper-susceptible as means of decreasing the risk instead of using environmental control for that purpose or employ the hypo-susceptible in more hazardous occupations since they are more able to cope with exposure beyond threshold levels. These two legal and ethical issues may be summarised under the rights of those monitored and the use of biological monitoring as a primary control strategy potentially replacing efforts to remove the toxic xenobiotics from the workplace. The latter issue is strictly ethically and professionally unacceptable.

Other practical impacts might extend to use of the biomarkers for making decisions about consumed products. For example, should a product be withdrawn from the market because few individuals are susceptible to adverse effects from the use of the product or should the individual be responsible for avoiding contact with the product?

With respect to long-term ethical issues questions like "Does society have an obligation to protect individuals beyond informing them of their risk?" and "Can an employee be forced to leave his or her job once a susceptible marker has been detected? have to be answered (Committee on Biological Markers, 1987).

In conclusion, the use of biological markers should abide with all the ethical requirements. Biological monitoring is considered as part of continuum of control measures and must be used only after primary prevention by environmental control and substitution has been applied (Harlperin *et al.*, 1986).

2.4.8 Limitations

Broadly, the limitations of biological markers are part of the issues discussed such as their use, selection, validation, variability, their quality assurance procedures and ethical considerations when they are being validated and used. Specifically, their limitations are practical or technical in nature.

There is a number of technical limitations in the use of biological markers. The most frequent technical limitations are sensitivity and the ability to detect changes in biomarkers levels, with external exposure at TLVs', over the baseline frequency observed in the general population (Lauwreys 1983). The other main limitation is specificity. The higher the marker's specificity is the lower is it's variability due to confounding factors. The best indicator of risk is not internal dose (uptake) but the biologically active dose at the target organ/tissue. Technical limitations in the direct measurement of the active-dose is pertinent to the inaccessibility of the target organ/tissue for routine sampling (Lauwreys, 1983).

There are several practical limitations in the use of biomarkers to be considered along with the technical ones. A major practical limitation is underlined in the need for sufficient understanding of the complex biological pathways and fate of the agent of interest (i.e. toxicological information such as toxico-kinetics and toxico-dynamics) for rational biological monitoring. Acquiring this information is usually time consuming and costly; however, it is essential for defining the relationships "a", "b" and "c" in figure 2.2 which is the ultimate objective of biological monitoring research (Kellard, 1991). Another limitation for example, is in the case of industrial chemicals, whereby one or more of the limitations apply to the potential biomarker, thus limiting the possibility of selective and biological monitoring (Lauwreys, 1983). This is one of the reasons that lead to the development and validation of only few biomarkers up to date (Schulte, 1991). An important practical limitation is that local effects and acute peak exposures can not be prospectively detected and evaluated through biological monitoring techniques (Lauwreys, 1983). Last but not least, is a limitation to the promise of the current generation of biomarkers. Most of the biological effect markers in practice so far have been associated with carcinogenesis; while biomarkers of other toxic effects have been unpopular and relatively neglected (Schulte, 1991).

2.5 A CONCLUDING REMARK ON OCCUPATIONAL MONITORING

The three means (AM, BM & BEM) of assessing exposure to hazardous agents are different. The relationships among external exposure, biomonitoring data and biological or health effects may be envisioned as each representing an apex of a triangle. Biological monitoring and biological effect monitoring represent different ends of the scale. So the combination of three together would give us the necessary knowledge to fully understand the relationships between external dose, internal dose, and health effects illustrated in figure 2.2. In addition, we might be able to understand the role of individual susceptibility and life styles in affecting the above mentioned relationships.

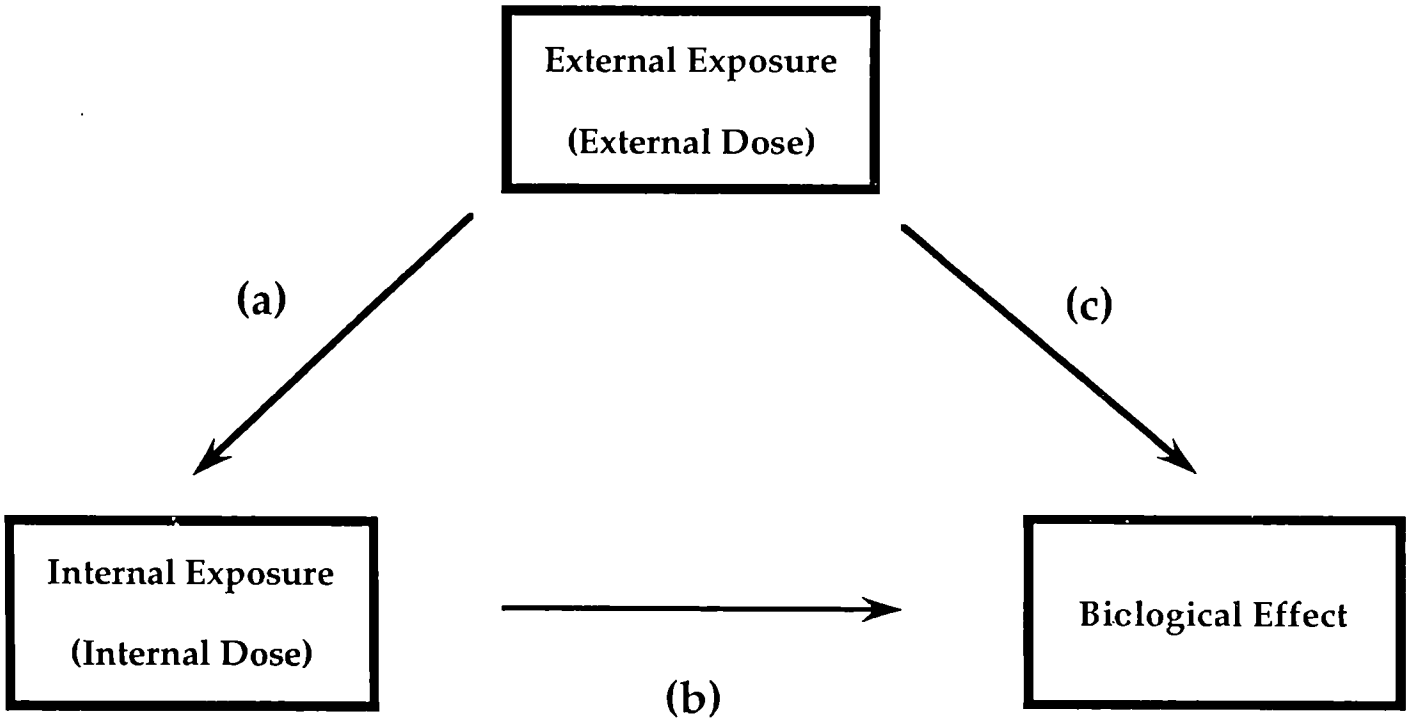

External Exposure
(External Dose)
(a)
(c)
Internal Exposure
(Internal Dose)
(b)
Biological Effect

Figure : 2.2

CHAPTER 3

POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

3.1 DEFINITION

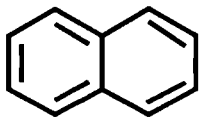
Polycyclic aromatic hydrocarbons (PAHs), also known as Polynuclear aromatics (PNA), are organic compounds consisting of two or more benzene rings, where certain carbon atoms are common to two or more rings. The simplest fused structure, containing only condensed aromatic rings is naphthalene (Sollenberg, 1983). Typical PAHs chemical structure are illustrated in figure 3.1.

3.2 SOURCES

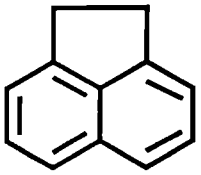
PAHs are widely dispersed in nature and are formed by pyrolysis or incomplete combustion of organic material and high-temperature processing of crude oil, coal, or other industrial carbon-containing compounds (Rom, 1983).

3.3 IMPORTANCE TO OCCUPATIONAL HEALTH

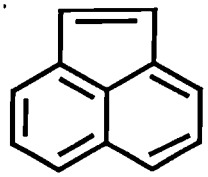
They are important in occupational health for several reasons: some are known to be potent carcinogens in man especially those PAHs containing 4-6 aromatic rings {i.e. benz(a)pyrene B(a)P, di-benz(a,h) anthracene DiB(a)A, benz(a)anthracene B(a)A}. There is strong epidemiological evidence that exposed groups have increased risks of lung, urinary tract, brain and skin cancers (Hansen, 1989; Hammond *et al.*, 1976 and Hansen, 1988) and many processes in a variety of workplaces are contaminated with PAHs (Jongeneelen *et al.*, 1988[c]).



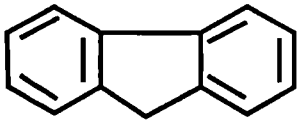
Naphthalene



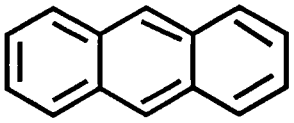
Acenaphthene



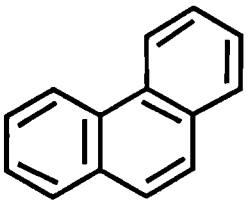
Acenaphthylene



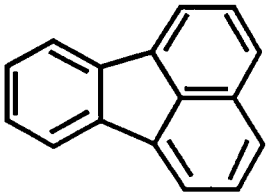
Fluorene



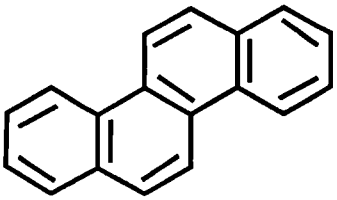
Anthracene



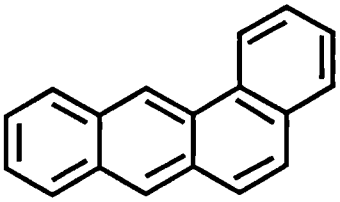
Phenanthrene



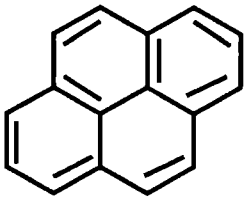
Fluoranthene



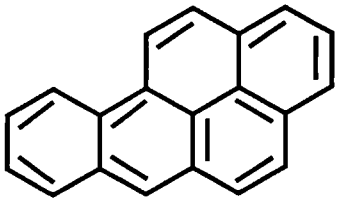
Chrysene



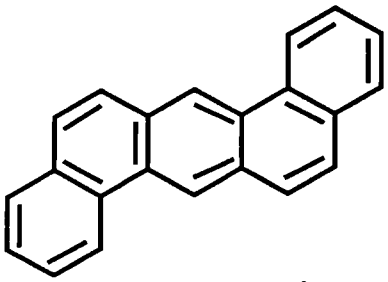
Benz(a)anthracene



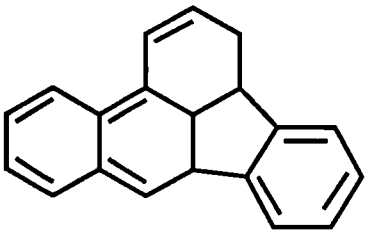
Pyrene



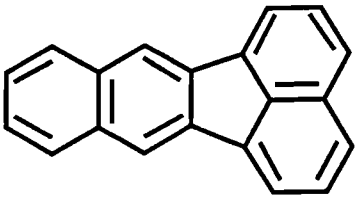
Benz(a)pyrene



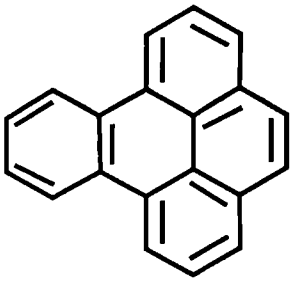
Dibenzo(a,h)anthracene



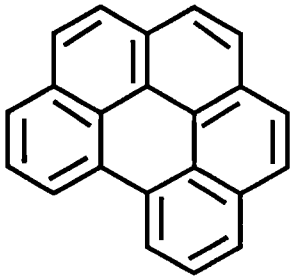
Benzo(b)fluoranthene



Benzo(k)Fluoranthene

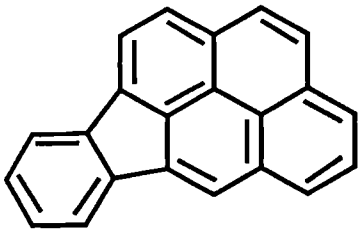


Benz(e)pyrene



Benzo(g,h,i)perylene

Figure : 3.1



Indeno(1,2,3-cd)pyrene

3.4 IN THE GENERAL AND OCCUPATIONAL ENVIRONMENT

PAHs can be found almost everywhere in air, soil, and water; originating from natural and anthropogenic sources. The contribution from natural sources like forest fires and volcanoes is minimal. The main source being industrial activities and other emissions caused by man (Karcher, 1992).

The burning of fossil fuel causes the main emissions of PAHs. Also, coking is one of the biggest contributor to PAHs in the environment among industrial processes. Other contributors come from combustion of refuse and wood. PAHs also occur in tobacco smoke and grilled, smoked and fried food. Table 3.1 shows the environmental exposure to B(a)P the most potent carcinogen of all PAHs. Table 3.2 presents a summary of B(a)P exposure levels in the occupational environment (Jongeneelen, 1987[a]).

PAHs emitted into the environment from the zone of burning are in the form of vapour. Due to their low vapour pressure most PAHs will spontaneously condense on dust particles or form very small particles themselves (Sollenberg, 1983).

3.5 TOXICOKINETICS

Literature available concerning the pharmacokinetics of PAHs in humans is limited. Considerable amount of studies have been done in animals making it possible to extrapolate to humans based on their results (Santodonato *et al.*, 1981).

3.5.1 Absorption

The absorption of PAHs into the human body can be through inhalation, skin absorption and ingestion.

Table : 3.1 Estimation of Daily Exposure to B(a)P

		Daily Intake		
Food		: 1-3	µg	Fritz *et al.*, 1971
		: 2-16	µg	Verhoeve and Compaan, 1980
		: < 0.5	µg	Vaessen *et al.*, 1984
		: 0.05	µg	Obana *et al.*, 1984
Water	< 50 ng/l	: 0.05	µg	Piet *et al.*, 1974*
Air Pollution	0-30 ng/m^3	: 0.45	µg	Brasser, 1976
Smoking 20 cigarettes		: 0.4	µg	Hoffman *et al.*, 1978
Daily intake		: **~ 0.5-16**	µg	

* Jongeneelen, 1987a

Table : 3.2 B(a)P 8 Hour-TWA Exposure Level in Different Occupational Environments

I. Very high B(a)P exposure (> 10 μg/m^3)

- Gas and coke work (topside work)
- Aluminium works (some job categories)
- Manufacturing of carbon electrodes
- Handling of molten tar and pitch (roofing, paving)
- Chimney sweeping (from top)
- Asphalting mixed with tar (some jobs)
- Optical industry **
- Refractory brick production **

2. Fairly high B(a)P exposure (1-10 μg/m^3)

- Gas and coke works in general (non-topside work)
- Blast furnace
- Steel works (some jobs)
- Manufacturing of carbon electrodes (in general)
- Aluminium works (in general)
- Asphalting mixed with tar (in general)
- Foundries
- Silicon carbide production **

3. Moderate B(a)P exposure (0.1-1 μg/m^3)

- Steel works (in general)
- Foundries (some jobs)
- Welding of rail on track
- Manufacturing of Soderberg electrode paste
- Rolling mill **
- Paving (using bitumen based asphalt) £

4. Low B(a)P exposure (0.01-0.1 μg/m^3)

- Automobile repair shops
- Asphalt manufacturing (from petroleim)
- Foundries (in general)
- Construction of tunnels and rock chambers
- Aluminum electrolysis halls woth prebaked electrodes
- Handling carbon black (**)
- Handling petroleum pitch (**)
- Road Paving (with bitumen)%
- Roofing (with bitumen based asphalt) $

5. Very low B(a)P exposure (< 0.01 μg/m^3)

- Iron mine
- Garages
- Road Paving (with bitumen)@
- Roofing (with bitumen based asphalt) $

* Lindstedt and Sollenberg, 1982
** Blome and Baus, 1983
£ Monarca, 1987
% Claydon, 1984
@ Brandt, 1992
$ WHO, 1985

i) Inhalation: This is a major route of exposure to PAHs. They generally reach the lungs under environmental and occupational conditions by absorption on carrier particles. This is a very important fact to note since it was shown that B(a)P will be cleared from the lungs in 20 minutes if inhaled in the absence of particles (Santodonato *et al.*, 1981). Knowledge of the distribution of PAHs in the air between particulate form and gaseous form is essential since the elution mechanisms of the chemical from particles play a major role in the bioavailability of PAHs which has a bearing on internal dose and ultimately biological effect. Several *in vitro* and *in vivo* studies have shown that the ability of PAHs to be desorbed or eluted from particles in the organism may vary greatly depending on the carrier particle (Becher *et al.*, 1984).

ii) Ingestion & Skin Absorption: The high lipophilicity of this class of compounds supports the observation that they are able to pass across epithelial membranes. The contribution of exposure to PAHs through these two routes to internal dose in occupational environments is not extensively studied; however, several researchers have indicated their importance; more specifically skin absorption, since it is hard to control exposure through that route due to the large area of contact with the environment (Jongeneelen *et al.*, 1990 and Wolff *et al.*, 1982 & 1989). This was confirmed by Jongeneelen *et al.*, (1988[b]), when they were able to demonstrate a dose dependent uptake through skin in urine samples of rats exposed to coal tar by looking at urine mutagenicity tests, 3-hydroxy-benzo(a)pyrene, and 1-hydroxypyrene. More recently, van Rooij *et al.* concluded that 75 % of the total amount of pyrene absorbed by coke oven workers is through the dermal route (van Rooij *et al.*, 1993).

As for the ingestion route Buckley *et al.*, 1992 illustrated that dietary exposure, which is nearly unavoidable, could be potentially a substantial contributor to PAHs internal dose as much as some occupational exposures. They reported that the mean elimination rates of 1-HP (6 - 17 ng/hr) are 10 times lower during the consumption of a low-PAH content meal than rates (69 - 189 ng/hr) during the consumption of a high-PAH content meal. They suggested a half-life of 4.4 hr and a t_{max} of 6 hr. Assuming that these parameters are accurate and that the suggested $t_{1/2}$ for dermal and inhalation exposure is 6 - 36 hr (Jongeneelen, 1992), implies that 1-HP output due to dietary intake might contribute

significantly to post-shift urine samples collected from workers. This suggests that dietary intake of PAHs could potentially confound the relationship between "external doses" arising from occupational exposures and internal dose of pyrene indirectly quantified using 1-HP.

3.5.2 Distribution

Regardless of the route of entry PAHs, once absorbed, become localised in a wide variety of body tissues.

Most studies on PAHs and substituted PAHs (e.g. di-methyl-benz-anthracene, DMBA) showed that they accumulate in body tissue after 24 hours from exposure. Bock and Dao in 1961 showed that unmetabolised B(a)P is extensively localised in the mammary gland and general body fat after a single feeding of (10-30 mg) of the carcinogen. Other animal studies showed that moderate levels do accumulate in the kidney, liver and mammary tissue. Animal studies in rats and mice with ^{14}C-B(a)P administered subcutaneously, intravenously, and intratrachealy, showed similar patterns of distribution reported in the literature (Santodonato *et al.*, 1981).

In general, the amount of carcinogen found in tissues is directly related to the dose administered and is dependent upon the use of lipid vehicle. This is important to note, if a comparison of PAHs distribution, among several studies, is to be done.

Limited research have been conducted with human subjects to characterise PAHs distribution in human tissue. Some studies did report similar findings to those in animals.

3.5.3 Metabolism

PAHs are metabolised by the cytochrome P-450 dependent microsomal mixed function oxidase (MFO system). The enzymes involved are classified into two broad categories. Phase 1 enzymes which catalyse oxidative reaction and phase 2 enzymes which catalyse

conjugative reaction of oxidised PAHs with endogenous compounds such as H_2SO_4, glutathione etc. (Jongeneelen *et al.*, 1987[b]).

The intermediate products of metabolism are reactive electrophiles (e.g. diol-epoxides), or "bioactivated" metabolites. They are formed having the capability for covalent interaction with cellular constituents like RNA, DNA, proteins etc. ultimately leading to tumour formation (Rom, 1983). Doull *et al.* in 1980 suggested that these metabolites can be further conjugated with glutathione (GSH), sulphuric or glucuronic acid making them more water soluble to be excreted in bile and urine (Ouyang *et al.*, 1994). Kari *et al.* and Kwei and Erwin mentioned in their articles that final metabolites of these detoxifying pathways are potentially toxic (Ouyang *et al.*, 1994). Grover in 1986 wrote, GSH conjugates of B(a)P can be excreted or enzymatically acted upon to form cysteinyl S-conjugates which are mainly eliminated in urine as their corresponding mercapturic acids (S-substituted N-acetyl-cysteine conjugates) (Ouyang *et al.*, 1994). Figure 3.2, adopted from WHO monograph volume 32 (1985), shows the metabolism of B(a)P one of the most potent carcinogens in the family of unsubstituted PAHs.

3.5.3.1 Metabolism of Pyrene

The metabolism of pyrene, a non-carcinogenic high MWt. PAH, was first studied by Chalmers and Peacock in 1941 (Harper, 1959). Further research by Elson, Goulden and Waren in 1945 showed that the excretion of unidentified metabolites of PAHs - one of which is pyrene - in both urine and faeces.

Harper concluded that the main metabolites of pyrene in urine are 3-hydroxypyrene mainly in free form but possibly conjugated to a small extent; 3:8 and 3:10-dihydroxy-pyrenes a little free but mainly conjugated and an identified pyrene precursor. Jacob, *et al.* (1982) concluded that pyrene is metabolised by liver microsomes of untreated rats to 1-HP, 4,5-dihydroxy-4,5-dihydropyrene, two different diphenols and a triol tentatively identified as 1,4,5-trihydroxy-4,5-dihydropyrene. They also mentioned that 1-HP is the major metabolite in normal rats. Induction of cytochrome P-450 and P-448 enzymes did not seem to alter the amount of 1-HP excreted suggesting alternative routes of metabolism with increased

enzyme activity. Up to date it is still not all clear what are the enzymes responsible for metabolising pyrene; however the most interesting and studied pyrene metabolite by researchers is 1-hydroxypyrene which is thought to be metabolised by cytochrome P-450 (fig. 3.3).

3.5.3.2 Metabolism of Carcinogenic PAHs

PAHs like many environmental carcinogens were realised as chemically unreactive and that their adverse biological effect were probably mediated by electrophilic metabolites capable of interacting with cellular macromolecule like RNA, DNA, proteins ultimately leading to tumour formation.

The most extensively studied carcinogenic PAH is the widespread environmental and industrial pollutant benz(a)pyrene (Gehly *et al.*, 1982). It is a good example of a chemical carcinogen which is not highly reactive with cellular macromolecules but exerts its activity through metabolites (e.g. diol-epoxides) that are biologically active and can modify macromolecules such as DNA and proteins.

One of the earliest studies on the metabolism of B(a)P was by Chalmers and Kirby in 1940. They found that, in the rat, 1% of a subcutaneous dose of B(a)P was excreted unchanged in the faeces and only trace amounts in urine suggesting that the latter route is of minor importance. They observed that a large amount of the injected B(a)P was converted to a fluorescent derivative which then was not identified.

Since several researchers studied the metabolism of B(a)P in microsomal preparations, cells and intact tissue of animals and humans (Autrup *et al.*, 1980). A number of reviews have also focused on B(a)P metabolism and particularly its metabolic activation (WHO, 1985).

B(a)P, a prototype of carcinogenic PAHs, is metabolised into a variety of oxygenated derivatives by cytochrome P450 monooxygenase (fig. 3.2). There are approximately 36 known metabolites of B(a)P. The one of most interest to biomedical and health scientists are the diol-epoxide metabolites. The principal ultimate carcinogenic metabolite of B(a)P is

Figure : 3.2

METABOLISM OF BENZ(A)PYRENE

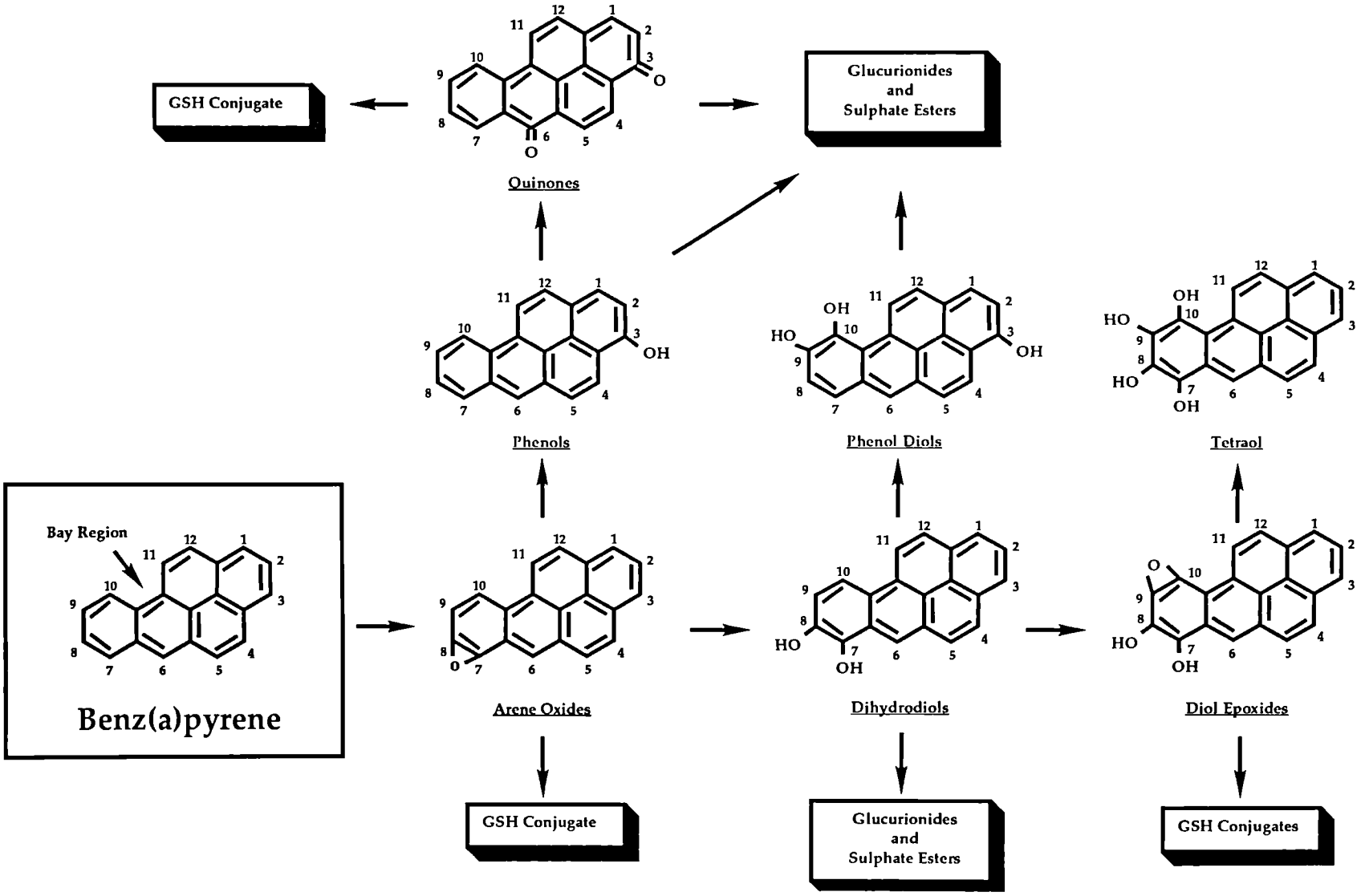
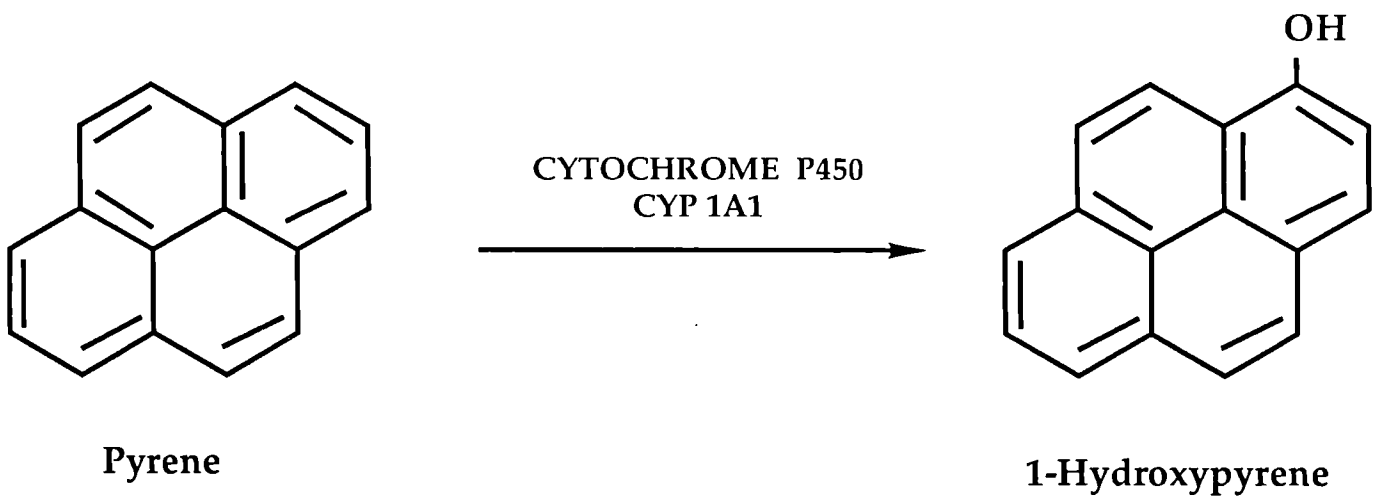


Figure : 3.3

METABOLISM OF PYRENE
TO
1- HYDROXYPYRENE



Pyrene

1-Hydroxypyrene

believed to be the bay-region benzo-ring diol epoxide one. Several studies have supported this observation whereby it was demonstrated that minimal metabolic formation of bay-region diol epoxide is consistent with the weak biological activity of benz(e)pyrene (WHO, 1985).

These bay-region benzo-ring diol epoxide metabolites of B(a)P exist as diastereoisomeric pairs in which the benzylic hydroxyl group and the epoxide oxygen are either *cis* {called, benz(a)pyrene diol-epoxide 1 (**BaPDE1**) or benz(a)pyrene syn-diol-epoxide (**BaP syn-DE**)} or *trans* {called, benz(a)pyrene diol-epoxide 2 (**BaPDE2**) or benz(a)pyrene anti-diol-epoxide (**BaP anti-DE**)} (WHO, 1985).

The BaPDEs are known to covalently bind with cell macromolecules. For instance they can bind non-enzymatically to DNA forming DNA-adducts (Rüdiger *et al.*, 1976). In the study by Weinstein *et al.*, (1976) it was observed that in human bronchus, trachea, colon and oesophagus cultured tissue the major BaP-DNA-adduct was formed by the trans addition of ± BaPDE 1 at the C-10 position to the exocyclic 2-amino group of guanine giving a structure of BaPDE-I-dG (Autrup *et al.*, 1980; Weinstein and Perera, 1982).

This mechanism of enzymatic activation of B(a)P by cytochrome P-450 and the formation of DNA-adducts is associated with its carcinogenicity. This phenomenon has been studied by a number of scientists whereby they examined the possible existing relationship between BaP-DNA-adducts and cancer through different designs of experimental and occupational toxicology and epidemiological studies. This will be dealt with in more detail in heading 7.4.3 of chapter 7 on methodology.

3.5.4 Excretion

It has been known since 1936 that PAHs are mainly excreted through the hepatobiliary system and the faeces (Peacock *et al.*, 1936 and Chalmers and Kirby *et al.*, 1940). With the advent of biotracer methodologies, researchers were able to illustrate that in intact rats maximum elimination occurs in faeces and the urinary excretion

contributes to about 10% of total excretion (Santodonato *et al.*, 1981).

There are several factors that affect excretion. The route of entry affects the process of excretion of chemicals by changing the route and speed of absorption and metabolism. Thus, in quantitative studies, looking at the rate of elimination of PAHs we must take the route of administration into consideration. Another factor that influence elimination of PAHs, when administered through the lungs, is bioavailability by affecting retention (Becher *et al.*, 1984). A predisposing factor in determining retention, and ultimately excretion routes and rate, is the concomitant exposure to agents which inhibit ciliary activity, one of the several defence mechanism of the respiratory system. The hindrance of this defence mechanism will help increasing time available for elution of PAHs from soot particles in the lungs.

3.6 EFFECTS OF EXPOSURE

The potential of PAHs to induce malignant transformation dominates the consideration given to health effects, resulting from acute exposure. Although, the emphasis on carcinogenicity is certainly justified when dealing with public health issues concerning PAHs exposure, one must not underestimate non-carcinogenic effects resulting from environmental or occupational contact.

The majority of health effects observed by occupational physicians involves the skin and eyes. Skin disorders include dermatitis, chronic-tar dermatosis, warts, cutaneous phototoxicity, chronic melanosis, folliculitis, and pitch acne. Many other effects are mentioned in the WHO monograph (Jongeneelen, 1987[a]).

In addition to those Jonos in 1943 described depression, weakness, severe headache, slight confusion, vertigo, increased salivation in small percentage of workers who developed burns from creosote (Jongeneelen, 1987[a]).

3.6.1 Mutagenesis

Mutagenic effects demonstrated in bacterial and mammalian cells following exposure to PAHs are generally related to the capacity to induce tumour formation. This relationship is based on the participation of a common electrophilic metabolite in producing the mutagenic/ carcinogenic event with common target site in the cell being DNA or other macromolecules (Santodonato *et al.*, 1981).

This link was strengthened by several researchers demonstrating that several petroleum derivatives have been found to contain mutagenic /carcinogenic compounds and among them are PAHs. Some of these micro-pollutants are mutagenic/carcinogenic and have been detected in bitumen and bitumen fumes (Monarca *et al.*, 1987).

3.6.2 Carcinogenesis

Polycyclic aromatic hydrocarbons were the first compounds ever shown to be associated with carcinogenesis. They were among the most potent carcinogens, especially 4-6 ringed ones, known to exist producing tumours by single exposures to microgram quantities in experimental animals. They act both locally and systematically (Santodonato *et al.*, 1981).

The mechanism of PAHs carcinogenicity is through metabolism to reactive intermediates which become covalently bonded to proteins and nucleic acids (Rom, 1983). Binding of carcinogenic metabolites to nucleic acids, particularly DNA, is now generally accepted to be a critical event which leads ultimately to tumour induction.

It is believed that the carcinogenicity of PAHs increases with electron richness and decreases with increase in planarity (Newman *et al.*, 1982). Their effect have been shown in almost every tissue and species tested regardless of route of administration in experimental animals (Santodonato *et al.*, 1981). The WHO stated that there is enough evidence that 11 PAHs are carcinogenic to animals.

3.7 PAHs IN CANCER EPIDEMIOLOGY

Most of the epidemiological studies trying to relate human cancer with PAHs exposure in asphalt work have major drawbacks in the study design or methodology, but they all indicate excess risk of developing cancer in exposed groups. This implies that further investigation is urgently needed to clarify whether or not there is a causal relationship (Chiazze *et al.*, 1991).

A search of the literature concerning carcinogenicity of PAHs exposure, clearly shows that cancer incidence in all types of tissues, organs and systems is in excess in all working populations exposed to PAHs at a variety of occupations, where the source of PAHs is different. On the other hand, there are very few studies that show no increase in cancer incidence among PAHs exposed population. Those observations are by no means sufficient to reject the association between PAHs exposure and carcinogenicity (Robertson and Ingalls, 1974).

Some of the most commonly reported cancers associated with exposure to PAHs are skin, lung, bladder, gastro-intestinal tract and brain cancers.

3.7.1 Skin Cancer

The era of scientific inquiry into environmental and occupational carcinogenesis began in 1775 when Sir Percival Pott described cancer of the scrotum in English chimney sweepers. The source of PAHs in that occupation is from vapour which has condensed on soot which in turn comes in contact with skin from contaminated clothing (Rom 1983).

In 1908 cancer of the scrotum received statutory recognition in England and was added to the third schedule of the Workmen's Compensation Act.

In 1910 Wilson described 35 cases of scrotal cancer, 25 of which were mulesspinners from cotton textile factories (Lee and McCann, 1967).

Scott (1922) reported 65 workers with epitheliomas among Scottish shale-oil workers between 1900-1921. In 1953 substitution of refined-petroleum lubricant oil for shale-oil was required in UK. and the use of spinning mules was virtually eliminated. In 1976 Hammond *et al.* reported excess mortality for cancers of the skin in roofers exposed to hot fumes of roofing material including coal-tar, pitch, and asphalt.

Although scrotal cancer has been adequately described and recognised one could still see cases of scrotal cancer like the one reported by Jarvis in 1980 of a pitch worker. This clearly indicates the lack in the degree of implementation of recommendations by post researchers and the adequacy of dissemination of such information.

3.7.2 Lung Cancer

A 33-fold increase in lung cancer risk among male workers exposed to tar fumes in a Japanese steel mill was documented by Kawai, *et al.* in 1967. Sir Richard Doll confirmed these observations in an epidemiological study done on a London gas company (Doll, 1952; Doll, *et al.*, 1965 & 1972). Milham, S. in 1979 found elevated standard mortality rates (SMRs) for lung cancer in a cohort at an aluminium plant reduction workers. He also found a dose-response relationship between lung cancer mortality and years of exposure.

Researchers in all disciplines are still enquiring about the relationship existing between all sorts of cancer incidence and exposure to PAHs. Hansen (1989) looking at an occupational cohort exposed to bitumen fumes found a high standard morbidity ratio for lung cancer. Again, Hansen (1991), found a significant increase in SMR for lung cancer in an epidemiological study investigating mortality of mastic asphalt workers exposed to bitumen fumes. Chong *et al.* (1989) showed that epidemiological data shows linearity between coke oven emissions and lung cancer. Findings reported by most authors are contradictory to few animal studies which failed to demonstrate any carcinogenic effect of bitumen fumes in rats and guinea pigs (Santodonato *et al.*, 1981). This controversy in research outcomes leaves scientists with a need to answer this question.

3.7.3 Bladder Cancer

The role of occupational agents in the aetiology of bladder cancer has been described since 1895 by Rehn (Bonassi *et al.*, 1989). Since the 1960's many studies have identified occupations at risk from bladder cancer, the majority of which involve exposure to PAHs. Some of these occupations are gas production, petroleum and aluminium reduction.

Sir Richard Doll observed a higher incidence of bladder cancer in a population of 2071 male pensioners with more than 5 years of employment as carbonizers. Hammond *et al.* (1976) confirmed Doll's observation in a mortality study of roofers exposed to hot fumes of roofing material. One of the recent case-control studies trying to associate bladder cancer with exposure to PAHs is by Bonassi *et al.* in 1989. He showed that workers who have a definite exposure to PAHs have an elevated risk of bladder cancer, even after adjustment for confounding factors.

3.7.4 Cancer of the Gastro-Intestinal Tract

In 1852 Redmond *et al.* found that coke plant workers suffered an excess mortality from cancer of the colon and buccal cavity. Another study indicating increased of mortality from stomach cancer in roofers exposed to hot fumes of roofing material (Hammond *et al.* in 1976). Exposed petroleum workers showed a 3-fold increase in cancers of the oesophagus and stomach (Hanis *et al.*, 1979). In 1985 Silverstein *et al.* observed a 2-fold excess of cancer of the digestive system in exposed metal workers.

3.7.5 Brain Cancer

This is one of the less observed types of cancer among PAHs exposed workers; however, several authors have noted an increase in brain tumours. Alexander *et al.*, 1981 reported 18 cases in a petrochemical plant. Hansen (1989) in one of her recent enquiries into cancer mortality discovered a substantial increment for brain cancer in an asphalt industry.

3.7.6 Other Types of Cancer

Workers handling pitch and asphalt suffered a substantial incidence of leukaemia (Hammond *et al.*, 1976). Metal workers exposed to coal-tar pitch volatiles (CTPV) showed a 4-fold excess of leukaemia (Silverstein *et al.*, 1981).

Redmond *et al.* (1976) reported an elevation in SMR from cancer of the genitourinary system in coke plant workers.

Cancer of the sinuses was described by Blot *et al.* (1977) in a population environmentally exposed to emissions from petroleum industry.

Elevated SMR for lymphatic cancers is reported by Milham (1979) in an aluminium reduction plant.

Lloyd (1971) showed excess mortality from cancer of the kidney in coke oven workers. He also observed an increased risk of kidney cancer with both duration of employment and level of exposure.

CHAPTER 4

OVERVIEW ON BITUMEN

4.1 DEFINITION OF BITUMEN AND ASPHALT

Bitumen was defined by the Institute of Petroleum in UK as "a viscous liquid, or solid, consisting essentially of hydrocarbons and their derivatives, which is soluble in carbon disulphide; it is substantially non-volatile and softens gradually when heated. It is black or brown in colour and posses water-proofing and adhesive properties. It is obtained by refinery processes from petroleum, and is also found as a natural deposit or as a component of naturally occurring asphalt, in which it is associated with mineral matter". The chemical definition of "bitumen", entered as "asphalt", in the European Core inventory issued in October 1981 is: "Bitumen, CAS No. 8052 - 42 - 4; a very complex combination of high molecular weight organic compound containing a relatively high proportion of hydrocarbons having carbon numbers predominantly greater than C_{25} with high carbon-to-hydrogen ratios. It also contains small amounts of various metals such as nickel, iron, or vanadium. It is obtained as the non-volatile residue from distillation of crude oil or by separation as the raffinate from a residual oil in a deasphalting or decarbonisation process (CONCAWE report No. 7/82, Bright *et al.*, 1982).

Bitumen has got the same definition in most parts of the world except for North America. In USA, bitumen is referred to as asphalt; while in Europe the term asphalt is used for a mixture of bitumen and mineral matter such as sand and filler (CONCAWE report No. 6/84, Claydon *et al.*, 1984). When carrying a risk assessment it is essential to clarify this loose use of this terminology. In this thesis the European terminology is utilised and the word "asphalt" refers to the mixture of bitumen and mineral matter.

4.2 BITUMEN: A SOURCE OF PAHs

PAHs are by-products of incomplete combustion of organic material. They could be emitted from a variety of sources. One of the many sources is hot bitumen, since it consists essentially of hydrocarbons

and their derivatives.

Emissions from combustion processes are extremely complex in their chemical composition. Each combustion process emits a different profile of PAHs. Lesage *et al.* (1987) demonstrated that workers in paving and roofing operations are exposed to different PAHs with a profile largely different from that of workers in aluminium refineries.

The PAHs profile found in the fumes emitted from different bitumen-containing material vary considerably. Brandt (1990) has demonstrated that under controlled laboratory conditions the PAH contents of condensed fumes from penetration grade bitumen, generated at 160 °C and of fumes from oxidised grade bitumen generated at 250 °C do differ however nonsignificantly. Factors, other than the type of bitumen, e.g. temperature of application, boiling point distribution affect the profile of PAHs emitted during the use of such material at elevated temperatures (Brandt, 1990).

Other external determinants such as type and concentration of inorganic particulate matter suspended in the air must be taken into consideration while assessing the workers' exposure. Also, weather conditions have considerable effects on the external exposures profiles of PAHs. In a study evaluating workers' exposure to PAHs, the ratio of particulate matter PAHs to those in the vapour form was shown to vary if sampling is carried out at different temperatures (e.g. 18 °C and 28 °C). It was observed that at low sampling temperature the particulate PAHs profile contributed to approximately 10% of the total PAHs concentration and ranged from 2- to 6- ring PAHs, while the gaseous profile constituted 90% of the total and was restricted to the 2- and 3- ringed PAHs. This distribution was altered when the sampling was carried out at high sampling temperature; the particulate PAHs profile contributed to approximately 30% of the total PAHs concentration and was solely dominated by the 4- and 5- ring PAHs, while the gaseous profile constituted 70% of the total and ranged from 2- to 4- ringed PAHs (Lesage *et al.*, 1987).

The results of the studies presented above demonstrate that it is necessary, for a multitude of reasons, to identify the characteristics of the

source and conditions under which it is giving rise to the PAHs.

The physical, chemical and toxicological characteristics of individual PAHs are significantly different. Therefore, the complex mixtures with different PAHs profiles have distinct toxicity potencies. This also means that their physical and chemical interactions with the environment under several conditions vary notably. For example, high molecular weight PAHs are less volatile than the low molecular weight ones and they tend to be present condensed on fine particulate matter rather than in vapour form (Brandt *et al.*, 1985).

All this stresses the fact that sufficient knowledge must be acquired about the source of PAHs of interest, and hence their profile, in order to be able to conduct a reliable health risk assessment for exposure.

The following section of this thesis is a mini-review on bitumen and bitumen fume. Its definition, occurrences, uses, toxicity and other related issues.

4.3 TYPES, OCCURRENCE AND USES

Bitumens can occur naturally. Natural seepage of bitumen can be intruded by mineral matter to give rise to natural asphalts. They can also occur as lake asphalts and rock asphalts (WHO, 1985). Bitumen used for different industrial applications are produced by a variety of methods of refining petroleum crude oils.

Bitumens and bitumen derivatives are normally classified into classes representing the main types most frequently used in industry. The classification used here is that of CONCAWE followed by the WHO way of classification by "class numbers".

Penetration Grade (Class 1): are classified by their penetration value ranging from 15 to 450 PEN. They are mainly used in road surfacing and roofing. Recommended temperature for handling is

90 - 220 °C.

Hard Bitumen (Class 4): are normally classified by their softening point ranging from 75 to 115 °C. They have a lower penetration value and a higher softening point compared to class 1 (i.e. more brittle). They are used for manufacturing of bitumen paints or enamels. Recommended temperature for handling is 160 - 230 °C.

Oxidised Bitumen (Class 2): are classified according to their softening point and their penetration value. They are less susceptible to temperature and have a higher resistance to different types of stress compared to class 1 & 4. They are used in the manufacture of roofing felt, water proof papers, electrical compounds and other products. Recommended temperature for handling is 150 - 230 °C.

Cutback Bitumens (Class 3): are bitumen derivatives. They are a mixture of class 1 & 2 with a suitable volatile solvent from petroleum crudes such as white spirit, kerosine, or gas oil. They are more fluid and easier to handle than class 1, 2 & 4 (with typical penetration value of 100 PEN and 8-14% kerosine). When the solvent evaporates the initial properties of bitumen are recovered. They are referred to as road oils in the USA. Their main use in road work is for surface dressing. Recommended temperature for handling is 65 - 180 °C.

Blended Grades (Class 6): are bitumen derivatives. Bitumen is blended with solvent extracts, thermally cracked residues or certain heavy petroleum distillates. Their final boiling point is 350 °C. Their high boiling point makes them useful in certain application.

Bitumen Emulsions (Class 5): are another type of bitumen derivatives. They are fine dispersions of bitumen in water. They have similar uses to class 3 but can be applied at ambient temperature or limited heating.

Modified Bitumen (Class 7): a derivative of bitumen where

additives (e.g. sulphur, polymers) alter the properties of bitumen. They are used in road construction and roofing.

Thermal Bitumens (Class 8): are not under the CONCAWE classification since they are neither manufactured in Europe nor in the USA.

Bitumen is an important product used in modern society and has been in use for thousands of years in many industrial and domestic applications (Chong, 1989). In 1938, the annual consumption of bitumen was 5 million tonnes by January 1985, it had increased to 60 million tonnes (WHO, 1985). Figure 4.1 shows the estimates of the world's annual use in 1938 and 1985 and estimates of quantities of bitumen used in developed countries like USA, Europe and others (Japan, New Zealand, Canada, and South Africa) between 1960 - 1985 (WHO, 1985).

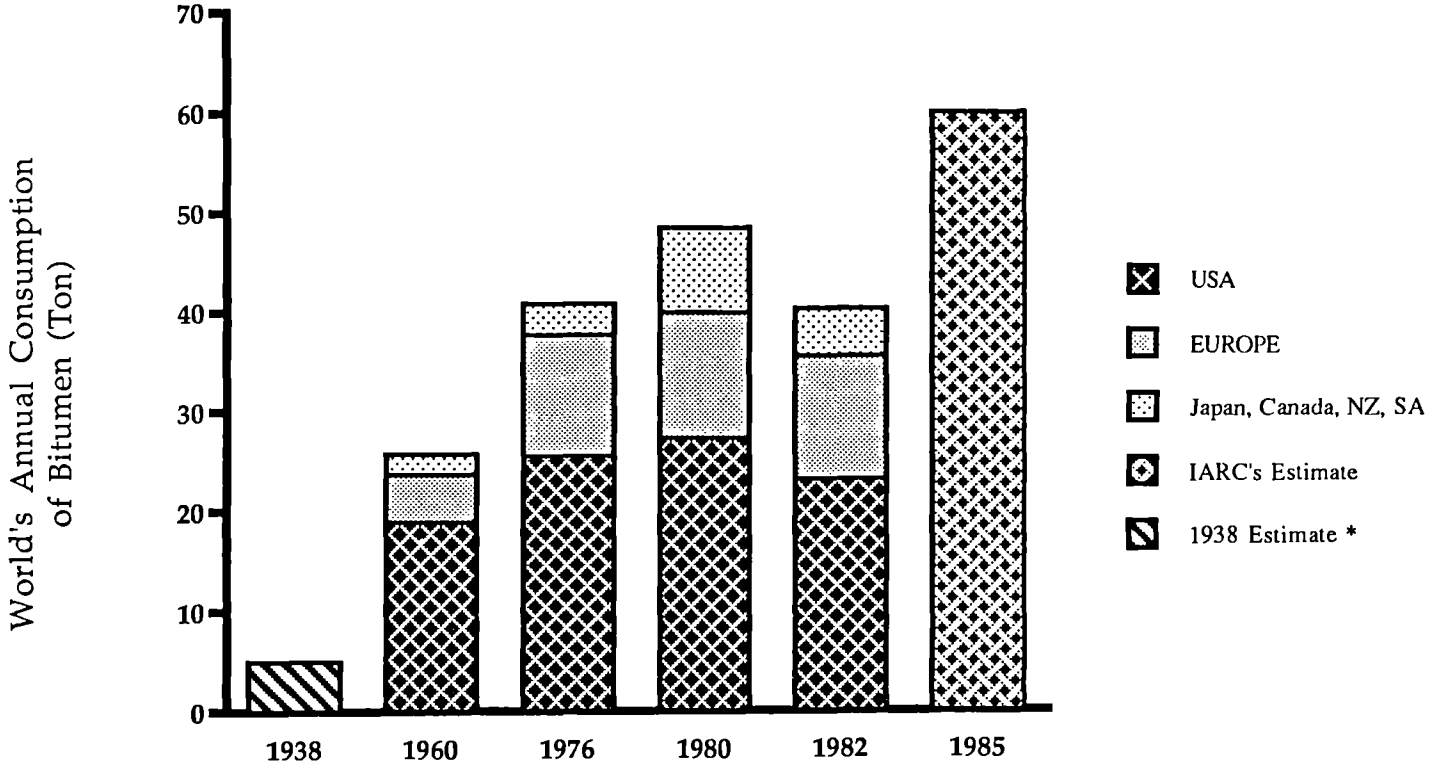
The major percentage (80%) of bitumen is used in road application (construction and maintenance) of that on average 85% is penetration grades, about 10% cutbacks and 5% emulsions. This percentage however varies from country to country. Other industrial applications such as waterproofing, roofing, flooring, impregnated felts, electrical and sound insulation, pipe coating, briquettes, agriculture etc., consume 20% of bitumen used. Sixty percent of their consumption is oxidised, 30% penetration and 10% hard and derivatives.

4.4 OCCUPATIONAL EXPOSURE TO BITUMEN FUMES

Monarca *et al.*, 1987 wrote that in 1978 a report on occupational exposure to asphalt fumes by the US Department of Health, Education and Welfare estimated that about five hundred thousand workers in the USA were exposed to bitumen fumes. In 1981, the National Institute for Occupational Safety and Health (NIOSH) estimated that two million workers were exposed to bitumen and bitumen fumes in USA (WHO, 1985).

Figure : 4.1

Estimate of the World's Annual Use of Bitumen in 1938 and 1985 and in the Main Users (Countries) Between 1960 and 1982



* The 1938 estimate is documented in Chipperfield, 1984. (WHO, 1985)

Occupational exposure to bitumen and its fumes may occur during production, handling, storage, transport and application of bitumen. Industries like highway and street construction, roofing and sheet metal work and blast furnaces and steel mills had the largest number of exposures to bitumen.

4.4.1 Occupational Exposure Limits

The threshold limit value for bitumen/asphalt (petroleum fumes) recommended by ACGIH is 5 mg/m^3. This TLV was based on the Baylor and Weaver study in 1968 (Bright *et al.*, 1982). The HSE recommended in the EH 40/94 an OES of 5 mg/m^3 and STEL of 10 mg/m^3. These current standards refer to total particulate matter (TPM), hence including inorganic part (i.e. dust) and other organic pollutants. These standards are not appropriate for assessing the carcinogenic exposure since the hazard is not related TPM however to the benzene soluble material BSM and to the PAHs content of the bitumen/asphalt fumes condensates (Brandt *et al.*, 1993).

The BSM ranges between 0.5 to 81 % of the TPM in paving operations and 10 to 86 % in roofing operations utilising penetration grade bitumen based asphalt and bitumen mastic respectively (Brandt *et al.*, 1985). Under control laboratory conditions it was found that Σ 14 PAHs constitute only 0.1 % of the BSM in bitumen fume condensates. It should be noted that this selection of PAHs represents only a proportion of the PAHs (unsubstituted and substituted) that can be present (Brandt, 1993). Assuming that contribution of the other PAHs is relatively negligible and these 14 PAHs are fully contributing to 0.1 % of the BSM; and knowing that the PAHs profile of the laboratory fumes fit that of fumes sampled on the paving operators, then on the basis of that, and taking the worse conditions, we can derive an empirical TLV-TWA for PAHs (represented by these 14 PAHs) in the paving and roofing industries utilising bitumen based asphalt. A suggested TLV of 4.3 $\mu g/m^3$ would reflect the TLV of 5 mg/m^3 for bitumen fumes. It is worth noting that this is 1 - fold lower than the PAHs' TLV-TWA of (40 μg PAH-TPM/m^3) used in Norway (Becher *et al.*, 1984).

The setting of these occupational exposure standards requires the review of the scientific literature searching for evidence regarding exposure to the toxic substances. The process of standard setting for asphalt exposure has been largely influenced by social, economical and political considerations which are beyond acknowledged scientific approaches. Chong *et al.*, 1989 suggest that there is a number of approaches possible for estimating the risk associated with exposure to bitumen/asphalt. In their article they discussed five different strategies for that purpose and concluded that none of the approaches is adequate for regulatory decision-making when used separately.

There are a few published papers on the toxicology of bitumen (Bright *et al.*, 1982). A limited number of molecular and classical epidemiological studies on the effects of bitumen/asphalt exposure are available (Chong *et al.*, 1989). This suggests these types of studies are urgently needed to investigate occupational groups exposed to bitumen/asphalt, like the one described in this thesis, in order to define the risk of exposure with greater accuracy and set standards with greater confidence (Chong *et al.*, 1989).

4.5 OCCUPATIONAL MONITORING OF EXPOSURE TO BITUMEN FUME

4.5.1 Ambient Monitoring

Earlier studies on ambient monitoring of bitumen fume used non-specific methods of analysis and reported their results as TPM or cyclohexane soluble material (CSM). The WHO Monographs, Volume 35, (1985) has two tables listing the different studies that measured bitumen fumes during refinery and road-paving operations as TPM and/or CSM and the levels recorded. Only few studies did PAHs analysis on air samples collected during paving and roofing processes like the one by Malaiyandi *et al.* (1982).

It is not until recently that studies on risk assessment of exposure

to bitumen fume utilising environmental monitoring stressed the need to conduct PAHs determination in air samples as well as with the bitumen fume analysis (Brandt *et al.* (1985), Monarca *et al.* (1987), Lesage *et al.* (1987) and Brandt and Cordingley (1992)). There are advantages of using the results collated from both analytical methods. Using the mass ratios like PAHs/BSM sheds knowledge on exposure toxicity by clearly identifying health risk factors varying between different industrial environments. It helps in detecting interferences. The differences between the ratios in certain environments can be hinted at as a criterion for the rejection of non-representative samples (Lesage *et al.*, 1987).

4.5.2 Biological Monitoring

The interest in biological monitoring of exposure to bitumen fume started in the late eighties. In fact only a few researchers have performed biological monitoring of workers occupationally exposed to bitumen. In 1988, Burgaz *et al.* observed a nonsignificant increase in the excretion of urinary thioethers in workers exposed to bitumen fumes in comparison to controls. Pasquini *et al.* (1989) monitored the urinary excretion of non-selective biological and biological effect monitors in workers exposed to bitumen fumes. They found that although the bitumen exposure is low it contributed to the urinary excretion of mutagens; however it did not affect the excretion of urinary thioethers and urinary d-glucaric acid. In 1992, Burgaz *et al.* evaluated the urinary excretion of non-selective (i.e. urinary thioethers) and selective (urinary 1-hydroxypyrene) biomarkers of exposure to bitumen fumes. Both biomarkers were elevated in the exposed group. Urinary thioethers, but not urinary 1-hydroxypyrene results were confounded by exposure to electrophilic substances from smoking.

4.6 EFFECTS OF EXPOSURE TO BITUMEN/BITUMEN FUMES

4.6.1 Acute Toxicity

4.6.1.1 Animal Studies

Not much is known about the acute toxic effects of bitumens. Only a couple of studies in the 1960's have studied the toxic effects due to inhalation of bitumen aerosols or fumes. Simmers in 1960 (Bright *et al.*, 1982), reported that the main toxic effects arising from exposure to bitumen aerosol through inhalation were congestion, acute bronchitis, pneumonitis, peribronchial round cell infiltration and loss of cilia in mice. Hueper and Payne in 1964 confirmed Simmers observations in another study on the effects of bitumen fume inhalation in rats and guinea pigs. They observed similar effects in the respiratory system (Bright *et al.*, 1982).

Acute effects on skin were epidermal hyperplasia, inflammatory infiltration of the dermis, cutaneous ulceration with abscess formation (WHO, 1985). In a very recent article studying the short-term toxicity of bitumen upgrading products in the rat following repeated dorsal skin exposure, Poon *et al.* (1994) observed moderate hyperkeratosis in female rats and moderate to marked epidermal hyperplasia in both sexes at high doses. Other effects were reduced food consumption and growth suppression of the spleen kidney and liver. A dose-related increase in absolute and relative liver weight. A drop in hematocrit and RBC and an increase in percentages of reticulocytes were observed. Histological changes were observed in thymus gland, the thyroid, liver, and spleen. The "no observed effect level" was 25 mg/kg bw/day (Poon *et al.*, 1994).

4.6.1.2 Human Studies

Numerous studies have been conducted on the acute effects of bitumen and asphalt. Most of them report acute effects primarily on the skin and eye including photo-toxic skin reactions, photophobia, keratoconjunctivitis (Chong *et al.*, 1989).

In 1950, Zeglio, P. studied the effect of bitumen vapour on the respiratory tract. Complaints from cough and phlegm, burning of the throat and chest, hoarness, headache and nasal discharge were recorded. Physical examinations revealed bronchitis, rhinitis, oropharyngitis, and laryngitis among the workers (WHO, 1985).

In the study by Emmett *et al.* (1977), keratoconjunctivitis was frequently observed among roofers working with both oxidised bitumen and coal-tar pitches (WHO, 1985).

Chronic eczema was observed by Chanial and Joseph in 6 out of 250 road workers using penetration bitumen (WHO, 1985).

Norseth *et al.* (1991) observed that workers handling asphalt reported symptoms like abnormal fatigue, reduced appetite, laryngeal and pharyngeal irritation and eye irritation. The authors did not record the type of asphalt used. This loose use of terminology is highly criticised since it does allow drawing relationships between effects and the specific type of substance used. In the same year an epidemiological study by Hansen indicated that there was an excess of bronchitis, emphysema, and asthma in a cohort of mastic asphalt workers exposed to bitumen fumes.

4.6.2 Mutagenicity

Not much is known about the mutagenicity of bitumen and bitumen fumes. Penalva *et al.* found that an extract of class 3 bitumen in dimethyl sulphoxide (DMSO) was mutagenic to *Salmonella typhimurium* TA98 in the presence of an Aroclor-induced rat-liver metabolic system (S9); however, vapours, particles and aerosols emitted at different temperatures collected in DMSO were weakly mutagenic both in the presence and absence of S9 in the TA98 and TA100 strains (WHO, 1985).

In the published literature there are less than a few articles providing toxicological data on the mutagenicity of bitumen. Levels of bitumen fumes recorded during road paving were found to be low, containing only trace amounts of PAHs and not mutagenic in the Ames Test using TA98 and TA100 strains with and without rat-liver enzyme

system (S9 mix) (Monarca *et al.*, 1987). There is a linear proportionality between the mutagenicity of bitumen fume condensates in the Mobile modified Ames Test expressed by mutagenicity index (MI) and the % of 3-6 ring PACs content in by DMSO extraction of samples (Brandt, 1993). So in general, the data available on the mutagenicity of bitumen/asphalt fumes is scarce and inconclusive.

The formation of DNA adducts in human skin *in vitro* treated with, coal-tar, creosote or bitumen has been studied by Schoket *et al.* in 1988. They found that levels of DNA adducts formed in human skin are similar to those formed in mouse skin after administration of doses known to be carcinogenic to the latter. They also observed that highest levels of DNA adducts were formed with coal-tar while the lowest were with bitumen.

4.6.3 Carcinogenicity

4.6.3.1 Animal Studies

i) Skin application: The CONCAWE report no. 7/82 in 1982 and WHO monograph volume 35 in 1985 both reviewed the published literature on the carcinogenicity of bitumen - mainly class 1 & 2 - by its topical application or subcutaneous injection in mouse skin. Local and systemic effect were observed. Epidermoid carcinomas, topical squamos-cell carcinomas, papillomas and subcutaneous leiomyosarcomas were observed on applying mixtures of bitumens (pure or dissolved in solvents, e.g. benzene) in different doses for various periods of time in the studied animals. In general the results presented are not strongly conclusive, but almost imply that bitumen has a weak carcinogenic activity especially when compared with coal-tar pitch.

ii) Inhalation: the number of studies on the toxicity of bitumen and bitumen fume by inhalation are scarce. A study by Hueper and Payne (1960) showed that fumes of oxidised bitumen did not produce lung tumours in either rats or guinea pig; however the administration of the fume condensates showed high carcinogenic potency when applied to mice skin or muscle.

4.6.3.2 Human Studies

Few reports are available relating to the effects of bitumen exposure on man. In 1947, Henry identified one case of skin cancer that could be related to bitumen exposure from 3753 cases recorded between 1920 and 1945 in UK (Bright *et al.*, 1982).

Bright *et al.* (1982) mentioned in the CONCAWE report No. 7/82 that studies conducted in 1963 and 1975 in Europe concluded that bitumen is biologically inactive and its vapours are non-carcinogenic. They quoted studies like the one by Bayler and Weaver who suggested that bitumen is not a hazard to human health. Another investigation by Baird observed no increase in lung cancer among petroleum bitumen workers. Nevertheless, Bright *et al.* (1982) regarded the literature, up to 1982, on the carcinogenicity of bitumen to man as inconclusive.

In 1985, the WHO wrote that there is no epidemiological studies available of workers exposed only to bitumen. Since then few epidemiological studies have been conducted on only bitumen exposed workers. Hansen (1989[b]), studied cancer incidence in unskilled workers in an asphalt industry. She found that they have a significant increase in brain cancer in comparison to controls. In the same year she published the results of another study on cancer incidence in an occupational cohort exposed to bitumen fumes. Significant increases were observed for cancer of the mouth, oesophagus, rectum, and lung. In a study on the mortality of mastic asphalt workers significant increases in lung and nonpulmonary cancer and liver cirrhosis were seen (Hansen, 1991).

A large scale study by IARC/WHO collated and examined the results of twenty epidemiologic studies describing cancer risk in asphalt workers, roofers and other bitumen exposed workers in various countries. They suggested that there is an increased risk of lung and stomach cancer, nonmelanoma skin cancer, and leukaemia in roofers. For pavers and highway maintenance workers the aggregate relative risk was consistently lower than roofers for cancers of the lung, stomach, bladder, skin and leukaemias. Only one study illustrated that the risk of skin cancer was elevated. For miscellaneous and unspecified workers an increased risk of lung cancer was reported. In the report the authors stressed that the data

was poorly focused for elucidating the bitumen-cancer controversy (Partanen and Boffetta, 1994).

CHAPTER 5

THE STUDY

5.1 BACKGROUND

Over the last two decades there has been an increased interest in the assessment of the health risks of populations occupationally exposed to chemical carcinogens.

Man is exposed to a variety of chemicals as contaminants both in the general environment and the workplace. Many of these chemicals are potentially harmful. The particular group of pollutants investigated here, the polycyclic aromatic hydrocarbons (PAHs) are an important class of chemical carcinogens.

PAHs are important to occupational health for several reasons: some are potent carcinogens (Harvey, 1991) and there is strong epidemiological evidence that exposed groups experience excess risk of lung, urinary tract, and skin cancers (Partanen and Boffetta, 1994). Secondly, many processes in a variety of workplaces are contaminated with them (Jongeneelen *et al.*, 1988[c]).

The acute and chronic toxicity of high and low doses of PAHs in animals have been well researched using *in vivo* and *in vitro* techniques (WHO, 1983 and Karcher, 1992). Many studies have looked at acute exposure to high levels of PAHs and its effects on human health, such as in coke oven workers. On the other hand, the literature focusing on the effects of long-term exposure to moderate and low levels such as those found in the roofing and road paving industries is scarce. For typical levels of the B(a)P time weighted average (TWA) exposures in these industries see table 3.2. Hence, estimates of the risk to health of chronic exposure to low levels of PAHs have not been made.

Similarly, a number of animal studies have been conducted on the toxicity of bitumen and bitumen fumes and their effects when administered through different routes. Again, the literature lacks on investigations assessing the risks to health of workers exposed to bitumen fumes. Few occupational health and hygiene studies have been conducted measuring workers exposure to bitumen/asphalt and their fumes. Table 5.1 lists a number of these studies and the levels of bitumen fumes

Table : 5.1 Continued

PROCESS/ BITUMEN	TPM (mg/m^3)	BSM@ or CSM% (mg/m^3)	Σ PAHs (ng/m^3)	B(a)P (ng/m^3)	AUTHORS
Paving (4.5 % bitumen)	0.15 - 0.52	0.03 - 0.06	268 - 813	--	Brandt and Cordingley, 1992
Paving	--	--	~ 12000Ω	--	Lesage *et al.* 1987
Roofing (Oxidised bitumen)	0.35 - 8.42	--	--	--	Claydon *et al.*, 1984*
Roofing (Oxidised bitumen)	--	0.2 - 5.4@	--	--	"
Roofing (Oxidised bitumen)	--	0.2 - 2.9%	--	< 0.05	"
Roofing	0.5 - 6.4	0.2 - 5.4	24 - 364	--	WHO, 1985£
Roofing	--	--	14500 - 112500	--	"
Roofing (Asphalt & tear off)	--	--	9.2 - 22.3	0.9 - 1.5	Wolff *et al.*, 1989
Roofing (Pitch tear-off)	--	--	13.4 - 17.2	0.9 - 1.5	"
Flooring (Mastic asphalt)	5.5 - 11.8	--	--	--	Claydon *et al.*, 1984*
Flooring (Mastic 11% Bitumen)	--	2.9 - 11.0@	--	--	"
Flooring (Bitumen)		0.3 - 38.9%	--	--	"
Flooring (Bitumen)		1.6 - 10.8%	--	--	"
Flooring (Bitumen mastic)	2.9 - 18.2	1.8 - 13.1	285 - 2971	--	Brandt, 1990

* Studies by several investigators reported by Claydon, 1984

£ Studies by several investigators reported by WHO, 1985

Ω Static "area" sampling during paving operations

Table : 5.1 Ranges of TWA (8 hr) Concentrations of TPM, BSM or CSM, Σ PAHs and B(a)P Reported in Studies Measuring Exposure to Bitumen Fumes in Paving, Roofing and Flooring Processes

PROCESS/ BITUMEN	TPM (mg/m^3)	BSM[@] or CSM[%] (mg/m^3)	Σ PAHs (ng/m^3)	B(a)P (ng/m^3)	AUTHORS
Paving (5 % bitumen)	0.15 - 5.61	--	--	--	Claydon *et al.*, 1984*
Paving (6.5 % bitumen)	0.3 - 1.0	--	--	--	"
Paving	0.1 - 0.3	--	--	--	"
Paving	--	0.1 - 0.23[@]	--	--	"
Paving (5.7 % bitumen)	0.1 - 1.2	--	--	< 0.05	"
Paving (4.4 % bitumen)	0.4 - 1.1	--	--	< 0.05	"
Paving (Gussasphalt)	--	0.1 - 0.27[%]	--	--	"
Paving (6.2 % bitumen)	0.1 - 1.3	--	--	< 0.05	"
Paving (Asphalt mix)	0.3 - 6.4	--	--	--	WHO, 1985[£]
Paving (Asphalt m ix)	--	--	4.32 - 12.99	--	"
Paving (Mastic asphalt)	--	--	0.122 - 0.185 ($x10^6$)	--	"
Paving (Asphalt cement)	--	0.22	--	--	"
Paving (Coarse asphalt)	--	0.38[%]	--	--	"
Paving	0.5 - 1.56	--	--	--	"
Paving operation	--	--	8.4 - 38.8[Ω]	7.5 - 10	Monarca *et al.*, 1987
Paving (Bitumen cutback)	0.2 - 15.1	0.1 - 0.3	--	--	Brandt, 1990
Paving (3.5 % bitumen)	0.23 - 0.73	0.02 - 0.1	--	--	Brandt and Cordingley, 1992
Paving (7.5 % bitumen)	0.78 - 5.71	0.52 - 3.97	--	--	"

recorded in various industrial process using different types of bitumen/asphalt. Likewise there is a limited number of epidemiological studies evaluating the health risks of workers exposed to these fumes only. The results from the both types of investigations accumulated in the literature are not sufficient to reach a reliable characterisation of the risks involved in such exposures. Monarca *et al.*, 1987 suggested that more extensive risk assessment studies are needed in several works situations where exposure to different types of bitumen/asphalt and their fume is taking place, through molecular epidemiology investigations using a coupled environmental and biological monitoring approach.

Complex organic mixtures containing PAHs are emitted during roofing and paving processes with hot bitumen, which contains essentially hydrocarbons and their derivatives. The emission can be in the form of vapours or particulate matter and may gain entry into the human body through inhalation, skin absorption, and occasionally ingestion. Airborne concentrations vary over a wide range depending on type of bitumen used and its temperature at application, environmental conditions and working methods and risk may vary quite widely between exposed individuals.

It is important to assess the risks to health of bitumen fume exposed workers and figure out the best way for utilising efficient control measures to protect the them from exposure to chemicals which may have an adverse effect on health in later life.

PAHs are the main toxic component of concern in bitumen/asphalt fumes. This study aims at estimating the adverse health effect risks due to PAHs exposure in these fumes. For that purpose a novel strategy combining ambient, biological and biological effect monitoring has been used. The study assessed:

1- External exposure "External Dose" is the measure of potential exposure to PAHs which was achieved by collecting samples of the air to which workers are exposed and quantifying the PAH profile.

2- Internal exposure "Internal Dose" as the amount of PAHs absorbed into the body (body burden) which takes into consideration

exposure through all routes of entry and was assessed through biological monitoring by biological monitoring 1-hydroxypyrene (1-HP) - a selective index of exposure to pyrene - and urinary thioethers (UTh.), a non-selective index of exposure to a variety of chemicals, which were quantified in urine samples of exposed and control groups.

3- Biological effect monitoring a measure of a biological response to PAHs exposure and achieved through measuring urinary output of d-glucaric acid (UDGA) - an index of hepatic enzyme activity - and cytogenetic assays such as sister chromatid exchanges (SCE) in lymphocytes, and micronuclei in exfoliated cells of the urinary tract (MN).

Results from the three different types of monitoring will be correlated; and the aim is to arrive at a quantitative estimate of risk associated with long-term exposure to moderate and low levels of airborne concentrations of PAHs in bitumen fumes. Knowledge of dose response relationship may allow review of current Occupational Exposure Standards (OES) and Biological Exposure Indices (BEIs), which are at present either unavailable or based on poor background scientific knowledge for most PAH compounds. As a consequence we hope to minimise health risk from exposure to hazardous substances and more specifically environmental carcinogens.

5.2 AIMS OF THE STUDY

The aim of this study is to estimate the adverse health effects due to PAHs exposure in bitumen/asphalt usage by assessing exposure in three ways:

1 - to assess external exposure to PAHs in a range of occupational groups by measurement of airborne concentrations of these substances .

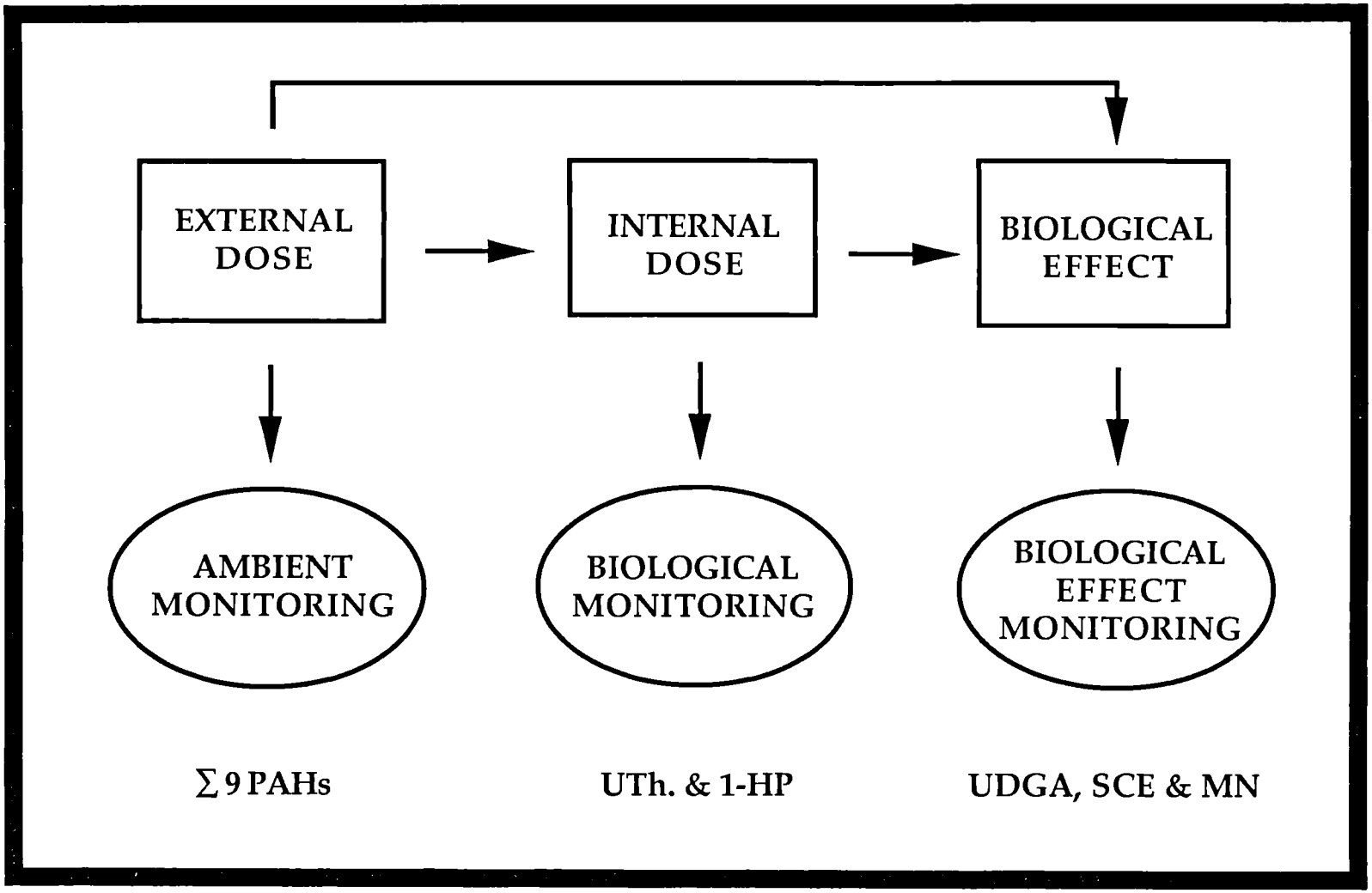
2 - to assess internal dose in occupational groups by quantifying urinary output of thioethers and PAH metabolites.

3 - to examine the effect of such exposure by measuring cytogenetic and biochemical changes in PAH-exposed workers.

4 - to establish the relationship between the above three measurements.

The strategy designed for this study and the methodology used for achieving the objectives of the thesis is presented in figure 5.1.

Figure : 5.1


EXTERNAL DOSE
INTERNAL DOSE
BIOLOGICAL EFFECT
AMBIENT MONITORING
BIOLOGICAL MONITORING
BIOLOGICAL EFFECT MONITORING
∑9 PAHs
UTh. & 1-HP
UDGA, SCE & MN

SECTION II

GENERAL METHODOLOGY

CHAPTER 6

AMBIENT MONITORING OF PAHs

6.1 AMBIENT MONITORING OF PAHs

One of the earliest surveys carried out to measure PAHs in the ambient atmosphere were conducted by the Public Health Service in the late 1950's (Dash and Guldberg, 1982). Since then a number of ambient air monitoring surveys are conducted every year measuring PAHs in environmental and occupational settings using different available techniques.

6.2 AVAILABLE METHODS

Several sampling and analytical methods are available for quantifying PAHs in air. This section will briefly describe, compare and contrast the most widely used ones for sampling PAHs in bitumen fumes.

6.2.1 Total Particulate Matter & Benzene Soluble Matter

This method is the most widely used. It is designed to measure of total particulate matter (TPM) and benzene soluble material (BSM) or cyclohexane soluble material (CSM).

Sampling: In U.S.A., the Occupational Safety and Health Administration OSHA and the National Institute of Occupational Safety and Health NIOSH recommended a method. In the UK. there is no legally acceptable published method for the sampling and analysis of bitumen fumes; however, in February 1990, the Health and Safety Executive published under the series of Methods for the Determination of Hazardous Substances the MDHS 68: a method describing the measurement of particulate and cyclohexane soluble matter in air from coal tar pitch volatiles. This is based on exactly the same principle as the OSHA and NIOSH methods.

The principle behind all these methods is that a known volume of air is drawn through a pre-treated and weighed filter, which is again weighed then extracted with the appropriate solvent. The solvent extract is filtered and part of it is evaporated to dryness in a pre-weighed sample, then the weight

gain of the cup is determined to the nearest μg. In theory all these methods are suitable for measurement of TPM and solvent-soluble matter (SSM) in personal and static air samples. Although all these methods are based on the same principle; however, the procedures are slightly different. For example, the OSHA and the NIOSH methods differ slightly with respect to the filter combination and sampler configuration (Brandt *et al.*, 1985).

6.2.1.1 Advantages and Disadvantages

These methods have got several advantages. They are easy and minimal expertise is needed to carry them out. They are off-the-shelf and time- and cost-effective. The literature contains several references which report TPM and the solvent fraction (Brandt *et al.*, 1985). This is important when one wants to compare and contrast his results to published research. Last but not least, there are several legally accepted published methods for measurement of bitumen fume.

Meanwhile there are several advantages favouring the use of these methods; they have a number of disadvantages one must consider. First of all they are empirical and need to be cross-validated for precision of results with other acceptable methods. They are not very sensitive (e.g. detection limit 0.1 mg/m^3, Claydon *et al.*, 1984). They suffer from inherent confounding factors. For example, non-solvent soluble fine particulate matter might be washed, plasticizer from the some filter holders might migrate to filters, and solvent-soluble fraction might contain a significant number of compounds which are not PAHs. Also, Schulte *et al.* (1975) addressed the fact that filters might disintegrate or the water content of the filter might change before and after extraction. All these interferences will lead to errors in weighing which will result in under- or over-estimation of the amount of bitumen fume. This will eventually give a wrong estimate of the external dose of the workers. This method is not accurate for risk assessment of exposure. The obtained results might over- or underestimate the true carcinogenic potential of the workers' exposure depending on specific PAHs present in the mixture (Chong *et al.*, 1989).

6.2.2 Analysis of PAH-Species

The alternative methods to TPM, BSM and CSM is to qualitatively and quantitatively analyse for specific PAHs of interest.

This method, for estimating the external dose of PAHs, is more widely used in recent research involving risk assessment of exposure to fumes containing PAHs from different sources (Becher *et al.*, 1984; Monarca *et al.*, 1987; Lesage *et al.*, 1987; Jongeneelen *et al.*, 1988[a]; Wolff *et al.*, 1989; Knecht, *et al.*, 1989; Jongeneelen *et al.*, 1990; Buchet *et al.*, 1992; van Rooij *et al.*, 1993, Ferreira Jr. *et al.*, 1994[a & b] and Burgess and Crutchfield 1995).

The major and most important difference between the published methods is the number and selected PAHs analysed. This hinders the close comparison of results from different studies. One of the widely acceptable methods is published in the NIOSH Manual of Analytical Methods (method no. 5515). The assay for PAHs analysis used in this study was based on this NIOSH method.

6.2.2.1 Advantages and Disadvantages

PAHs analysis has several advantages over BSM and CSM assaying. In general, all the reported methods are sensitive (e.g. detection limit of B(a)P was reported as 0.01μg/m^3 in a air sample volume of 960 L, Lesage *et al.*, 1987). This depends mainly on the type of detector used; UV- and mass-spectrometers being at the low and high sensitivity spectrum respectively. The results obtained from such analysis will provide qualitative and quantitative profiles of the PAHs studied. These profiles can be used to identify the source of PAHs. They can also be used to develop statistical models to predict PAHs levels in air which will eventually save time and money in characterising urban aerosols (Dash *et al.*, 1982). Using these profiles computer models can be developed to predict the potential potency of a mixture (Milliken *et al.*, 1982). This is essential for risk assessment studies whereby, information regarding correlation between external dose and internal dose of PAHs, and the biological response is needed. This will aid in better characterisation of the biological and toxicological properties of PAHs (Glass *et al.*, 1991). Measuring PAHs in particulate and vapour form

separately will help in understanding the bioavailability factor which is most crucial when studying correlations mentioned above.

These methods, as all others, have a number of disadvantages. The methods available are time consuming and are a difficult task to carry out. The initial capital cost needed to purchase the analytical equipment as well as the running cost of the method are very high. This makes the possibility of these methods to be used on routine basis in the near future quite remote. This of course will lead to a slow increase in data available for research and comparison of results from independent studies. Careful attention to details of procedures is required. So training, practice and experience are needed before the analyst is expected to carry out the analysis successfully.

6.2.3 Biological Approach

The concept of biological activity of particulate matter containing PAHs was used to develop a basically different method for the quantification of PAHs in air samples. Short-term assays, such as Ames test, were utilised to accomplish that. A good example of a study that utilised two types of monitoring methods is by Monarca *et al.* (1987), where he followed a coupled biological and chemical approach by analysing environmental samples for mutagenicity with salmonella/microsome test and for PAHs contents with an HPLC method.

The biological approach has yet not been routinely used because of insufficient knowledge on dose-response relationships. If properly researched and developed, might prove to be useful; especially when results can be compared to *in vitro* mutagenicity assay utilising human tissue or fluids. Other researchers have used this approach and commented on its potential in being a good measure for risk assessment of exposure to PAHs (Scassellati-Sforzolini *et al.*, 1986).

6.2.3.1 Advantages and Disadvantages

This technique for ambient monitoring of mutagenic/carcinogenic pollutants has got mainly two advantages. First, it has got no capital cost and the running expenses are quite affordable. Secondly, it provides knowledge

on the *in vivo* formation of mutagens and have the potential to detect exposure to unrecognised mutagenic/carcinogenic substances and the synergistic effects of other environmental elements (Scassellati-Sforzolini, *et al.* 1986).

The disadvantages of this assay quite overweigh its benefits. Obviously, it has got all the disadvantages of mutagenic assays used in biological monitoring programs which are mentioned in heading 7.2.1.1 of this thesis. It is not sensitive enough for low levels of exposure to mutagens/carcinogens (Scassellati-Sforzolini, *et al.* 1986). It does not provide any information on the amount and profile of pollutants. This requires its coupling with other analytical methods, like analysis of PAH-species, for obtaining this information. This renders the investigation more costly and time-consuming. A standardised procedure for this method for quantifying pollutants has not been achieved yet and is quite difficult to design and proper interpretation of the use of mutagenicity in ambient monitoring schemes as a measure of exposure requires further study and validation. All this made its use in ambient monitoring and risk assessment studies less frequent, thus leaving the scientist with very little knowledge regarding its usefulness.

6.3 SELECTION OF THE METHOD (ANALYSIS OF PAH-SPECIES)

In this study individual PAHs have been measured for five reasons. 1- The study is aimed at assessing the risks from exposure to PAHs in bitumen fumes and not other components of the fume. So by utilising the above method we will have a good measure of the qualitative and quantitative PAHs profile. 2- Since the ambient air levels are expected to be low to moderate in the paving and roofing industry a sensitive method was required. 3- Since the urinary metabolite 1-HP was our choice of a selective biomarker of exposure to PAHs the measurement of ambient pyrene, the only parent compound, was complementary. The measurement of pyrene will assist in the risk assessment and ultimately in drawing the correlation between the external dose of PAHs or pyrene and the internal dose mirrored by the excretion of 1-HP. 4- Qualifying and quantifying the profile of the

carcinogenic PAHs, such as B(a)P, would help in explaining the results of the SCE and MN the cytogenetic biological markers chosen for this study. This will eventually make the risk assessment a more comprehensive one. 5- Last but not least, was the availability of the equipment and the expertise to develop and run the assay which in several instances is the main reason behind the choice of the methods.

6.3.1 Principle of the Method

Briefly, polycyclic aromatic hydrocarbons in the air are in vapour form or condensed, due to their low vapour pressure, on suspended particulate matter. The particulate matter is sampled onto a filter and a the vapour onto a sorbent tube. After addition of an internal standard the PAHs are extracted separately from the filter and the sorbent tube using dichloromethane (DCM). The samples are concentrated by evaporation and a part of it is analysed. Nine PAHs were quantified using a gas chromatography/mass spectrometry. Appropriate correction are applied as determined by the recovery of the internal standard.

The method used is based on the NIOSH method no. 5515 issued on May 15, 1985. The detailed procedure of the method is found in chapter 10.

CHAPTER 7

BIOLOGICAL MONITORING OF PAHs-EXPOSURE

7.1 BIOLOGICAL MONITORING OF PAHs

As mentioned in the introduction of the thesis, biological monitoring techniques can be classified into two types. They are selective (specific) and non-selective (non-specific) biological monitoring. Each approach has got its advantages and drawbacks. These two approaches should not be regarded as opposite however on the contrary as complementary especially when risk assessment from exposure to complex mixtures of xenobiotics is concerned.

In 1988, Bos and Jongeneelen wrote that there are few methods available for biological monitoring of exposure to PAHs. Since then several potentially useful biomarkers have been looked at in depth and methods have been developed and validated for such purposes. The following section will describe and discuss the most widely used selective and non-selective biomonitoring methods for assessment of exposure to PAHs.

The vast array of specific and non-specific biomarkers available made it feasible for researchers to adopt a strategy involving the simultaneous use of both types of biomonitoring approaches for risk assessment of exposure to chemicals. In 1988 Bos and Jongeneelen used this strategy for biomonitoring workers and patients exposed to coal-tar products. In 1991, Burgaz *et al.* addressed the issue in the Eurotox Congress. They measured UTh. and 1-HP in workers exposed to bitumen fumes. Another study by Burgaz *et al.* in 1992 re-addressed the same issue. In a cross-sectional epidemiological study looking at exposure to PAHs in the coke oven and graphite-electrode-producing plants the same approach, as the one utilised by Burgaz *et al.* (1991) was followed. Urinary thioethers, 1-HP as well as other biological markers were also used by Ferreira Jr. *et al.*, (1994[a]).

Although researchers do strongly recommend, where possible, the use of both approaches simultaneously; this does not mean that utilising either technique separately should be disregarded. There is a general consensus in the literature that selective biomonitoring when assessing exposure to PAHs is effective; while, the issue of the usefulness of non-selective biomarkers is open ended.

7.2 NON-SELECTIVE BIOMONITORING OF PAHs

The main two non-selective biomarkers used for estimating exposure to PAHs have been urinary thioethers and mutagenicity assays. Clonfero *et al.*, (1987, 1988 and 1989), Venier *et al.*, (1987), DeMeo *et al.*, (1987), Bos and Jongeneelen (1988[b]), Pasquini *et al.*, (1989), Reuterwall *et al.*, (1991), Burgaz *et al.*, (1991 and 1992) and Ferreira Jr. *et al.*, (1994[a]) have all used UTh. and mutagenicity assays as non-selective biomonitoring of exposure to PAHs. Each of the methods was either used alone, together or in parallel with environmental monitoring, selective biomarkers or and/or biological effect monitoring.

7.2.1 Mutagenicity Assays

The principle of the method is fairly simple. Suspected mutagens in human urine is tested for their mutagenic activity towards bacterial population which are cultured in standard plates. A classical example is the Ames test. This test has proven to be a useful means to identify genotoxic exposures. The results are usually presented as revertants per ml of urine and tested for statistical significance between exposed and control populations.

7.2.1.1 Advantages and Disadvantages

This method has all the advantages of non-selective biomonitoring mentioned for urinary thioethers in the section 7.2.2.1 of this chapter; however, its main advantage is that it estimates directly the uptake of mutagenic compounds or those with mutagenic metabolites.

The major drawback of this approach (e.g. Ames test) is the strong confounding factor of smoking and diet. Another disadvantage is that the analysis of results must take into consideration synergistic, anta-gonistic, additive and inhibitory effects of compounds in complex mixtures like PAHs. In most instances, knowledge about the interaction between the different ingredient of the mixtures is not available making it extremely hard to interpret the results. Also, the different bacterial strains used in different studies makes comparison of results not feasible and interpretation even

harder. It has been reported that there is great inter-laboratory variation resulting from slight changes, like pH and exposure to visible light, in testing procedures. The article by Ellenberger and Mohn (1980) discusses in detail several aspects of the mutagenicity testing which is beyond the scope of this document.

7.2.2 Urinary Thioethers

Sulphur-containing compounds were first observed in the dog's urine by Jaffe in 1879 and Baumann and Preusse in 1789 (van Doorn *et al.*, 1981) after administration of halogenated aromatic compounds like bromobenzene. It is not until recently, late 1970's, that urinary thioethers started being quite frequently used as a non-selective biological monitoring tool for occupational exposure to electrophilic agents - a class of substances including mutagens and carcinogens - or their precursors.

Researchers have used UTh. as a biomarker in several chemically-exposed occupational groups such as studies by van Doorn *et al.* (1981) looking at UTh. excretion in workers exposed to methylchloride. Seutter-Berlage *et al.* (1979) quantified UTh. excretion in pesticide-exposed workers. Table 7.1 lists several other early studies which examined UTh. in occupational monitoring (Edwards, 1990).

Initially, scientists used this biomarker on its own to assess exposure to electrophillic substances. Results from these studies were not conclusive about the usefulness of UTh. for non-selective biomonitoring of exposure to chemicals in a variety of occupational environments. This led Aringer and Lidmus in 1988 to suggest that it is essential for researchers to conduct well designed and controlled studies looking at the excretion of UTh. in which exposure to the electrophiles is characterised both qualitatively and quantitatively. They suggested that this approach will aid in answering the question raised whether the determination of thioethers excretion in urine is a good index of exposure to genotoxic compounds in the work environment.

Only in more recent studies assessing exposure to PAHs from different sources, scientists started utilising UTh. simultaneously with environmental monitoring, other selective and non-selective biomonitoring

Table : 7.1 Occupational Toxicology Studies Which Utilised Urinary Thioethers as a Non-selective Biomarker of Exposure to Electrophiles Since 1979

AUTHORS	YEAR	TYPE of EXPOSURE	INDUSTRY
Seutter-Berlage *et al.*	1979	Pesticides	Pesticide factory
van Doorn *et al.*	1980	Methylchloride or Carbon disulphide	Not specified
van Doorn *et al.*	1981	Unknown mixture of electrophiles	Chemical waste incinerator
Scassellati Sforzolini *et al.*	1986	Asphalt fumes (PAHs)	Road pavers
Bayhan *et al.*	1987	Cytotoxic drugs	Nurses (Oncology)
Bos and Jongeneelen	1988	Coal-tar (PAHs)	Creosote impregnation plant & Dermatological Patients
Pasquini *et al.*	1988	Bitumen fumes (PAHs)	Road pavers
Burgaz *et al.*	1988	Bitumen fumes (PAHs)	Road pavers
Karakaya *et al.*	1989	Bitumen fumes (PAHs)	Asphalt plant & Road pavers
Pasquini *et al.*	1989	Anaesthetics	Operating room personnel
Thiringer *et al.*	1991	Cytostatic drugs	Nurses (Oncology and Haematology)
Reuterwall *et al.*	1991	PAHs	Coke-oven workers
Burgaz *et al.*	1992	Bitumen fumes (PAHs)	Road pavers
Edwards and Priestly	1993	Mixture of hydrocarbons	Petroleum retailers
Ferriera *et al.*	1994	PAHs	Graphite-electrode production plant & Coke-oven workers
El-Gazzar *et al.*	1994	Styrene Aniline & Benzidine	Plastic & Battery industry Chemical & Dyeing industry

markers as well as biological effect monitoring. Pasquini *et al.* (1989) carried out biological monitoring of the mutagenic/carcinogenic hazard associated with exposure to bitumen fume during paving operation. UTh. excretion was higher in non-smoking exposed when compared to non-smoking controls, however not significantly different non-smoking unexposed subjects. Contradictory findings were reported by Burgaz *et al.* (1992) where significant increase in the excretion of UTh. was observed in road paving workers exposed to bitumen fumes when compared to control after stratifying the population with respect to smoking habits.

Up to date results of research on UTh. as a non-specific biomarker of chemical exposure is not conclusive and its usefulness in risk estimation of exposure to PAHs is still controversial (Ferreira Jr. *et al.*, 1994[a]).

7.2.2.1 Advantages and Disadvantages

The thioether assay has several advantages especially when assessing exposure to complex mixtures (e.g. PAHs). They could be even of extreme value especially if the components of the mixture and their characteristics are unknown. Non-selective biomarkers, such as UTh., are also useful as a first approach in developing selective biomarkers (van Doorn *et al.*, 1981). The assay is cheap and minimal expertise is needed to carry out the analysis.

Although, in some occupational settings, it is appropriate to apply the thioethers assay for assessing the workers' exposure to chemicals it is important to consider its limitations. The baseline of UTh. excretion in the general population is high. This renders the assay insensitive for quantifying exposure to low levels of an array of chemicals. Aside to insensitivity are the multitude of confounding factors such as diet, smoking, medicine intake, and endogenous electrophiles (e.g. prostaglandin and oestrogen) which all contribute to the excretion of mercapturates. This introduces significant inter- and intra-individual variation, again not aiding in the observation of differences between exposed and control groups.

So little is known about the excretion of UTh. One study, by Aringer and Lidmus in 1988, observed a peak for UTh. excretion at 18:00 and reverts to baseline at 6:00 next day. This was never extensively researched and there is no sufficient evidence available for accurate predictions about the optimum

time of sampling. This limited knowledge about the kinetics of thioethers formation and urinary excretion does not allow us to utilise it in the optimum way. In complex mixtures the kinetics of uptake, absorption, distribution, metabolism and possible toxicity of individual components and their interactions are not known. This fact makes the interpretation of UTh. complicated since they assess the integrated exposure of the whole mixture. Results from UTh. can not be compared to legal occupational limits which makes the assay not worth utilising for occupational health and hygiene purposes. There are other limitations related to the methodology. For instance, some oligopeptides, which resemble thioethers, do not precipitate in an acidic medium. They will hence contribute to the background absorbance readings and over-estimate the actual exposure. The pH of the sample upon addition of DTNB/citrate reagent is very critical to avoid colour development due to presence of free hydroxyl group.

7.2.3 Other Methods

There are a few other non-selective biomarkers used for monitoring exposure to alkylating agents. A good example is methylated purines, amino acids in haemoglobin of erythrocytes, haemoglobin itself as dosimeter of exposure etc. (van Doorn *et al.*, 1981).

7.3 SELECTION OF METHOD (URINARY THIOETHERS)

Since the chemical exposure we are trying to examine is a complex mixture it was essential to utilise a non-selective biomarker to be able to draw some conclusions on the toxicity of the mixture. UTh. was our choice for two main reasons. First, it has been recently used in occupational toxicology studies where workers were biologically monitored for exposure to bitumen fume in the paving industry. Secondly, its suggested peak excretion is relatively short which can provide us with information about the previous day's exposures to chemicals in the working and domestic environments.

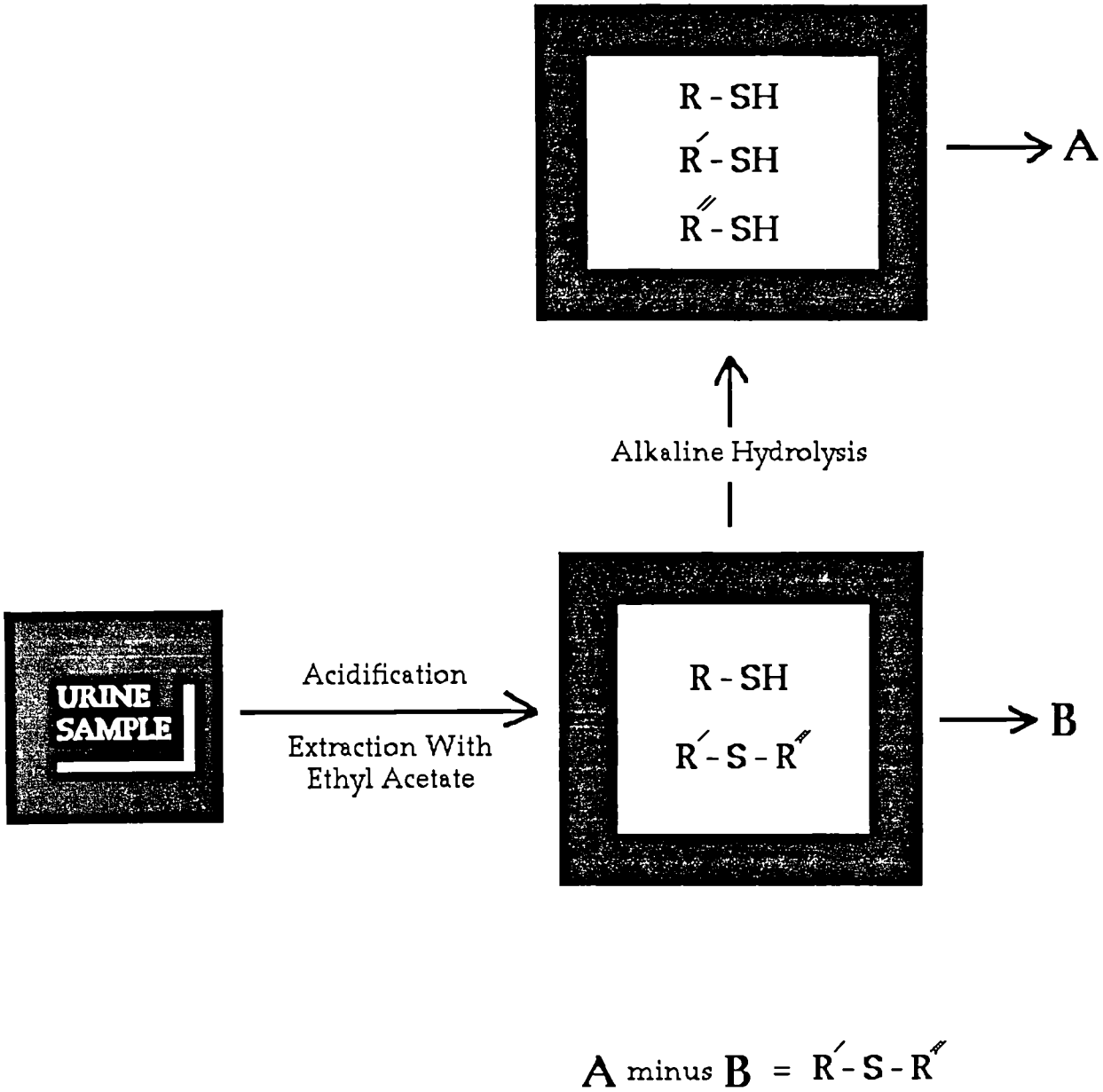
7.3.1 Principle of the Method

Electrophiles are absorbed into the body and are conjugated by nucleophilic glutathione to give rise to chemical-GSH conjugate. These conjugated chemicals can be further metabolised to N-acetylcystiene derivatives (thioethers) to be excreted in urine.

Urine samples are acid treated, solvent extracted, evaporated, and then re-constituted in water. An aliquot of the extracts are then alkaline hydrolysed. The absorbance recorded from the non-hydrolysed aliquot subtracted from the hydrolysed one will give the net concentration of thioethers (R'- S - R"). The method is schematically illustrated in a modified diagram (van Doorn *et al.*, 1981) in figure 7.1.

A detailed method procedure is found in chapter 10.

Figure : 7.1 URINARY THIOETHERS *Ex - Vivo*



7.4 SELECTIVE BIOMONITORING OF PAHs-EXPOSURE

This is a topic that has been extensively researched and reviewed in the recent literature. As discussed before in the introduction of the thesis, several assay have been developed and validated for specific biomarkers of exposure to a variety of chemicals. This section focuses on describing the available selective biomarkers of PAHs exposure. The major three ways of applying selective biomonitoring in assessing exposure to PAHs are analysing for parent PAHs excreted in body fluids, their selective metabolites or specific DNA, protein or haemoglobin adducts of chosen PAHs. These three methodologies will be discussed and their advantages and disadvantages highlighted.

7.4.1 Analysing for Parent PAHs

The chemical reduction of PAHs' urinary metabolites to their parent compounds and the subsequent quantification of specific PAHs by different techniques was one of the earliest selective biomonitoring techniques used for assessment of PAHs exposure. This way of monitoring the real exposure to PAHs by analysing body fluids was utilised by several scientists. Becher and Bjørseth in 1983 developed an analytical procedure for measuring PAHs in urine. They assessed its applicability by applying it to urine samples from non-occupationally exposed controls and aluminium workers. No significant difference was observed between the two groups and smoking was reported to be as a major contributor to the internal dose; thus confounding the observation of potential differences between the two groups arising from occupational exposure. Another study monitored the internal dose of PAHs by analysing for total PAHs in the urine of a group of workers expose to coal-tar in a primary aluminium industry (Clonfero *et al.*, 1987). In that study, Clonfero and co-workers found that the low MWt. PAHs contributed to 46-86% of the total dose while the high MWt. carcinogenic PAHs increase slightly after occupational exposure and add up, in some instances, to 14% only of the excreted dose. This was suggested to be due to their predominant excretion in the faeces (Clonfero E. *et al.* 1987). In 1989 Clonfero *et al.* compared the sensitivity of this assay to mutagenicity assays and 1-HP the selective urinary biomarker of pyrene exposure. They found that the

mutagenicity assay was the least useful for assessing exposure of psoriatic patients to a complex mixture of PAHs in coal tar. Analysis of total PAHs was less sensitive than to 1-HP due to the method used in the study. The method used was adopted from Grimmer *et al.* (1991) which was reported to be inefficient in separating conjugated metabolites and reducing them to their parent compounds.

7.4.1.1 Advantages and Disadvantages

The main advantage of this method is that it provides a great deal of information about the profile of the internal dose of PAHs. This will help in the understanding of the possible interactions between the components of the mixture and the toxicokinetics of individual PAHs. There are as many as a hundred different PAH compounds identified in the workplace and each of them is metabolised to a range of metabolites. Analysing for all those metabolites in order to quantify the total internal dose of PAHs is impractical. In that case analysing for the total dose of parent compounds in urine proves to be of more use.

The disadvantages however are considerable. The method is not as sensitive as other available selective biomonitoring techniques. This is due to the inefficiency of several analytical steps in the method. It is time consuming and impractical for routine use (Becher and Bjørseth, 1983). Smoking and other non-occupational exposure to PAHs do heavily influence their internal dose, thus confounding the results of occupational exposure studies.

7.4.2 Analysis of Metabolites of PAHs

More recent research focuses on this methodology to assess exposure to PAHs in environmental and occupational setting. This is due to the fact that the measurement of all the components of PAHs mixture is impractical to carry out on routine basis and the way around the problem of PAH-exposure assessment is to research into the use and suitability of a potential dosimeter representative of the mixture.

The first attempt to determine urinary metabolites of PAHs was by Keimig and Morgan of the Department of Preventive Medicine and

Environmental Health at the University of Iowa College of Medicine in USA in 1982. Their criteria for choosing a specific PAH as an indicator of absorbed dose in occupationally exposed people was to determine its minimum oral dose or what they called "dose-detection threshold". That minimal dose should be less than or equal to the reported occupational exposure for that PAH to be considered as a potential biomarker. Since naphthalene and pyrene are the most abundant in occupational environments polluted by PAH they were chosen to be studied. 1-naphthol and 1-HP were identified as the metabolites of naphthalene and pyrene respectively. The authors recommended in their article that further research should be done to refine their method and test it in exposed workers.

In 1987, Jongeneelen and co-workers published a method for determining hydroxylated metabolites of PAHs in urine. They studied with psoriatic patients exposed to coal-tar. Using HPLC with fluorescence detection they identified and monitored 1-HP, 3-hydroxybenzo(a)anthracene and 3-hydroxybenzo(a)pyrene the respective metabolites of pyrene, B(a)A and B(a)P.

Since that time a large number of articles have been published describing experimental, occupational and environmental studies using 1-HP as a biomarker. Table 7.2 shows a fairly extensive list of these studies. Researchers developed interest in looking at other selective metabolites of specific PAHs. In an unpublished work Shealy *et al.* described the development of a method for measuring phenolic metabolites of chrysene (1- and 3-hydroxychrysene). Grimmer *et al.* (1991) measured five isomeric hydroxyphenanthrenes which are selective metabolites of phenanthrene. They measured hydroxyphenanthrene metabolites as well as dihydrodiols in coke oven workers. The vast amount of research done on 1-HP is very promising. It is elucidating more and more facts about the toxicokinetics as well as other characteristics of that pyrene metabolite. Jongeneelen and co-workers are intensively researching this topic and they are trying to suggest Biological Exposure Index (BEI) for occupational exposure to coal-tar by using 1-HP as the biomarker of choice.

7.4.2.1 Advantages and Disadvantages

Selective biomonitoring of PAHs such as 1-HP determination has proven to be of good value. The methodology has several advantages such as

Table : 7.2 Occupational Toxicology Studies Which Utilised Urinary 1-hydroxypyrene as a Selective Biomarker of Exposure to Pyrene and/or total PAHs Since 1985

AUTHORS	YEAR	OCCUPATIONAL / ENVIRONMENTAL*
Jongeneelen *et al.*	1985	Creosote impregnation plant
Jongeneelen *et al.*	1986	Coal-tar distillation
Jongeneelen *et al.*	1988a	Wood preservation plant
Jongeneelen *et al.*	1988b	Creosote impregnation plant
Jongeneelen *et al.*	**1988c**	**Road pavers**
Zhao *et al.*	1988	Traffic police & Coke-oven
Clonfero *et al.*	1989	Dermatological patients*
Clonfero *et al.*	1990	Anode plant
Jongeneelen *et al.*	1990	Coke-ovens
Tolos *et al.*	1990	Aluminium reduction plant
Zhao *et al.*	1990	Coal-burning*
Buchet *et al.*	1992	Graphite-electrode & Coke-oven plants
Buckley *et al.*	1992	Diet*
Burgaz *et al.*	**1992**	**Road pavers**
Gardiner *et al.*	1992	Carbon black manufacturing
Jongeneelen *et al.*	1992	Coke-ovens
Cenni *et al.*	1993	Pyrite mine Railway tunnel under construction Earthenware factories
Granella and Clonfero	1993	Automotive repair
Grimmer *et al.*	1993	Coke-oven
Kanoh *et al.*	1993	Diesel gas*
van Rooij *et al.*	1993a	Coke-oven
van Rooij *et al.*	1993b	Wood preservation plant
Boogaard *et al.*	1994	Petrochemical plant
Ferreira Jr. *et al.*	1994a	Graphite-electrode production Coke-oven
Ferreira Jr. *et al.*	1994b	Steel foundry Graphite-electrode production
Hansen *et al.*	1994	Iron foundry
Jongeneelen *et al.*	1994	PAHs*
Omland *et al.*	1994	Iron foundry
Quinlan *et al.*	1995	Coal liquefaction

being fairly cheap and relatively easy to conduct. Its high sensitivity with the detectors available makes it applicable to occupational as well as environmental exposure studies. Its sensitivity for monitoring exposure to PAHs was studied by Clonfero E. *et al.* (1989) in comparison to other techniques. It was found to be the most sensitive.

7.4.3 DNA Adducts

DNA-adducts result from covalent binding between a genotoxic alkylating xenobiotic or its reactive metabolite and DNA in target cells. This reaction is viewed as a key event in genotoxic mechanisms (Watson *et al.*, 1991). It is thought to be the initiating event triggering the cascade of biochemical change eventually leading to malignancy is what Weinstein wrote in 1988 (Hemminki, 1992).

It is only during the last two decades scientists made use of this endpoint in the study of genotoxic xenobiotics' metabolism in different animal and human tissue. Currently, DNA-adduct formation is used as an endpoint dosimeter of exposure to electrophilic alkylating substances. In the case of mutagens/carcinogens the carcinogen-macromolecule adducts, represented by DNA-adducts, could be used as tools for the determination of carcinogen dosimetry at molecular level (Weston *et al.*, 1989). This biomarker, although normally measured in surrogate tissue, is useful for risk assessment of exposure as a particularly relevant measure of the biologically effective dose of initiating carcinogens (Vähäkangas and Yrjänheikki, 1990). DNA-adducts can also be used as a biomarker of susceptibility to carcinogens. It was suggested, by Perera *et al.* (1989), DNA-adduct formation reflects on the ability of individuals to activate carcinogens and repair DNA damage. DNA repair rate influences the steady state adducts level (Vähäkangas and Yrjänheikki, 1990).

There are several methods for measuring DNA adducts. These are broadly classified under three categories: (1) immunological assays (2) ^{32}P-postlabelling (3) chemicophysical assays. Under category (1) comes the ultrasensitive enzymatic radioimmunoassay (USERIA), enzyme-linked immuno- sorbent assay (ELISA) and competitive radioimmunoassay (RIA); category (2) includes the ^{32}P-postlabelling assay and finally category (3)

covers synchronous fluorescence spectrometry (SFS) and GC/MS techniques. Each of the above methods have its own advantages and disadvantages; however, all are sensitive enough to detect PAH-DNA-adducts for the purpose of experimental, occupational and environmental exposure studies. Table 7.3 compares and contrasts all these methods.

7.4.3.1 Experimental Toxicology

Experimental toxicologists have been studying DNA adducts formation in animal and human tissue *in vitro* and *in vivo* for a number years. For instance recent studies looking at PAHs-DNA adducts formation in mouse and human skin explants found that adducts profiles were qualitatively similar. When comparing B(a)P-DNA adducts in mouse skin *in vitro* and *in vivo* , it was observed that the former was 50% of the latter. This study suggested that short-term mouse skin culture systems are an acceptable model for B(a)P metabolism *in vivo.* It also insinuated that human explants systems also provide an accurate model for B(a)P metabolism *in vivo* (Watson *et al.*, 1987).

Phillips *et al.* (1990) studied (by ^{32}P-postlabelling) PAH-DNA adducts formation in mouse skin exposed to one of a number of PAH containing compounds *in vivo* (e.g. bitumen, creosote, Juniper tar, coal-tar ointment). Similar levels and patterns of adducts were observed in human and mouse skin *in vitro* when exposed to coal-tar ointment and Juniper tar. Multiple dosing of skin with bitumen resulted in a steady-state accumulation of adducts during the first 3 weeks followed by an approximately steady-state levels of adducts. For creosote and coal-tar, levels of adducts were higher than bitumen. This is expected knowing that PAH content of bitumen is considerable lower than coal-tar and creosote. Their conclusion supported that of Watson *et al.* (1987) and that human skin explants have a metabolic capacity that resembles that of skin *in vivo* . B(a)P-DNA adducts in Sprague-Dawley rats exposed intraperitoneously to 100 mg/kg body weight exhibited similar patterns in lung, liver and PBLs tissue. They reached a maximum in 3 to 4 days followed by a decrease to 56 days. This suggested that PAH-DNA adducts in PBLs as a surrogate tissue may predict the biologically effective dose at target tissue such as lung and liver (Nesnow *et al.*, 1993).

Table : 7.3 Advantages and Disadvantages of Methods Used for Quantifying DNA and Protein Adducts

Advantages	Disadvantage
32P-Postlabelling	
* Highly sensitive (1 adduct/10^9 - 10^{10} nucleotide)	* Technical complexity
* Non-specific (can assess exposure to complex unknown mixtures)	* Handling radiolabelled carcinogens
* Moderately costly	* Non-specific (can't assess exposure to a single substance)
	* Under-estimates of DNA adducts
	* Useful for research and screening purposes
	* Technique not fully investigated and validated
	* Not used for protein adducts
Immunoassays (USERIA, ELISA, RIA)	
* Can be specific	* Needs rigorous characterisation and standardisation of monoclonal anti-bodies and specific antisera
* Relatively simple technique	* Under-estimates of DNA adducts
* Suitable for large # of samples	* Quite sensitive (1 adduct/10^7 - 10^8 nucleotide)
* Less costly	
Physicochemical Assays SFS and GC/MS	
* Highly specific (e.g. BaPDE-DNA adducts)	* Less sensitive (1adduct / 10^7 nucleotides)
* Simple and straightforward methodology	* Difficulties in interpretation of spectra
* Suitable for large scale studies	* High quality reagents needed (Interference from impurities)
	* High capital cost

% Farmer *et al.* (1991)
@ dell'Omo and Lauwreys (1993)
$ Vähäkangas and Yrjänheikki (1990)
* Shugart *et al.* (1982)

7.4.3.2 Occupational Health

DNA adducts are being used more frequently in occupational and environmental exposure studies. Studies using the different analytical techniques available investigated DNA-adduct concentration in population exposed to a variety of alkylating agents. Only those looking at PAHs-DNA adducts are pointed out here.

PAH-DNA adducts have been studied by different analytical methods in different groups of workers occupationally exposed to PAHs in several industries like coke ovens, foundries, roofing, fire fighting and aluminium plants. The article by dell'Omo and Lauwreys (1993) has a very good summary of the results reported by more than twenty studies.

Only those studies monitoring PAH-DNA adducts in roofers will be reported here in detail. Seven out of 28 roofers biomonitored had detectable levels of adducts in their PBLs (Shamsuddin *et al.* 1985). Herbert *et al.* (1990) biomonitored 12 roofers and 10 matched controls. Eighty three percent of the roofers had detectable levels of aromatic DNA adducts while the controls had only 17%. Mean adducts levels in roofers was significantly higher than those of the referents. The adducts levels in the exposed did not correlate with concentrations of total PAHs or B(a)P in personal air samples. On the other hand, total PAHs and B(a)P levels in post-shift skin wipes correlated with the median adduct levels in 8 roofers. No correlation was found between either dietary PAHs consumption in the month before sampling or smoking habits and adducts. These two studies imply that PAH-DNA adducts are potentially a good biomarker of roofers exposure to PAHs. The number of undetectable levels of adducts in PBLs reported for some roofers could be due to a number of reasons. Good hygiene practices may lead to a low external dose of PAH. Similar external doses entering the body through various routes of exposures may lead to different doses in target or surrogate tissues. Intra-tissue variability in adducts levels is another factor as well as inter-individual variation in metabolism of PAH and adduct formation. PAH-DNA adducts have not been measured in pavers exposed to bitumen fumes.

Effort from several disciplines are aiming at answering various important questions. Is DNA adducts a useful biomarker for predicting the internal dose of a genotoxicant? Is it closely linked with external exposure?

What are the main factors affecting their formation? What are the major confounding factors? Is the mechanism of DNA adduct formation linked with clinical cancer?

Once a firm link is established between this indicator and clinical cancer, then it can serve as an endpoint surrogate for cancer. Therewith linking different established endpoints to each other and to various exposures would be the appropriate strategy to follow for cancer prevention.

7.4.3.3 Advantages and Disadvantages

The advantages of using DNA adducts as a biomarker of exposure to mutagens/carcinogens are several. There is a number of different types of highly sensitive assay available for their analysis. Depending on the methodology used the biomarker can be either selective or non-selective in evaluating exposure to complex mixtures. The high sensitivity of the methods allows the studying of this biomarker in workers with low levels of exposure to chemical mutagens/carcinogens.

They can be monitored in most biological tissue. Depending on the tissue being analysed DNA adducts can provide knowledge about past exposures or current ones. For instance Nesnow *et al.* (1993) reported that women have two populations of lymphocytes short-lived ones (3-4 days) and long-lived ones (530 days). Their ability to quantify current exposure renders them suitable for evaluating preventive control measures used in occupational hygiene intervention studies.

Various advantageous characteristics of this marker gives it the potential to supply information on carcinogenic mechanisms. Those features also provide an approach to the problem of understanding the role of metabolism in risk models and man which is particularly of importance when several bioactivation pathways are possible, such as in PAHs, where discrimination between genotoxic and non-genotoxic metabolites is difficult (Watson *et al.*, 1987). Monitoring DNA adducts aid in understanding toxicokinetics, possible mutagenic/carcinogenic mechanisms and other features of the studied chemical hazard since they can be studied *in vitro* and *in vivo* . The ability to evaluate the mutagenic/carcinogenic dose at the target tissue using this biological indicator removes the non-linearity introduced by

extrapolating from whole-body dose to the effective dose at the target tissue and helps in establishing clearer dose-response relationships. This ability of dose assessment at target tissue may aid in elucidating carcinogenic mechanisms and in prediction of cancer risk. It can also give an idea about the ability of the individual to activate carcinogens and repair DNA damage.

Although the advantages of DNA adduct monitoring are very appealing to toxicologist from different disciplines there is a considerable number of disadvantages to account for. The equipment used for the available methodology is rather expensive and a certain amount of expertise is needed to carry out the complicated analysis with confidence. Method standardisation and rigorous validation is needed especially that standards are prepared in house and none are commercially available. The interpretation of results is sometimes difficult and cross comparison of results between various laboratories using the same or different method of analysis is yet not feasible. For example, the variability is the utilisation of an immunoassays (e.g. the variation in the preparation and use of specific anitsera or monoclonal antibodies introduces appreciable inter-laboratory variation in the results obtained. In addition, direct comparison of adducts levels determined using the miscellaneous techniques available may be misleading because they assess distinct end-points. The results found in the literature regarding the agreement in the results between assays is conflicting. While Hemminki (1988) found a strong correlation between postlabelling and immunoassays; Vähäkangas and Yrjänheikki (1980) and Haugen *et al.* (1986) reported a poor correlation between physicochemical and immunoassay techniques.

7.4.4 Protein Adducts

The chemical modification of proteins is a well-established phenomenon. Ehrenberg and his colleagues conducted the first piece of work on the use of proteins adducts as dosimeter (Wogan, 1988). Since then protein adducts (e.g. albumin adducts and haemoglobin (Hb) adducts) have been used as a tool for biomonitoring exposure to genotoxic compounds. The latter was suggested by Osterman-Golkar *et al.* in 1976 as a suitable dose-monitoring protein (Wogan, 1988). Up to date most of the literature concerns studies on Hb alkylation and it is still the most popular protein for study by

virtue of its ready accessibility and long life (Farmer *et al.*, 1991). In general, binding to proteins (albumin and Hb) correlates with that of DNA suggesting the usefulness of protein adducts as a surrogate for DNA adducts in assessing the internal exposure to alkylating agents when target tissues are not available (Hemminki, 1992). Watson *et al.* (1991) has alluded to few examples of chemicals which bind to proteins without detectable binding to DNA. They did not negate what Hemminki (1992) wrote in his article; however they stressed that knowledge of the mechanisms and nature of interaction of the chemical in question with the respective molecule is essential when it comes to concluding on the usefulness of protein adducts as an indirect tissue DNA dosimeter.

The methods of analysis for protein adducts are similar to those of DNA adducts. This usually means that analysis of both biomarkers can be carried out in the same laboratory facilitating the execution of studies designed to compare both biomarkers.

Few reports have been published regarding PAH-protein adducts and the knowledge available concerning them is very much limited if compared with that of DNA adducts. No published studies have looked at protein adducts in animals and a limited number on populations exposed to PAHs. dell'Omo and Lauwreys (1993) in their quite extensive review on adducts to macromolecules mentioned two studies which looked at PAH-protein adducts in occupationally exposed groups and one in non-occupationally exposed individuals. One of the occupational exposure studies looked at foundry workers and the other at foundry workers and roofers. In both studies, the investigators found significant increase in PAH-protein adducts in comparison to controls. No correlation was found between protein adducts and DNA adducts in the latter study. Recently, PAH-Hb adducts were determined in steel foundries and graphite electrode plant workers. The results were similar to the studies mentioned in the text above, whereby the BaPDE-Hb adducts were significantly higher than those of the controls (Ferreira Junior *et al.*, 1994[b]). The authors found a correlation between BaPDE-Hb adducts concentrations and airborne PAHs levels. They also observed that tobacco consumption is a confounding factor to be controlled for. In 1994, Omland *et al.* measured BaP-albumin adducts and 1-HP in iron foundry workers and controls. They reported, no difference in BaP-albumin adducts while controlling for smoking; while 1-HP was sensitive enough to show

significant differences between the biomonitored groups. The non-significant results observed for BaP-albumin adducts was thought to be due to possible high air pollutant PAH exposure in the control group. The authors highlighted in their article the pitfalls in the study design which did not assist them in concluding on the usefulness of BaP-albumin adducts as a biomarker of workers' exposure to PAHs in iron foundries.

The significant increases in PAH-protein adducts observed in different groups exposed to PAHs in a variety of industries implies on the mileage this biomarker holds in being of real value for exposure and risk assessment. More validation is needed perhaps by closely examining it with DNA adducts, cytogenetic endpoints such as SCE and chromosomal aberrations and other susceptibility (Ferreira Junior *et al.*, 1994[b]).

7.4.4.1 Advantages and Disadvantages

Using protein adducts as a biological marker has got certain advantages. Blood proteins (e.g. albumin and Hb) are easy to obtain since they are in large quantities. The wide spectrum of half-lives for different proteins render them suitable for measuring an integrated exposure over relatively long-term periods of time or more recent exposure. In the case of albumin, it is synthesised in the liver which is the main sight of metabolism for most xenobiotics and particularly PAHs. This means they are readily available for binding with reactive metabolites (e.g. PAH-diol-epoxides). One of their advantages over DNA-adducts is that they are not influenced by large inter-individual variations due to differences in the activity of DNA repair systems and no known enzymatic repair mechanism exists for Hb and serum proteins. This characteristic makes the interpretation of the results more straightforward in comparison to DNA adducts (dell'Omo and Lauwreys, 1993).

Although this biomarker seem to have a distinct advantages over other indicators few occupational toxicology studies have made use of it which leaves the scientists with very little knowledge about its applicability and usefulness for risk assessment of exposure to alkylating agents. Another drawback is that the analytical methods used for quantifying protein adducts are the same as those used for DNA adducts implying that they carry the

same technical disadvantages.

7.5 SELECTION OF METHOD (URINARY 1-HYDROXYPYRENE)

Our choice fell on 1-HP for a number of reasons. First, there was more known about its absorption, metabolism and excretion in animals and humans. Its concentration in urine is ~ nineteen times higher than that of 3-hydroxybenz(a)pyrene which makes it easier to measure even when exposure to pyrene is low (Zhao *et al.*, 1990). Its sensitivity, good analytical recovery and stability favours its use. Its precursor, pyrene, is one of the most abundant high MWt. PAHs in occupational and environmental airborne PAHs. The correlation found between 1-HP and ambient total PAHs external exposure and its significant correlation with dermal absorption to coal-tar derived road tar on the wrist and hands of pavers makes it more interesting to utilise it as a biomarker. It has also been well researched using *in vitro* assays with human hepatic preparation and *in vivo* in animals (Jongeneelen *et al.*, 1987[a]).

7.5.1 Principle of the Method

Figure 3.3 show the metabolism of pyrene to 1-HP. 1-Hydroxypyrene is excreted free and conjugated in urine. The conjugated metabolite is enzymatically hydrolysed in the presence of ß-glucuronidase/arylsulfatase. The free and unconjugated 1-HP are extracted from the hydrolysed urine sample and quantified using suitable analytical techniques such as HPLC coupled with a fluorescence detector.

A detailed method procedure is found in chapter 10.

CHAPTER 8

BIOLOGICAL EFFECT MONITORING OF PAHs-EXPOSURE

8.1 BIOLOGICAL EFFECTS MONITORING OF PAHs-EXPOSURE

Biological effect monitoring can be categorised as selective and non-selective. Further classification of biological effect markers include those indicating a biochemical change or a cytogenetic effect due to chemical, physical, biological, and psychosocial hazards. PAHs, radiation, viruses, stress and lack of sleep are good examples of these types of hazards respectively.

In 1987, Rubin addressed the topic of biological indices of enzyme induction, as markers of hepatic enzyme activity, and their usefulness in assessing human exposure to low levels of environmental and occupational xenobiotics which can lead to adaptive changes in the liver. In the same year, Colombi wrote about his experience of testing microsomal enzyme induction in occupational and environmental medicine. He discussed in detail the general limitations of these kinds of indices. From the literature we can see that most of these indices are non-selective with very few selective ones such as increased zinc protoporphyrin in erythrocytes due to exposure to lead (Zielhuis and Henderson, 1986).

Cytogenetic biomarkers are extensively employed in chemical exposure risk assessment studies. There are several non-selective indices available for biological monitoring of populations exposed to genotoxicants. Table 8.1, although not comprehensive, lists several of those cytogenetic end points.

The general reasons for studying cytogenetic markers in human population include: i) to determine if a new chemical with a mutagenic potential has been introduced or an increase in ones already present has occurred; ii) to evaluate mutation frequencies with termination of exposure to the suspected or known mutagens; iii) to identify groups in the general population with high frequency of mutations and attempt to identify causes; iv) to identify the susceptible individuals to genotoxic agents within the population.

Table : 8.1 Cytogenetic Assays Available for the Biomonitoring of Human Populations Exposed to Xenobiotics

Mutagenic exposure	Somatic cell effects
Mutagens in body fluids	*Non-cytogenetic effects
Determination of chemicals and their metabolites in biological samples	Haemoglobin alkylation
	Damaged DNA bases in urine
	DNA adducts
Determination of conjugation compounds	DNA strand breaks
	DNA sequences
	Unscheduled DNA synthesis
Somatic cell effects (sperm)	DNA repair
Sperm morphology	*Cytogenetic effects
DNA adducts in sperm	Sister chromatid exchange
DNA strand breaks in sperm	Chromosomal aberrations
Unscheduled DNA synthesis in sperm	Micronucleus test
DNA quantitative distribution in sperm	**Genetic damage in offspring**
	Spontaneous abortion
	Congenital defects
	Cytogenetical effects

* Adapted from Hogstedt, 1986.

This part of the chapter comments on the available methods for biological monitoring of biochemical changes and cytogenetic effects arising from chemical exposure. It also points out the biomarkers of choice and the reasons for picking them for the estimation of the risks involved in exposure to PAHs.

The two types of biological effect biomarkers have been used to monitor workers' exposure to chemicals both separately and simultaneously. Their simultaneous use might be useful in some instances; however, it is rarely that biochemical changes are inter-related or linked with cytogenetic endpoints which implies that the use of either in isolation of the other could still be of use. Both types of biological effect monitors have been used to assess risk from a variety of single chemical or complex mixture exposures.

Approximately, 12% and 5% of the biological monitoring studies conducted between 1981 and 1985 included agent-specific non-adverse effects and cytogenetic monitoring respectively (Yager, 1991). This indicates that there is a general interest in biological effect monitoring of exposure to chemicals. This volume of research is not sufficient to answer and solve all the queries and problems regarding these biochemical effect markers and cytogenetic endpoints. Future research must focus on further validation of the methodology in humans by investigating baseline values in non-occupationally exposed population. Dose-response relations must be determined as well as the relationships existing between the bioindicator and disease in chronic animal studies. These may elucidate potential for prediction of health effects. The assessment of possible relations between endpoints and health outcomes in a longitudinal cohort studies would be of great value.

8.2 BIOLOGICAL MONITORING OF BIOCHEMICAL CHANGES

There are many indices to monitor biochemical changes. It is beyond the scope of this thesis to discuss them all. The interesting one for this study are those that could be employed in the risk assessment of

exposure to PAHs. The most widely used ones are those capable of monitoring cytochrome P-450 activity upon exposure to complex mixture of PAHs, known hepatic enzyme inducers, are UDGA and urinary 6ß-hydroxycortisol (6ß-OHF). More recent experimental techniques include measurement of hepatic blood flow, serum lipids and lipoproteins and the activity of specific isozymes of P-450 in human lymphocytes by radioimmunoassay with monoclonal antibodies (Rubin, 1987). Sherson *et al.* (1992) used measurements of activity of cytochrome P-450 IA2 as a biological effect marker to predict interactions of smoking and uptake of PAHs in foundry workers. The enzyme activity was assessed by measuring the metabolic ratios of dietary caffeine.

8.2.1 Urinary 6ß-hydroxycortisol

6ß-hydroxycortisol is a polar, non-conjugated, hydroxylated, and minor metabolite of cortisol formed in the endoplasmic reticulum of hepatocytes by the mixed function oxygenases and is excreted by the kidney (Roots *et al.*, 1979 and Saenger *et al*, 1984). It constitutes approximately 1-2% of the total daily cortisol secretion. This bioindicator has not been employed for biological effect markers of exposure to PAHs; however, could potentially be a promising biological effect marker, since it reflects on the activity of cytochrome P-450 enzymes.

8.2.1.1 Advantages and Disadvantages

The assays main advantages are sensitivity, non-invasiveness, and cheapness. It is relatively easy to conduct with moderate expertise. Its main drawbacks as a biomarker of enzyme activity, like most other bioindicators, is that it is hampered by large inter- and intra-individual variation and is confounded by several factors like smoking, alcohol, diet, etc.). A more specific disadvantage of this biological effect biomarker is that it is affected by diurnal and cortisol production variations. This phenomenon made it necessary for users of this marker to correct for this variability by expressing their data emanating from such an experiment as the ratio of 6ß-OHF to 17-hydroxycorticosteroid. Better dose-response relationships and differences between exposed and control groups were observed when data was expressed as a ratio compared to absolute

concentrations of 6ß-OHF. The limited knowledge about its behaviour in PAHs exposed populations does pose minor difficulties when interpreting the results which can not be related to studies in the literature.

8.2.2 Urinary D-Glucaric Acid (UDGA)

Human exposure on long-term basis to low levels of many environmental and occupational chemicals may stimulate the activity of enzyme - induction - which may cause proliferation in the endoplasmic reticulum of liver cells. This adaptive biochemical change, the enhancement of metabolism by *de novo* synthesis of enzymes, serves to increase the clearance of xenobiotics from the body. From this point of view, the biochemical change is considered to be a useful response. On the other hand , prolonged biochemical change may result in undesirable consequences such as a clinically appreciable enlargement of the liver, disturbance of the anticoagulant control and osteomalacia (Hunter *et al.*, 1971 and Colombi *et al.*, 1987). Therefore it is essential to try to understand how the main detoxifying organ in the body, the liver, reacts to different types of environmental chemicals. It is crucial to find possible biomarkers for monitoring these adaptive reversible changes resulting from exposure to a single or a mixture of chemicals. These biomarkers can supplement and preferably replace the available more crude techniques that only detect liver damage at virtually non-reversible stages.

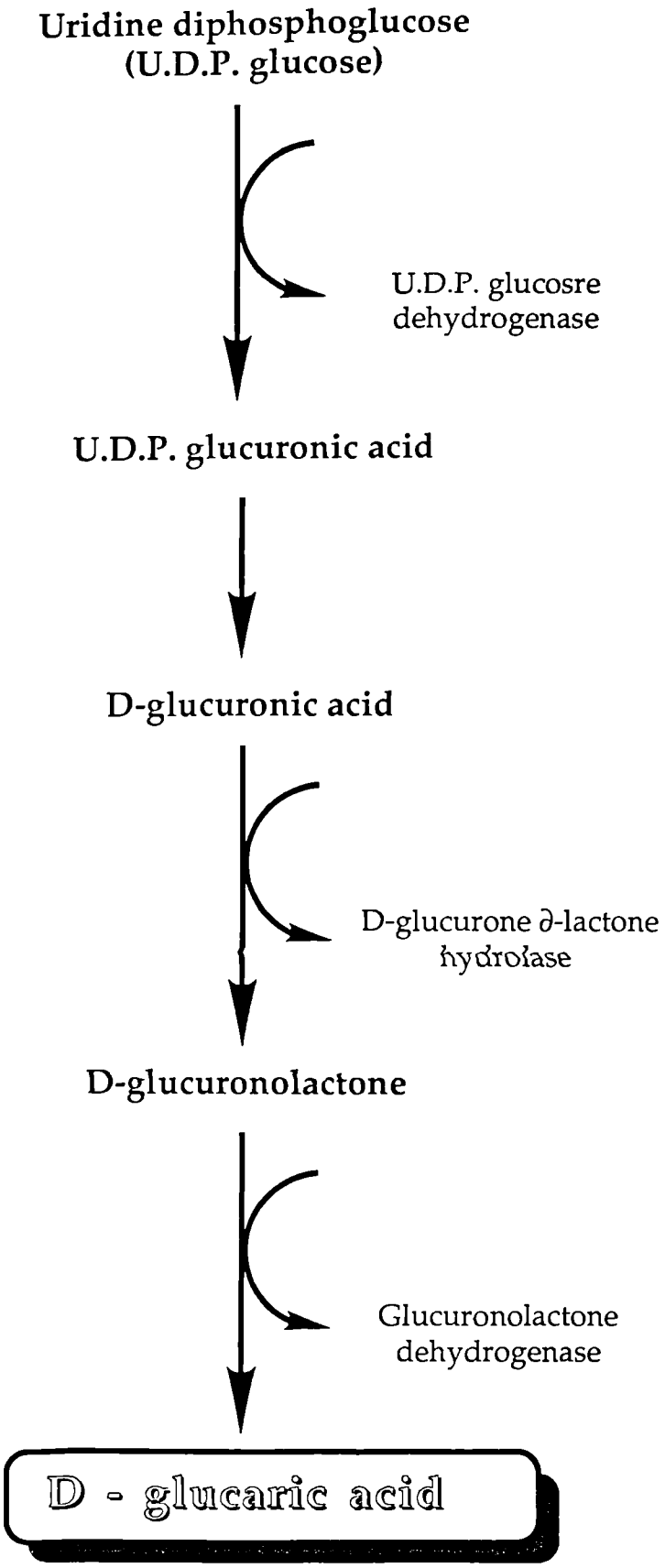
The most obvious for biological monitoring of hepatic enzyme activity are liver biopsies. This of course is impossible to employ in occupational chemical studies for the obvious ethical and practical reasons; however, they might be very useful under legitimate medical purposes. The applicability of several other indirect approaches to assess enzyme induction in humans have been studied. Each and every methodology has its own advantages and drawbacks.

The ultimate aim of biological monitoring programs is the risk assessment of hazard levels that individuals are exposed to. This is based on the relationship existing between biological indicators and exposure. The evaluation of this relationship and the validation of the biomarkers in controlled experimental studies is by no means sufficient to fully

validate the biological indicator. This relationship must be tested in field studies where the uncertain nature of exposure, the multitude of confounding factors, matching of control population, inadequacy of dose indicators to assess exposure and many other factors can effect the investigated relationship. In support of the above, where many scientists were successful in observing a cause-effect relationship between enzyme inducers and the biological indicator in controlled clinical studies few others have failed to demonstrate any relationship existing between occupational chemical exposure and the same biomarker under study. The reason for the absence of a cause-effect relationship between the biomarker and exposure; found in the limited literature investigating inductive effects as a consequence of occupational exposure, could be attributed to many factors (Colombi, 1987). Limitations in the planning of the study, non-sufficient understanding of the pharmacokinetics of the inducer leading to an inappropriate sampling strategy, the definite links between inducers and the biomarker of choice, knowledge of confounding factors, intra- and inter-individual variation, poor performance of the test, can all separately and/or collectively hinder the observation of a cause-effect relationship.

D-glucaric acid, one the most commonly used biomarkers of enzyme activity, is the derivative of endogenous biotransformation of d-glucuronic acid. It represents the main metabolic end product in the glucuronic-acid pathway (fig. 8.1), one of the most important route in drug metabolism (Hunter *et al.*, 1971 and Colombi *et al.*, 1987). It was first identified in the urine of mammals by March in 1961. After that many animal, clinical and occupational exposure studies have looked at the cause-effect relationship between stimulatory effects of drugs and chemicals and the excretion of DGA. Despite the fact that the subject is extremely reviewed the mechanism by which DGA synthesis and excretion is increased by chemicals remains to be unfolded. According to Latham and Sweeney (1976) DGA production is linked with mixed function oxidase system; on the other hand, scientists like Park and Breckenbridge, as reported by Colombi *et al.* (1987), doubt that DGA is a real index of induction since its increased excretion on exposure to inducing xenobiotics could be explained by inhibition of glycogen synthesis and an increase in carbohydrate flux which may play a role in stimulating the glucuronic pathway. In addition, not enough knowledge

Figure : 8.1



The main steps in the glucuronic-acid pathway in the liver in man and the guineapig (Hunter *et al..*, 1971)

of the behaviour of UDGA excretion in control and exposed population is available. This limited use of this test did not yet establish or exclude it from the list of useful biological monitoring markers in occupational and environmental studies. Scientist have utilised UDGA to biologically monitor exposure to variety of chemicals including exposure to PAHs in bitumen fumes. Franco and Fonte (1994) used it as a index of effect for evaluating liver response in operating theatre personnel. Before that a similar study was conducted by Pasquini *et al.* (1989) which determined UDGA excretion in operating room personnel exposed to anaesthetics. Other researchers, like Seutter-Berlage *et al.* (1979), Colombi *et al.* (1987) and Morretto and Lotti (1990), utilised this biological effect indicator in assessing exposure to pesticides, polychlorinated biphenyls and toluene respectively. More recent studies by Pasquini *et al.* (1989) and Ferreira Jr. *et al.* (1994[a]) monitored the excretion of UDGA in populations exposed to PAHs from different sources including bitumen fumes. Therefore more investigations is needed to be done to answer all the questions raised about UDGA as a potential biomarkers of exposure to a complex mixture of hepatotoxic xenobiotics.

8.2.2.1 Advantages and Disadvantages

It is cheap, rapid, non-invasive, and need minimal expertise for the analysis of the samples. This does not imply that this method has not got any drawbacks. A high coefficient of variation and intra- and inter-individual variation and the multitude of confounding factors are the main feature of this assay as well as the limited knowledge and queries raised in the literature on the optimum way of employing it in occupational studies.

8.3 SELECTION OF METHOD (URINARY D-GLUCARIC ACID)

Urinary excretion of UDGA as a measure for microsomal enzyme induction was chosen as an indicator for biological effect monitoring of exposure to PAHs. The relevance of such an exposure to roofers' and pavers' health status is often unpredictable and maybe impossible to

discover by means other than the assessment of enzyme activity one of the earliest, adaptive, reversible biochemical changes the liver undergoes on exposure to hepatotoxic xenobiotics. Therefore an urgent need for better validation of tests, such as UDGA, is required to be able to use induction as an early indicator of liver alterations or liver response to chemical hazards. Another major reason for utilising this biomarker in this study is its advantages.

8.3.1 Principle of Method

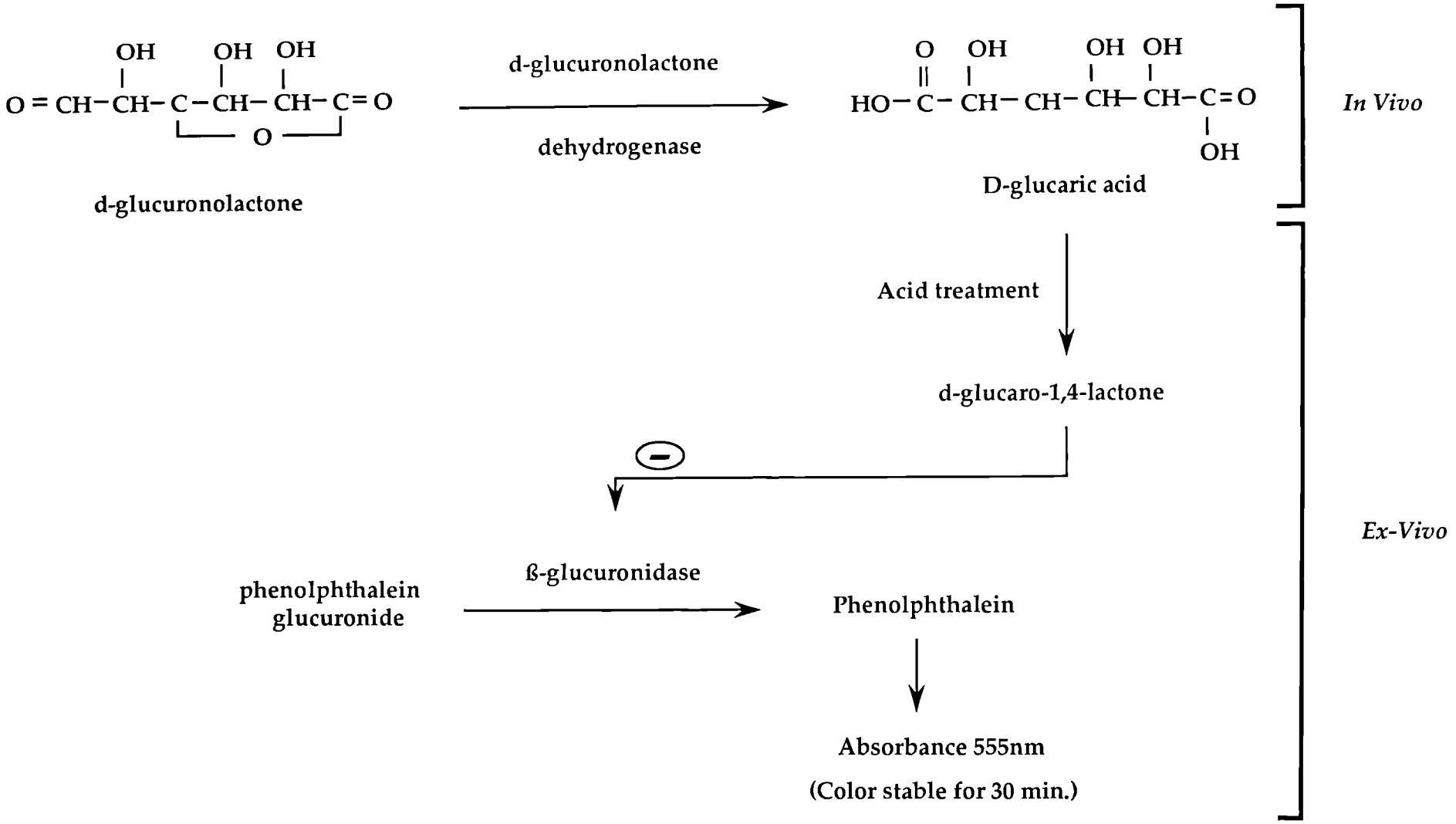
Figure 8.2 gives a schematic diagram of the *in vivo* and *in vitro* steps involved in UDGA determination. Briefly, *in vivo*, d-glucuronolactone is transformed, by d-glucuronolactone dehydrogenase, to d-glucaric acid to be excreted in urine. *Ex-vivo*, urinary D-glucaric acid is transformed to d-glucaro-1,4-lactone by acid treatment. Upon the addition of d-glucaro-1,4-lactone to a basic solution containing phenolphthalein glucuronide and ß-glucuronidase the enzyme is inhibited thus decreasing the amount of free phenolphthalein available for colour formation upon the addition of glycine (pH 12). The absorbance of the sample is measured at 555 nM using a UV spectrophotometer. On the other hand, a base treatment of urine does not allow the conversion of d-glucaric acid to d-glucaro-1,4-lactone. The absorbance measured for this sample reflects the amount of enzyme inhibition due to endogenously formed inhibitors not accounted for in the acid treated sample. The absorbance of the base treated sample subtracted from the acid treated sample will indicate the concentration of UDGA.

8.4 BIOLOGICAL MONITORING OF CYTOGENETIC EFFECTS

Cytogenetic end-points and mutational assays such as chromosomal aberrations, sister chromatid exchange, and micronuclei have been used extensively in occupational toxicology studies for revealing effects of exposure (Sorsa, 1984). These are thought to be early phases or indicators of disease which are reversible with termination of

Figure : 8.2

D-glucaric acid (DGA)



exposure to mutagens/carcinogens (Hogstedt, 1986).

The highlight of cancer research is the early recognition of a need for experimental systems for risk identification and screening methods for early stages of a cancer. One of the earliest reports of cytogenetic biomonitoring for determining risk in the occupational environment was that by Dr. Ehrenberg and his colleagues in 1959-60 performing a health risk assessment on ethylene oxide exposed workers. They found an increased number of chromosomal aberrations in PBLs in accidentally exposed individuals (Hogstedt, 1987). Since, a rapid development of a big number short-term assays for biological effect monitoring of exposure to genotoxicants was under way. This was also complemented by experimental animal studies which assisted in the identification of environmental/occupational mutagens/ carcinogens and the prevention of cancer incidence.

This part of the thesis will be addressing, discussing and commenting on the most widely used cytogenetic biological effect monitors and more importantly those utilised for risk assessment of exposure to PAHs.

Genetic damage can be assayed at molecular, functional gene, and chromosomal levels. The introduction to cytogenetic monitoring will concentrate on the latter type since our choice of biomarkers (SCE and MN) are classified as chromosomal abnormalities.

At chromosomal level, genetic damage, is identified as a change in the chromosome number or structure. Consequences of numerical changes in somatic cells are less characterised. The structural ones, such as SCE and structural chromosomal aberrations are more widely used end-points for evaluating somatic mutations in humans. There are some end-points studied, like micronuclei, which can result from numerical or structural chromosome aberrations. The understanding behind the formation of these "abnormalities" is essential but what is equally important is to comprehend their mechanistic link to clinical cancer. Once the link of cytogenetic marker to cancer is established the biomarker can serve as a surrogate for cancer (Hemminki, 1992). Further linking of these surrogate endpoints to various exposures would be a tool for cancer

prevention.

Cytogenetic end-points can be classified mainly into four types: i) Numerical chromosomal aberration which can be studied in most tissues; ii) structural chromosomal aberrations which are mainly studied in blood lymphocytes; iii) sister chromatid exchange, these are usually analysed for in PBLs and sometimes bone marrow; iv) micronuclei in non-dividing cells or more recently in PBLs and epithelial cells.

8.4.1 Chromosomal Aberrations

8.4.1.1 Background

Chromosomal aberrations end-points are non-agent-specific and may represent a cumulative exposure to a variety of chemicals. They are useful in assessing exposure to ionising radiation due to their stability to that type of mutagenic/carcinogenic insult. As for smoking and other chemical they are less applicable for exposure monitoring due to their low stability. High doses are required to produce an observable response in the exposed when compared to the general population (Garner *et al.*, 1991).

This indicator of exposure has been used but results are sometimes contradictory or negative even when a population is known to be exposed to carcinogens. The reason for these conflicts are usually methodological like timing of sampling or interpretation of end-points and the general applicability of statistical analysis. Nevertheless, Knudson (1985) and Forni (1987) reported that at a group level an increase in chromosomal aberration is associated with risk of cancer (Hemminki, 1992).

8.4.1.2 Structural Chromosomal Aberrations

This a simple and informative assay system for human population monitoring providing information on suspected mutagenic/carcinogenic exposure (W.H.O. 1985).

The most frequently used tissue for chromosomal aberrations are

human lymphocytes due to their ease of availability. Occasionally, bone marrow cells are used and less frequently cultured skin fibroblasts (Evans, 1986). Chromosomal aberrations are usually analysed at metaphase although sometimes at anaphase as well (Evans, 1986). There are numerous ways described in the literature for culturing human lymphocytes for chromosomal aberration analysis. The less variable ones are described in the W.H.O. environmental health criteria 46, published in 1985.

Old and more recent research on cancer have measured chromosomal aberrations in human populations exposed to environmental/occupational clastogens. In 1979, Kucerova *et al.*, evaluated the frequency of chromosomal aberrations in workers occupationally exposed to vinyl chloride, a known human mutagen/carcinogen chemical. In another interesting study in 1980 Lambert A. and Lindbald A. measured chromosomal aberrations in laboratory personnel. More recent research by the Nordic Study Group (1990) attempted to correlate levels of such changes with future cancer morbidity. Another study tried to correlate this type of abnormality to increased risk for cancer (Jacobsom-Kram *et al.*, 1993). This line of research is recommended for future work in order to explore the usefulness of this end-point and increase its value as an indicator of mutagenic/ carcinogenic exposure.

8.4.1.3 Numerical Chromosomal Aberrations

Numerical chromosomal aberrations in most instances result from events occurring during gametogenisis in a parent or at the time of fertilisation; this however is not strictly true and evidence on the time of origin is not really known.

Although, there is not a great deal of occupational toxicology or molecular epidemiology studies attempting to link this type of abnormalities to exposure to environmental clastogens we must not disregard the potential of these outcomes to be related to exposure to possible mutagens. Due to their limited use in risk assessment studies similar to the one described in this thesis we will not attempt to describe the methodology involved.

8.4.1.4 Advantages and Disadvantages

One of the main highlights of chromosomal aberrations is there non-specificity thus allowing for assessment of the summation of various exposures to many agents. In some instances, such as exposure to radiation they are known to be highly stable and very sensitive. Another advantage is that concurrent controls might not be necessary and individuals can be used as self-controls especially if baseline frequency are evaluated prior to exposure to mutagens/carcinogens taking place. In general they are cheap and do not need highly technological equipment. The literature got a vast number of studies on chromosomal aberration which aid in the interpretation of obtained results in perspective of other similar work. In the case of lymphocytes being the tissue under study, chromosomal aberration evaluation might indicate an insult imposed on the organism over a considerable long period because of the long life span that these cells have got. This does mean that the persistence of these events can be predicted with great certainty disregarding factors like duration of exposure, agent, and other factors. They can be studied in cycling cell population be it in target tissue or surrogate like peripheral blood lymphocytes. They can be studied *in vivo* in humans for prediction of exposure to mutagens/carcinogens or *in vitro* to predict mutagenicity/ carcinogenicity of a chemical/physical hazard.

The disadvantages of these assays are quite important to consider. Although they are not expensive they are time consuming and require a great deal of expertise especially when it comes to scoring sample slides under light microscopy. Structural aberrations are observed as either chromosomal-type or chromatid-type aberration. The former could be of five forms while the latter of eight forms. The several types of structural aberrations that can be observed makes the task of the scorer quite cumbersome and introduces methodological variability due to the human error factor. The disadvantages in the scoring step has been resolved with automated scoring using computers; however this is difficult to get hold of when research budgets are limited and more essential equipment is needed to be purchased. The culturing and preparation of samples must be performed straightway after sampling. This restricts time of sampling which is important in occupational studies. Since sampling is often invasive ethical approval is needed. Last but not least the outcome's

relation to development of disease is not usually clear which decreases the value of these abnormalities as an indicator of exposure to clastogens.

Table 8.2 gives a comparison regarding relevance, practical availability, sensitivity, specificity and scoring techniques between both types of chromosomal aberration and the other two cytogenetic end-points discussed below.

8.4.2 Micronucleus Test in Exfoliated Epithelial Cells

8.4.2.1 Background

Ninety two percent of all human cancers occurs in epithelial tissue (Rosin *et al.*, 1993). They are the logical sites for analysis of genotoxic damage in humans exposed to carcinogens. An increased frequency of micronuclei in exfoliated human cells provide good evidence of carcinogen exposure in the tissue from which cancers will develop. These cells are continuously exfoliated from the surface of the epithelium and are replaced by cell division in basal cells of the tissue. They can be collected by non-invasive methods from several sites in the body (e.g. bladder, buccal mucosa, oesophagus etc.)

8.4.2.2 Kinetics of Micronuclei Formation

It is important to understand the kinetics of micronuclei formation in epithelial tissue, in order to be able to utilise it as biological effect monitor and draw valid conclusion from any results obtained.

Epithelial tissue are of three types. The basal cell layer containing the stem cells. These give rise to more mature non-dividing cells called intermediate cells which in turn reach the surface to tissue as superficial epithelial cells.

It is believed that several anomalies can lead to the formation of micronuclei. The reason for their formation is chromosomal damage caused by prolonged exposure of basal cells to mutagens/carcinogens. Chromosomal fragments or entire "aberrant" chromosomes which lag behind in newly formed daughter nuclei form their own membrane and

Table : 8.2 Qualitative Comparison Between Four Cytogenetic End-points

End-Point	Relevance	Practical availability	Sensitivity (detection)	Specificity (range)	Scoring
Chromosomal structural aberration	Mutations. Oncogenic. Lethal.	Blood. Bone M.? Sperm.?	High.	Clastogens only.	Specialised. Tedious.
Chromosome numerical changes	Mutations. Oncogenic. Lethal.	Blood. Bone M.? Sperm.?	Not so high.	Clastogens. Protein-damaging.	Less specialised. Less Tedious.
Micronuclei	Lethal. Oncogenic.? Mutations.?	Epithelia. Blood. Bone M.? Sperm.?	Not so high.	Clastogens. Protein-damaging.	Even less specialised. Less tedious.
Sister chromatid exchange	?*	Blood. Bone M.?	Very high.	Wide.	Non-specialised. Less tedious.

? Not very frequently used.
?* Not defined
Table adapted from Evans (1986)

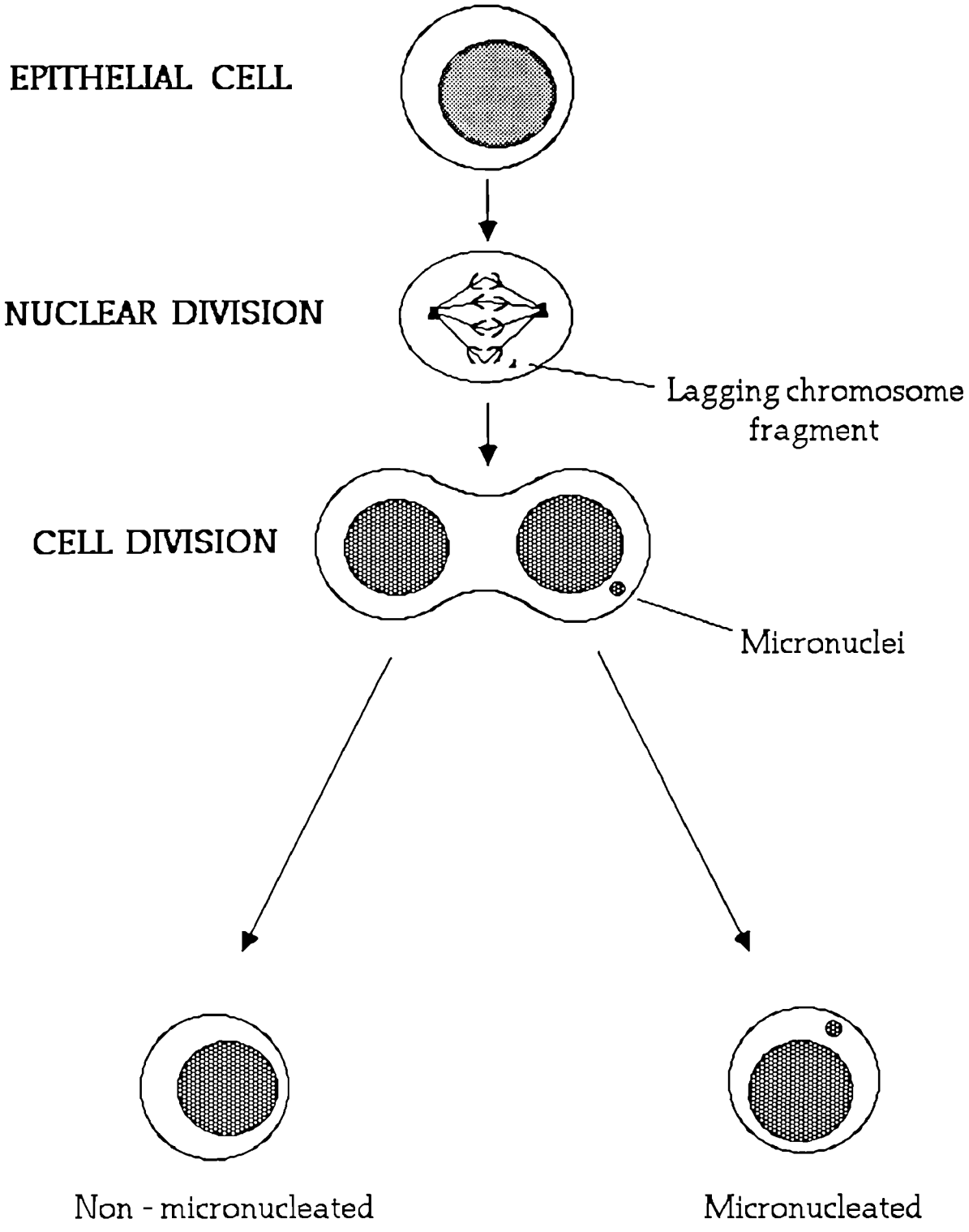
appear as Feulgen-specific bodies termed micronuclei in the cytoplasm. Figure 8.3 is a schematic diagram explaining the formation of micronuclei in replicating cells.

In addition to understanding the kinetics of formation, knowledge about the type of carcinogen and patterns of exposure are important to know before using the micronucleus test as a biological effect marker in a population exposed to mutagenic/carcinogenic chemicals. This information will ensure that exfoliated cells will be sampled at the right time so that waves of MN production in response to acute exposure would not be missed. On the other hand in case of chronic long-term exposure, as most usually the case in occupational studies, that will not be of limited significance.

8.4.2.3 Advantages and Disadvantages

As any other biological marker the micronucleus test has its own advantages and disadvantages. It is usually useful to consider the characteristic of any biological marker before employing it in any occupational study. The highlight of the MN test, aside to the characteristics listed below, is that it combines all the advantages of *in vitro* short-term tests for genotoxic and carcinogenic agents with those of using an intact organism *with all its defence mechanisms* (Stich and Rosin, 1983).

It is simple, non-invasive, rapid, and economic. Has got all the advantages of biological effect markers (see table 2.1). Represents a change that is biologically relevant to the process of carcinogenesis. Micronucleus frequency changes in response to carcinogens exposure, does not have all the disadvantages of short-term *in vitro* genotoxicity assays, can give a quick feedback on clinical intervention studies when compared to epidemiological ones looking at a decrease in frequency of carcinomas in a certain population on the introduction of control or preventive measures and could be coupled with cytopathological diagnosis which provide information on the frequency of genotoxic aberration at various pre-neoplastic stages (Stich *et al.*, 1985).



Micronuclei in Exfoliated Epithelial Cells

Figure : 8.3

Micronucleus test has got all the disadvantages of a biological effect marker (see table 2.1), but it cannot be used in the female populations, because samples might contain exfoliated epithelial cells originating from the vagina (Stich *et al.*, 1985). Time and skill requires to score the preparations, which could be done away with at later stages of the development by automated scoring. Of course automated scoring will give the assay more resolution to pick small differences in frequency by counting bigger number of cells. Szirmai *et al.* (1993) have developed a computerised automated image analysis for determining of micronucleus frequency. The method is still in its validation stage but is giving promising results regarding its applicability on large scale studies. MN testing might under-estimate total cytogenetic damage at high doses. On other occasions it might over-estimate the value of the risk involved; since it is difficult to decide whether the cells with multi-chromosomal aberrations are moribund or still have proliferative capacities to give rise to a group of genetically altered cells. Timing is very critical especially in acute exposure.

8.4.3 Sister Chromatid Exchange

8.4.3.1 Background

The history of SCE studies can be linked to Taylor's study in 1957 where the mode of DNA label segregation in sister chromatids after their labelling with ^{3}H-thymidine was discovered. If we are more precise we can trace the history even further back to McClintock's study in 1938 in which it was proposed that the possibility of full exchanges between sister chromatids was concluded from the observation of the behaviour of dicentric ring chromosomes in somatic maize cells (Zakharov, 1982). Although further work on SCE were not directly related with SCE phenomenon observed in ring chromosomes the above initial steps should be forgotten.

Briefly, sister chromatid exchanges (SCE) reflect the interchange at mitosis of DNA replication products between sister chromatids at homologous loci Edwards (1990). As means to detect carcinogens, SCE was found to correctly identify 46% of carcinogens and 86% of non-carcinogens

(Purchase and Ashby, 1982). A good correlation was drawn between SCE and mutagenicity and/or carcinogenicity on the grounds of 200 compounds tested (Deen *et al.*, 1989). This high degree of correlation and carcinogenicity prediction was interpreted as indicating that the SCE is an extremely sensitive cytogenetic endpoint in detecting exposure to mutagenic and/or carcinogenic xenobiotics (Evans, 1986). This may not be true in the case of physical hazards, like ionising radiation, producing DNA damage by forming double strand breaks which the SCE test may not pick (Garner, 1991).

The sister chromatid exchanges are thought to involve DNA breakage and re-union. The actual mechanism of SCE formation is still to be understood by genetic toxicologist.

Several explanations are proposed for the understanding of SCE formation. In 1974, Wolff *et al.* reported that SCE formation appears to be tightly coupled to DNA synthesis. Comparing SCE induction and late replication patterns indicated a need for DNA synthesis in a given chromosome region, subsequent to DNA damage, for SCE induction to occur (Latt and Scherck, 1980). Shafer in 1977, postulated that SCE formation involves the bypass of DNA cross-links during replication. The following year Stetka and colleagues observed persistently elevated SCE levels in rabbits exposed to mitomycin C which was thought to be due to the unremoved alkylating damage (Latt and Scherck, 1980).

Genetic toxicologists are still far from understanding the mechanisms of SCE formation and putting facts behind their hypothesis. More research is needed to accumulate data on correlation between DNA damage and SCE formation and the fate of the former during the formation of SCE. Additional information about possible involvement of special types of DNA sequences and SCE formation will help in discerning the mechanisms of SCE formation (Latt and Scherck, 1980).

SCE takes place upon the occurrence of double strand breaks in the DNA followed by an interchange of newly replicated double strand DNA which will preserve the polarity (Lambert, 1986). This statement is supported by the positive correlation observed between SCE frequency and structural modification of DNA. Also, results of *in vitro* studies in

mammalian cells illustrating that a numerous well-known mutagens and carcinogens that give rise to chromosomal aberrations and DNA damage also increase SCE frequency. Numerous models have been suggested by genetic toxicologists regarding the mechanisms of SCE formation, these are plausible under different conditions; however, with the current technology it is not possible to ascertain the actual mechanisms. The exchanges may be considered to be manifestations of recombination at the cytogenetic level and a marker of genotoxic and possibly clastogenic effects (Deen *et al.*, 1989 and Lambert, 1986).

The toxicity of PAHs and their genotoxic effects has been studied in animal and human peripheral blood lymphocytes (PBLs) *in vitro*. It was found that B(a)P, one of the more potent carcinogenic PAHs, increases SCE frequency *in vitro* in Chinese hamster cells only after metabolic activation using post-mitochondrial (S-9) fraction prepared from rat liver plus an NADPH-generating system together forming what so called as S-9 mix. On contrary, in humans lymphocytes *in vitro*, B(a)P was able to double SCE frequency alone (Takehisa and Wolff, 1977). Other studies, like the those reported by Waalken *et al.* (1980), reported that B(a)P - the indirect acting mutagen - can cause SCEs in cultured human and rabbit lymphocytes. Kligerman *et al.* (1985) illustrated that B(a)P can increase SCE frequency in PBLs of mice *in vivo*; however in an non-linear dose-response fashion. They explained the biphasic curve observed by a possible saturation of metabolic activating system or solubility problem encountered with high concentrations.

A large number of studied have looked at SCE in human PBLs as a biological effect biomarker of exposure to genotoxicants. Sinués *et al.* (1991) observed an increase in SCEs and micronuclei in PBLs as well as inhibited cell kinetics of workers exposed to vinyl chloride in the plastic industry. They reported that SCE was the most sensitive endpoint for indicating a biologic response. Zober *et al.* (1993), conducted a cytogenetic study on workers exposed to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dixoin (TCDD) and found that SCE was the only cytogenetic effect biomarker out of a panel of markers that showed any increase in frequency. Hospital workers exposed to low levels of 1 ppm (OES for ethylene oxide is 5 ppm) of the well-known carcinogen, ethylene oxide, showed significant increases in SCE frequency over the control after correcting for smoking habits and history

(Tomkins *et al.*, 1993). In the tyre industry, workers are occupationally exposed to a mixture of biologically active chemicals emitted from combustion processes containing PAHs. This group of workers were biomonitored by Sasiadek (1993). In her study SCE was found to be the most sensitive cytogenetic test for assessing exposure to such a mixture of chemicals. SCE was frequency was significantly elevated in operating room personnel as compared to controls (Sardas *et al.*, 1993).

SCE frequency has also been studied in large non-occupationally exposed populations with emphasis on confounding factors such as active and passive smoking, alcohol consumption, age, sex etc. (Hirsch *et al.*, 1992; Park *et al.*, 1992; Motykiewicz *et al.*, 1992; Anderson *et al.*, 1993 and Laztuka *et al.*, 1994).

A wealth of literature is available looking at the usefulness of SCE as a biomarker of exposure to mutagenic/carcinogenic xenobiotics of all sorts and potencies. However, very few studies have utilised SCE methodology to cytogenetically monitor populations exposed to PAHs and virtually none in populations exposed to PAHs in bitumen fumes. Reuterwall *et al.* (1991) assessed genotoxic exposure in coke-oven workers using several biological indicators, one of which was SCE in PBLs. He found an absence of biologic indication of genotoxicity using SCE in PBLs as dosimeter. Another study by Liou *et al.* (1989) employed SCE in PBLs as a cytogenetic endpoint to monitor fire fighters exposed to PAHs. Their results showed that frequency of SCE in PBLs is peripherally related to exposure. SCE in PBLs was incorporated in a biological monitoring protocol in a research evaluating the exposure to PAHs in an aluminium plant by Becher *et al.* (1984). They found that the high PAHs exposure was not significantly reflected in when comparing exposed with referents. There was no significant difference in SCE frequency between control and workers exposed to petroleum products (which contain PAHs) in a large oil refinery (Khalil *et al.*, 1994). In general, it seems that SCE is not a suitable biomarker for assessing the genotoxic effects imposed of PAHs' exposure. A investigation by Edwards and Priestly (1993) illustrated that SCEs in PBLs in petroleum retailers were found to be higher in driveway attendants than in self serve personnel. This observation by the authors could be due to the relatively low baseline frequency reported in the methodology used or possibly due to exposure to benzene which is known

to be a very potent carcinogen. These studies strictly imply that additional research should be carried out to answer the question on the usefulness and reliability of SCE in PBLs to detect genotoxic exposure, especially to low to moderate levels of PAHs.

8.4.3.2 Advantages and Disadvantages

It is cheap, simple, sensitive and agent non-specific. It allows the risk assessment of exposure to a single agent (e.g. *in vitro* cell culturing studies) and complex mixtures (e.g. occupational exposure studies). The *in vitro* studies allows rapid determination of the chemicals capable of increasing SCE frequency, dose-response relationships and the lowest dose of SCE-inducing agents that permit the detection of an increased SCE frequency. It offers advantages for animal *in vivo* cytogenetic studies. In some species PBLs can be removed nonlethaly allowing individual animals to serve as own control and permitting analysis over time. It is useful in assessing chronic long-term exposure to genotoxicants since mature PBLs do not replicate and most populations are long lived which allows the accumulation of DNA lesions thus reflecting such an exposure (Kligerman *et al.*, 1985). A valuable point to consider is that this biomarker has revolutionised the cytogenetic approach to the identification of the biologically effective dose (Archer and Livingston, 1986) and access to an extensive amount of studies and reviews is possible through databases which is most useful when comparing, contrasting and interpreting ones results. For example, many studies have investigated the effect of several confounding factors on SCE frequency in the general population which assists the researchers evaluating the usefulness of this endpoint for their type of work.

The main practical disadvantage of the method is the handling of human tissue samples. This requires special precautions to be taken while performing the assay to minimise the risk of infection to the laboratory personnel. Scoring the sample slides requires considerable expertise and is time consuming. In occupational studies ethical approval is needed since sampling is invasive. The fact that biological samples are not readily available and culture failures are possible, it is still not possible to use this assay on routine basis for assessing the risk of workers' exposure to mutagenic/carcinogenic chemicals.

The interpretation of results of this biological effect marker should be done with great caution. There is no consensus on whether SCE is a useful in assessing exposure to acute short-term or chronic long-term exposure to genotoxic xenobiotics. This is a disadvantage when trying to establish correlation between SCE frequency with external or internal exposure. This highlights the importance of the time of sampling. Another time consuming step is acquiring knowledge about the baseline frequency, the sources of variation and type of DNA damage that SCE analysis might detect in the human tissue of interest. This information is crucial before major population studies are undertaken and is necessary for results interpretation. A pitfall of this effect biomarker is that the effect of DNA damaging agents which do not cause SCE at G_0 lymphocytes go undetected because lymphocytes in the body are at G_0 stage.

8.4.3.3 Confounding Factors

There is a multitude of confounding variables to take into consideration. Several researchers have extensively studied the effects of each factor on SCE frequency. There are several reviews on the SCE test to refer to (Crossen, 1982).

Although all these factors have been thoroughly addressed in the literature it is worthwhile to briefly address them in this thesis due to their relevance to our work. Table 8.3 has a thorough but not comprehensive list of all the confounding factors.

Culture techniques differ between laboratories. This give rise to considerable variability in the baseline SCE frequency due to different ingredients and their concentrations used in the culture media. One of the most necessary ingredient for the identification of SCEs is 5-bromo-2'-deoxyuridine - analogue of thymidine - which in itself is an SCE inducer. The use of micro-cultures (whole blood cultures), where foetal or calf serum is usually used, yield lower frequencies compared to macro-cultures where autologus human serum is utilised. Cell proliferation is an important determinator. It was illustrated that rapid cell proliferation yield lower SCE frequency and there is a significant negative correlation between the two variables (Lambert, 1983). Temperature at which culturing takes place has been shown to be an important factor that should

Table : 8.3 Confounding Factors Influencing SCE frequency

Physical factors
Culture medium (type)
Culture conditions (e.g. pH)
Serum (e.g. autologus serum, foetal bovine serum etc.)
Antibiotics (e.g. type and concentration)
BrdU (e.g. concentration, duration of exposure etc.)
Mitogen (e.g. concentration)
Anticoagulant (type and concentration)
Culture duration and cell cycle kinetics
Blood storage conditions
Growth temperature
Biological Factors
Inter-donor variation (age, gender, race etc.)
Inter-cell variation
Menstrual cycle & Pregnancy
Social habits (smoking, drinking, diet etc.)
Drug therapy (e.g. radiation, genotoxic drugs)
Environmental exposure to xenobiotics
Several diseases
Polymorphism (e.g. glutathione conjugation)
Haematological Indices (e.g. white blood cell count)
Staining and scoring protocols

* Adapted from Edwards, 1990

be controlled. A drop of 2 °C from 37 to 35 °C increased SCE frequency and lengthened the cell-cycle duration significantly. An increase of 2 °C from 37 to 39 °C yielded only a significant increase in SCE frequency and not prolongation of cell-cycle (Abdel-Fadil *et al.*, 1982).

Results of studies relating to the effect of age and sex of the donors are not conclusive. This suggests that close matching of controls is necessary.

The increase of SCE frequency in cigarette smokers was first reported by Lambert *et al.* (1978). Since then several researchers have reported this observation but some others failed. This required that recruited donors to be interviewed with respect to their smoking history and current habits. It would be even more appropriate to biologically monitor them for tobacco consumption utilising selective biomarkers such as urinary cotinine and nicotine. In addition, it is advisable to investigate possible exposure to environmental tobacco smoke (ETS) by passive smoking although it has been reported to have no effect on SCE frequency (Gorgels *et al.*, 1992).

The effect of medication such as therapy using cytostatic drugs (e.g. cyclophosphamide, mitomycin C, etc.) or x-ray is well documented and was shown to induce SCE (Crossen, 1982). In a study by Sinues *et al.* (1992) the authors observed an increase of SCE frequency in asthmatic patients receiving continued therapy with theophylline. The health status of the donor is a confounding factor of concern. The role of viral infections and vaccination in influencing baseline SCE frequency in PBLs is not known. Ortiz *et al.* (1994) concluded that severe malnutrition might lead to an increase in SCE frequency and that the effect of bacteria infection is not much less associated with SCE induction. Even sleep deprivation for a day showed a considerable increase in SCE rate (Bamezai *et al.*, 1992).

Polymorphism displayed in an unknown GST in human erythrocytes influenced the SCE frequencies evaluated in whole blood samples of "conjugators" (possessing this specific activity) and "non-conjugators" cultured in the presence of one of either of three genotoxic agents (methylbromide, ethylene oxide, dichloromethane). Lymphocytes

of non-conjugators, but not those of conjugators, suffered an increase in SCE frequency when tested with all the substances; hence showing the protective effect of GST activity in human erythrocytes for cytogenetic toxicity of these chemicals (Hallier *et al.*, 1993).

8.5 SELECTION OF METHOD (MICRONUCLEUS TEST)

For this study our choice of cytogenetic end-point fell on MN. The rational behind our choice of this biological effect marker is discussed separately below.

Knowing that workers exposed to bitumen fumes and consequently polycyclic aromatic hydrocarbons, are at a higher risk of developing bladder cancer it was proposed to utilise the MN test in exfoliated epithelial cells of the urinary system as a biological effect indicator , as part of the strategy for assessing the genotoxic risk involved during their exposure. It would be the only biological effect indicator in this study utilised to assess the risk of exposure to PAHs at one of sites they deliver their insult to.

8.5.1 Principle of the Method

Micronuclei are formed due to chromosomal damage in the basal cells of the epithelium of the urinary system. These cell migrate to the top layer of the epithelium and then are exfoliated in the urine. They are collected from urine samples by centrifugation. They are fixed, dropped on slides, stained and scored using light microscopy. Three thousand cells from each sample are scored and the frequency of MN are recorded.

8.6 SELECTION OF METHOD (SISTER CHROMATID EXCHANGE)

SCE was chosen for this study for various reasons. Aside to its advantages such as sensitivity and relatively easy procedure compared to other short-term genotoxicity assay; the toxicity of PAHs *in vitro* has been thoroughly studied using this short-term genotoxicity assay. This allows indirect comparison of possible *in vivo* dose-response (external dose - SCE frequency) relationships observed in our study with ones reported in the literature. Since SCE is a non-specific biomarkers of effect of exposure to genotoxicants and since the populations being studied are exposed to PAHs mixture it was reasonable to utilise it for this study. In addition, sensitive and PAH-specific biomarkers of effect are not yet available.

8.6.1 Principle of the Method

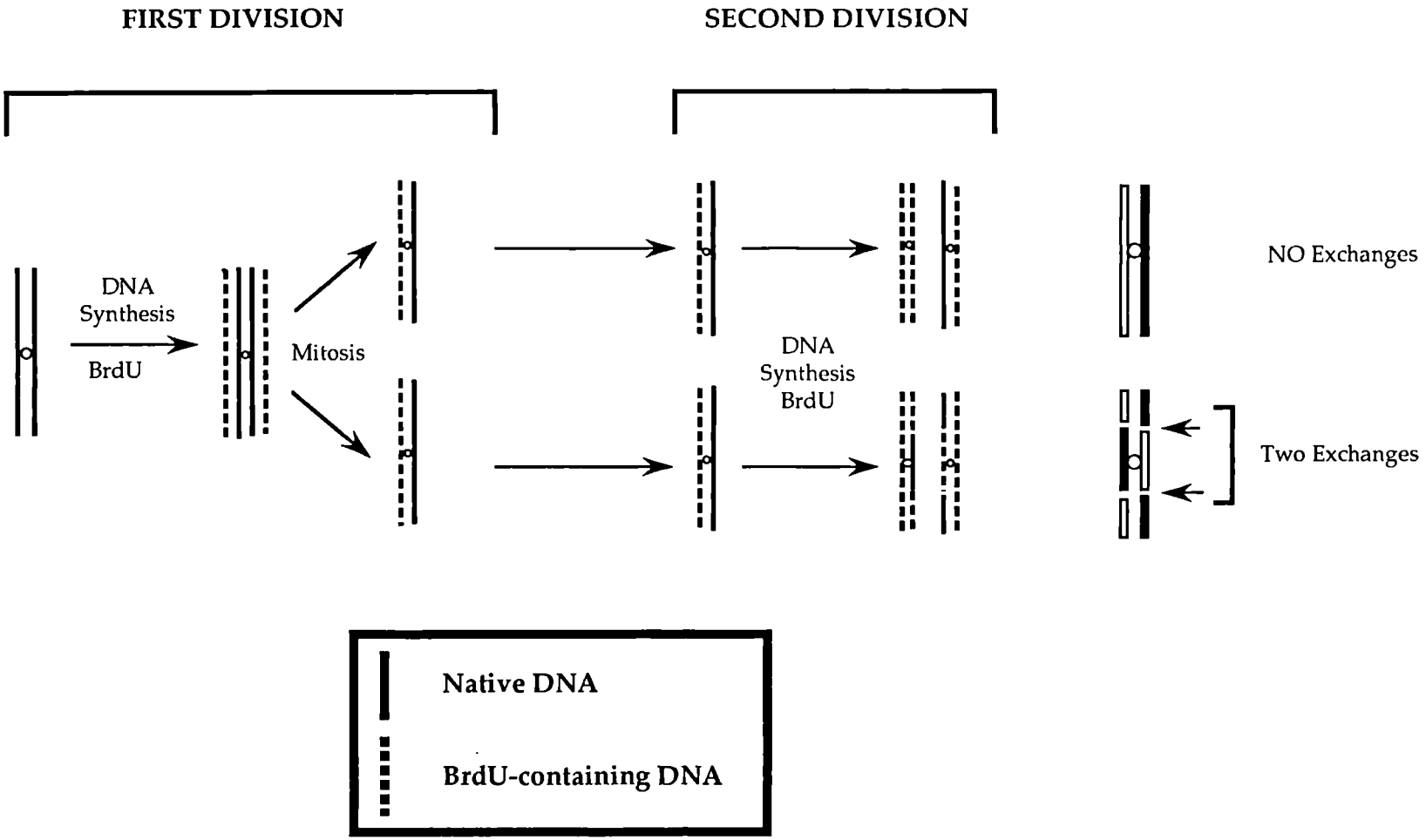
In order to look at SCEs in chromosomes it is necessary to differentiate between the two sister chromatids. The method used in this thesis is based on the ability of the chromatids to uptake 5-bromo-2'-deoxyuridine (BrdU) a base analogue of thymidine and the halting of the cell cycle using a spindle poison (e.g. colcimed) while cells are at the second-division metaphase, to be giemsa stained and scored under light microscopy.

Figure 8.4 is a schematic diagram explaining the principle behind the method for SCE analysis in PBLs. Dividing cells are left to cycle twice in the presence of BrdU, which in the first cycle will get incorporated into daughter chromatids upon a template of native DNA. In the second cycle BrdU is included into chromatids containing either native DNA or BrdU containing-DNA. Finally, halting the cell cycle there will be one unifiliary and another bifiliary substituted sister chromatids. The latter will stain dark with giemsa while the former will stain lightly. Any exchanges which took place during the two replicative cycles can then be observed using the light microscope.

The method also allows the scoring of cells that have replicated once (M_1), twice (M_2) or three times in culture since the chromosomes of

Figure : 8.4

Sister Chromatid Exchange (SCE)



lymphocytes in the first mitotic division stain uniformly with geimsa and those in their third or more cycle have an non-uniform staining patterns which are non-symmetrical between sister chromatids. This phenomena of differential staining could be used to study lymphocytes proliferation kinetics, as an increase in the proportion of cells in the first and second mitotic division (M_1 and M_2) relative to third mitotic division (M_3) may indicate a reduction in the proliferative capacity, an early marker of cytotoxicity. This is acheived by estimation of the replicative index (RI).

CHAPTER 9

URINARY CONCENTRATIONS OF BIOMARKERS

9.1 CORRECTION OF URINARY CONCENTRATIONS OF BIOMARKERS

Urine has a well established and historic role in toxic dose assessment. It is one of the most readily available biological tissues for biological monitoring of exposure to xenobiotics and estimating their "internal dose". Its collection is generally easier and more acceptable than the collection of blood, faeces, expired air from individuals encountered in the practice of occupational and environmental health.

Urinary concentrations measurements are utilised for qualifying and quantifying internal doses of substances as well as health effects of exposure. Regulatory agencies use urinary dose for the assessment of exposure and health risk involved, for acquiring information for decision making and design of preventive control measures. This requires the information to be accurate, reliable and properly interpreted.

Unfortunately, the literature conflicts regarding the usefulness of the different methods of adjusting for factors introducing wide variability in urine concentrations of biomarkers. This renders the issue of interpretation of urinary concentrations a difficult task and a topic of poor agreement. The disagreement is not only on what is the most appropriate method to use but also on how to use the parameter's results for better data interpretation.

Exogenous factors like varying hydration resulting from increased fluid intake, insensible losses such as sweating and diuresis affect the urinary concentrations of substances measured. Obviously, diluted urine samples will give apparently low values of the biomarker and visa versa, thus leading to inaccurate evaluation due to over- or under-estimation of internal doses.

Ideally, daily urine collection (24 hour urine sampling) is the way to overcome the effect of dilution. This approach is feasible in clinical research; however it is highly impractical and hard to obtain in occupational settings. This is not the only reason for its infrequent use. Araki *et al.* (1986) found that 24 hr total excretion of specific biologic substances is influenced by daily urinary volume (UV).

The alternative to the impractical daily urine sampling is urine

collection for a shorter well-defined period of time (timed excretion). This type of sampling is elaborative for routine monitoring of workers (Lauwreys, 1983) and suffers from inaccuracies in timing and incomplete emptying of the bladder (Pryde and Gompertz, 1994). The author of the thesis and his colleagues' personal experience in this matter support the above. On the other hand, Araki, S. *et al.* 1986, suggested the opposite. They wrote that it is not difficult to ascertain exactly from volunteers the start and termination times of urine collection if the technique is described to the subjects in advance. This approach has other drawbacks. Lauwreys has mentioned that there are compounds for which the expression of urinary results in excretion rate does not improve the accuracy of exposure estimate. Good examples are solvents rapidly metabolised *in vivo*. Sedivec *et al.* in 1981 suggested that methanol is better assessed by expressing its urinary concentration in mg/L instead of excretion rate mg/hr (Lauwreys, 1983).

Although 24 hr and timed excretion urine collection have been used in occupational settings and are considered to be acceptable; it seems that the most frequently used method when biological monitoring is to be conducted in occupational toxicology studies is spot urine sampling. This is because it is more practical and less effort is needed by the volunteers for compliance with collection of complete urinary collection and accurate time recording. It is common practice to make adjustments to the analytical results in order to compensate for hydration and dilution effects in spot urine sampling (Pryde and Gompertz, 1994). For that there is a number of methods reported in the literature which we will be discussed in the section below.

9.2 AVAILABLE METHODS FOR CORRECTION

There is mainly four types of procedures used for correction of results obtained from analysis of spot urine samples. Their use is based on recognition that the variability in the parameter's output is less than the that of the volume of fluid output. It is also obvious that for using those methods the characteristics of the biomarker that is to be adjusted must be diametrical to those of the parameters; whereby, they should be affected by diuresis and should be vary with dilution of urine (Alessio *et al.*, 1985). The most regularly

used methods are **specific gravity (SG)**, and **urinary creatinine (UCr) concentrations**. Other ways have been used such as **osmolarity** and **urinary volume (UV)**. The latter has been suggested as the most appropriate method of all the available ones to use (Araki, 1980 and Araki *et al.*, 1986). The following discussion will focus on mainly on SG and Cr since they are the most commonly used in occupational toxicology studies involving biological monitoring.

9.2.1 Specific Gravity of Urine

Specific gravity of a substance is the ratio of its mass to the mass of an equal volume of water at 4 ^{o}C. It is a unitless value. In the case of urine SG is related to urinary total solute concentration. It is used only to eliminate the influence of dilution of urine samples on urinary concentrations of substances. The adjustment is done by referring the results of urinary concentrations to a selected value of SG, which is usually a value prevalent in the general population (Alessio *et al.*, 1985). The simplicity and cheapness of the technique is its main advantages; however, sometimes its use for correction of urinary concentrations results is of limited value. For instance, it gives erroneous results in the case glucosuria and probably proteinuria Lauwreys, 1983). It is not reliable for very dilute and very concentrated values (SG < 1.010 and SG > 1.030). The use of samples with these values is unsuitable for analysis of biological indicators. In addition, Araki *et al.* (1986) have illustrated that SG is not independent of UV.

9.2.2 Urinary Creatinine

Creatinine (Cr) is an endogenous substance of myocyte origin. The amounts excreted of it reflect the volume of fluid filtered at the glomerulus since it is virtually uninfluenced by tubular secretion or resorption (Greenberg and Levine, 1989). Its excretion rates is dependant upon body weight, body size, and muscular mass, confounded by diet and is assumed to be stable despite the variability of urinary flow rates (Greenberg and Levine, 1989). It is worth noting that the confounding variables are usually ignored in studies utilising xenobiotics' urinary concentrations for biological monitoring as a tool for risk assessment. Its uses are similar to specific gravity; however,

there is not enough date to conclude whether adjustment of biological indices in spot urine samples is equally valid for both tests in biological monitoring program. In other words, a question is raised whether these two parameters can be used indifferently for adjustment of biomarkers' urinary concentration. The answers in the literature are inconclusive. Berlin *et al.* (1985) concluded that for adjusting the urinary cadmium concentrations there was no advantage offered by the use of either Cr excretion or SG. In a study by Alessio *et al.* (1985) the correlation of Cr with SG was tested in 207 individuals and it was found to be relatively weak (correlation 0.49) which suggested that these two parameter can not be invariably used for adjusting spot urine samples and they partly express the same phenomenon. This made the authors recommend that actual usefulness for either of the parameters should be tested and validated for each biological indicator. It is worth mentioning here that it should be remembered that different analytes have different toxicokinetics profiles which should be carefully considered when evaluating the use of concentration correction parameters.

The classical way of expressing the amount of Cr concentration is mmol/L or g/L. The average normal value of creatinine concentration excreted in a general population is 10 mmol/L or 1g/L. This means that data expressed either as amount of biomarker per litre or amount per gram Cr can be converted to amount per mmole Cr by dividing by 10 (Pryde and Gompertz, 1994).

9.2.2.1 Advantages and Disadvantages

Several techniques are available for analysis of creatinine in urine. They are relatively cheap and easy to carry out. Using creatinine values to correct for adjustment does not only eliminate the influence of dilution of urine samples but can also be used to obtain data comparable to those that would be obtained if the indicator had been determined in 24 hr urine samples (Alessio *et al.*, 1985). This is true since it is generally accepted that the amount of creatinine excreted in 24 hr is constant and little influenced by abnormal increase in excretion of urine (i.e. diuresis) (Alessio *et al.*, 1985). Another main advantage of using creatinine arises from the fact that most of the published literature utilises this method; hence allowing the researchers to compare their data to pervious studies.

Despite these merits, utilising this method of urinary concentration adjustment has got few disadvantages. Although occupational studies have shown that Cr is more suitable than SG for the adjustment of the results obtained on very dilute and concentrated urine, the use of Cr is still regarded as unreliable for urine sample with Cr concentrations < 0.3 g/L and > 3.0 g/L. van Rooij *et al.* (1993) excluded urine samples with Cr concentrations beyond the range of 4 - 34 mmol/L from his urinary 1-hydroxypyrene data analysis. Another important feature which is usually ignored are the confounding factors that are hard to control for. Creatinine's diagnostic importance is based on the supposition that its elimination is stable. This assumption however has been tested by several researchers and found to be debatable. The studies by Thompson *et al.* (1990), Araki *et al.* (1986) and Greenberg and Levine (1989) described below discuss this issue. The optimum way of expressing the biomarkers' urinary concentration results in relation to Cr is not yet fully understood.

9.2.2.2 Appropriate Usage of Urinary Creatinine Results

The reliability of this method has been challenged in relation to its use in adjusting for urinary cotinine (UCot) levels (Thompson *et al.*, 1990). The applicability of the method was tested by whether it improves the correlation between non-adjusted UCot and serum cotinine. It was shown that expressing UCot levels as a ratio to urinary creatinine (UCr) did not increases the correlation. As a consequence, the authors suggested based on the correlation between non-adjusted UCot and UCr and the mean UCr level of the sampled population a better way of expressing UCot concentrations. They observed that if UCot is adjusted using the following equation:

$$\log\{ UCot_{adj} \} = \log\{ UCot_{non\text{-}adj} \} - \S \times \{ \log(UCr) - \log(\bar{x}\ UCr) \}$$

whereby;

$\log\{ UCot_{adj} \}$, is the log of the adjusted urinary cotinine concentration
$\log\{UCot_{non\text{-}adj}\}$, is the log of the non-adjusted urinary cotinine concentration
§, is the slope of the regression line of the correlation between $UCot_{non\text{-}adj}$ and UCr
$\bar{x}$ UCr, is the mean UCr of the sampled population

the new $UCot_{adj}$ value improved the correlation significantly. From that they concluded that dividing $UCot_{non\text{-}adj}$ by UCr is not a satisfactory method of correcting UCot levels and does not decrease the imprecision introduced by variation in urine dilution. They also suggested that the described method of regression adjustment can be applied to other biomarkers. It is important to note that this adjustment method utilised does not account for factors that might affect excretion creatinine introducing inter-individual variation (e.g. body size, muscular mass, meat consumption etc.).

Greenberg and Levine (1989) addressed the validity of the assumption that Cr excretion is stable. They wrote that Cr elimination is not stable, the mechanisms responsible for the instability of elimination are not known, and that it is significantly dependent on urinary flow. They postulated that this instability is related to fluctuation in renal blood flow and variation in tubular Cr handling which may occur as a result of alteration in hydration status, change in cardiac output - due to exercise or stress - or posture. As a result they proposed a simple method to correct Cr concentration in spot urine samples for the effect of varying states of hydration. The mathematical correction proposed was based on the log-log regression solution of Cr excretion rate. From that an adjusted UCr can be calculated and used for correcting biomarkers' concentration as in the traditional use of non-adjusted UCr values. The formula used for adjusting UCr concentration was given by:

$$UCr_{adj} = \frac{UCr_{non\text{-}adj}}{(V / T)^{0.67}}$$

whereby;

UCr_{adj}, is the adjusted urinary Cr concentration
$UCr_{non\text{-}adj}$, is the non-adjusted urinary Cr concentration in mg/dL
V, is the volume of the urine sample in ml
T, time over which the sample of urine was voided in minutes

This mathematical model has not been fully validated and needs to be evaluated in many settings before it could used as a method for Cr adjustment. Its main highlights is that its wide acceptance is possible because no population based evaluation is needed (an exponent determination) for each substance under study. Of great importance is that it allows continued

application of the available clinical and occupational hygiene literature where urinary concentrations of a number of xenobiotics are expressed as a ratio to non-adjusted creatinine values (Greenberg and Levine, 1989).

This method of correcting has got all the characteristics of the traditional Cr adjustment method except for one drawback which has got to do with the extra effort needed by the workers to achieve an acceptable degree of compliance regarding urine collection and accurate time recording of voiding.

So the conclusion on the use of Cr as a mean of adjusting urinary concentration of biomarkers, and the way of expressing the results is still controversial. More investigation should be done to answer several queries shedding vagueness on the issue.

Prior to proposing alternatives to the classical application of Cr measurement for correction of substances urinary concentrations, it is required first to review the methods available to adjust urinary concentrations of xenobiotics.

Ideally, a proposed method should allow the evaluation of spot urine specimen rather than requiring extended urine collections. It should fully account for the effect of variable urinary dilution on biomarker's concentration. It should provide an estimate of internal dose independent of confounding factors. Cumbersome calculations or several chemical measurement should not be part of it. The units used ought to be understandable and comparable to established levels for control (normal) and exposed populations (Greenberg and Levine, 1989). Its effectiveness should prove worthy in a variety of physiologic, demographic and occupational settings. It other words it must be applicable to the population studied, non-invasive and practical under different settings.

9.3 SELECTION OF METHOD (URINARY CREATININE)

Our choice fell on creatinine for practical reasons. Aside to the fact that it is cheap and easy to conduct like other available methodology, the

needed equipment was readily available at the department. Also, almost all of the literature on biological monitoring of PAHs make use of urinary biomarkers apply creatinine in its traditional manner for urinary concentration adjustment. Of course this will allow the data to be related to similarly collated ones.

CHAPTER 10

METHODS

10.1 MEASUREMENT OF PAHs-SPECIES

10.1.1 Air Sampling

List of Material

1- Pump (SKC Inc.)
2- PVC tubing
3- Closed-faced filter cassette [37 mm] (SKC Inc.)
4- ORBO 43 sorbent tube (Supelco Inc.)
5- Teflon filters [37 mm, pore size 2μm] (SKC Inc.)
6- PTFE support [37 mm] (SKC Inc.)
7- Rotameter (SKC Inc.)
8- Aluminium foil
9- Ten ml glass vials
10- Forceps

Procedure

The sampling equipment illustrated in figure 10.1 was prepared on the day before sampling took place. The PTFE support and the filters were installed in the closed-faced cassettes and kept capped. On site, the cassettes were uncapped connected in series to the ORBO tube, after uniformly breaking its sealed ends, and the pump to form a sampling train. The cassette and the ORBO tube were wrapped with brown vinyl. The pumps were then set at the recommended flow rate of 2 ml/min, using a calibrated rotameter. The pumps were hung on the workers' waist and the cassette on their lapels. Sampling was carried out over the whole of the working shift from the subject's breathing zone. At the end of the working shift the flow rate was checked and recorded. A drop of >20% of the initial flow rate resulted in discarding the sample. The average flow rate was considered if the drop in flow rate was <20%. The cassette and the ORBO tube were capped and labelled after dismantling the sampling train. The samples were all transported in opaque boxes. In the laboratory the filters were carefully transferred into 10 ml glass vials using clean forceps. The glass vials and the ORBO tube were wrapped with aluminium foil and stored at -70 °C and analysed separately within one to two months. A control sample (filter and ORBO tube) was included for every ten samples. It was treated exactly the

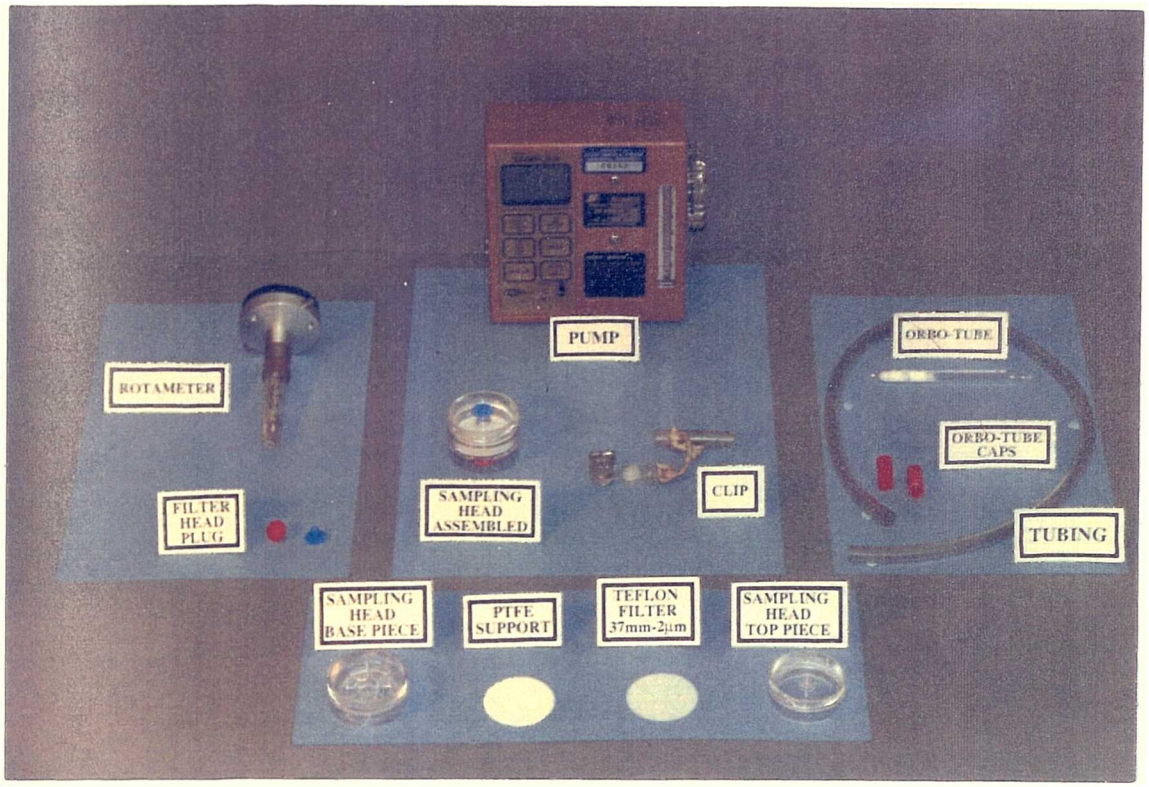


Figure : 10.1

"Sampling Kit" Used for Ambient Monitoring of PAHs

same as the exposed samples, however without drawing air through the pumps and keeping them capped until analysis.

Static air samples were handled exactly as the personal air samples except that they were not hung on the workers, but instead mounted on stands at head height in areas in the buildings were the office workers and departmental staff worked.

10.1.2 Chemical Analysis

List of Materials

1- TCL Polynuclear aromatic hydrocarbons (Supelco Inc.)
2- Fluoro-biphenyl - internal standard - (Supelco Inc.)
3- HPLC grade Dichloromethane (DCM) (Fisons plc)
4- 10 ml Screw cap glass vials
5- Glass pasteur pipettes
6- Anti-static gun - Zerostat 3 -
7- Sonicator
8- Micro-syringe (5, 25, 100 µl)
9- 10 ml glass tube
10- Hot plate
11- Nitrogen (BOC group plc)
12- 1 ml Amber vials with aluminium caps
13- 250 µl vial glass inserts
14- Crimper
15- Hewlett Packard GC-5890 SERIES II
16- VG Trio-1 mass-spectrometer
17- PTE-5 fused silica capillary GC column - 30 m x 0.25 mm i.d. (Supelco Inc.)
18- Helium (BOC group plc)
19- NaOH (2.5M) and $KMnO_4$ (1.5M)
20- UV lamp

Procedure

The filter and ORBO tube were spiked with 20 µl internal standard

(stock concentration 100 μg/ml). An anti-static gun was used to remove the static charge on the tube. The adsorbent beads were emptied into a 10 ml screw top glass vials. 5 ml of DCM was added to all vials containing either the filter or the beads. The vials were sealed and sonicated for 2 hours. DCM was transferred to a glass tube and evaporated under a gentle stream of nitrogen to 200 μl. The residual volume was transferred into an amber vial with 250 μl vial inserts and capped tightly. 1 μl of sample was automatically injected in splitless mode on to a Supelco PTE-5 fused silica capillary GC column using helium as a carrier gas at 10 psi. The oven temperature ramp was 85 ^{0}C for 3 min., 85 ^{0}C to 300 ^{0}C at a rate of 6 ^{0}C/min then kept at 300 ^{0}C for 5 minutes. A bench top VG Trio-1 mass-spectrometer (MS), in EI^+ mode with selective ion recording (SIR), was used for analysis. The MS was tested for leaks at an oven temperature of 80 ^{0}C by observing the percentage of helium, nitrogen and water. Both nitrogen and water were < 1 % and the helium at 100 %. The MS was then calibrated. Only a dynamic calibration with a standard deviation of < 0.02 was acceptable. An output of such a calibration is illustrated in figure 10.2. The concentrations of the following 9 PAHs (naphthalene, acenaphthene, phenanthrene, pyrene, benz(a)anthracene, benz(b)fluoranthene, benz(k)fluoranthene, benz(a)pyrene, dibenz(a,h)anthracene were calculated by comparison to standard curves. Initially, one of the high standard was injected using the MS in EI^+ mode scanning from mass 50 to 550 with a scan time of 0.55 sec. and interscan time of 0.05 sec. in order to determine the retention time of the 9 PAHs which are listed in table 10.1. Figure 10.3 shows the chromatogram obtained for the 1000 ng standard and figures 10.4a, 10.4b, and 10.4c show that acquired for a personal air sample both analysed with SIR mode on. The peaks were all quantified manually. For analysis of samples Time weighted average (TWA) concentrations for individual PAHs were calculated using the formula:

$$\frac{\text{PAHs Concentration (ng)}}{\text{Flow Rate(l/min) x Time of sampling (min)}}$$

and expressed as ng/m^3. The selection of PAHs for analysis is based on two criteria. The number of benzene rings to represent all families of 2 to 6 ringed PAHs, which are the most abundant in ambient air samples from processes involving the use of bitumen (Brandt *et al.*, 1985). Secondly, their carcinogenic potential was chosen to vary from non-carcinogenic in experimental animals (e.g. naphthalene) to potent carcinogens in human (e.g. benz(a)pyrene). Table

Table : 10.1 Selected PAHs quantified in static and personal air samples of the monitored population (retention times and correlation coefficient of standard curves

PAH Species	MWt.	Retention Time (min.)	r^2 (range)	CV %
Naphthalene	128	7.88	0.996 - 0.999	2.2
2-Flouro-biphenyl*	172	11.38	*	*
Acenaphthene	153	12.67	0.995 - 0.998	1.2
Phenanthrene	178	16.46	0.990 - 0.997	1.4
Pyrene	202	20.05	0.994 - 0.999	0.6
Benz(a)anthracene	228	23.13	0.995 - 0.997	2.7
Benz(b)flouranthene	252	25.65	0.995 - 0.997	6.0
Benz(k)flouranthene	252	25.70	0.995 - 0.997	6.0
Benz(a)pyrene	252	26.38	0.982 - 0.996	2.8
Dibenz(a,h)anthracene	278	30.46	0.989 - 0.994	9.7

* Internal Standard

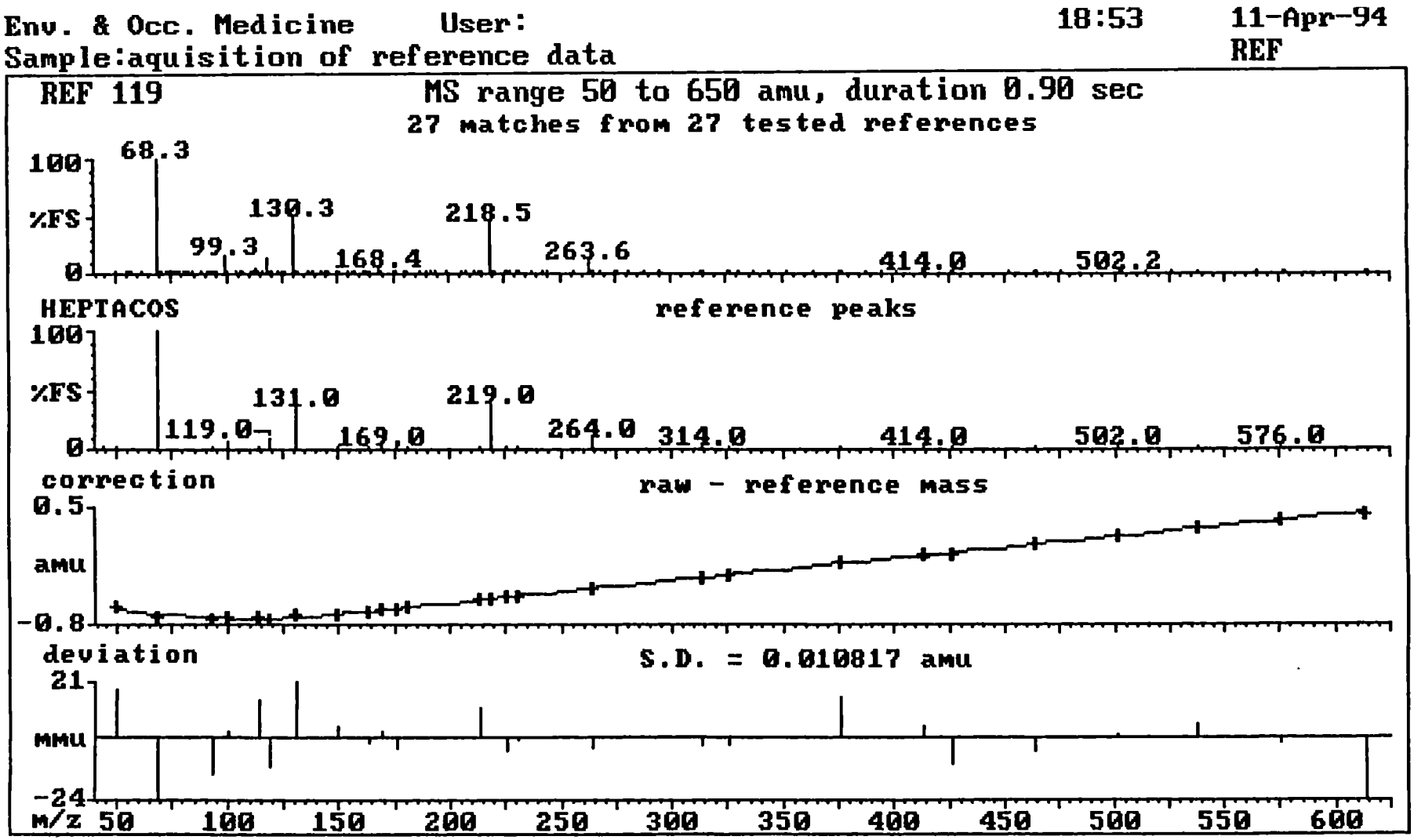


Figure : 10.2 A hardcopy output of an acceptable dynamic calibration of the GC/MS while in Scan Mode with a SD equal 0.010817

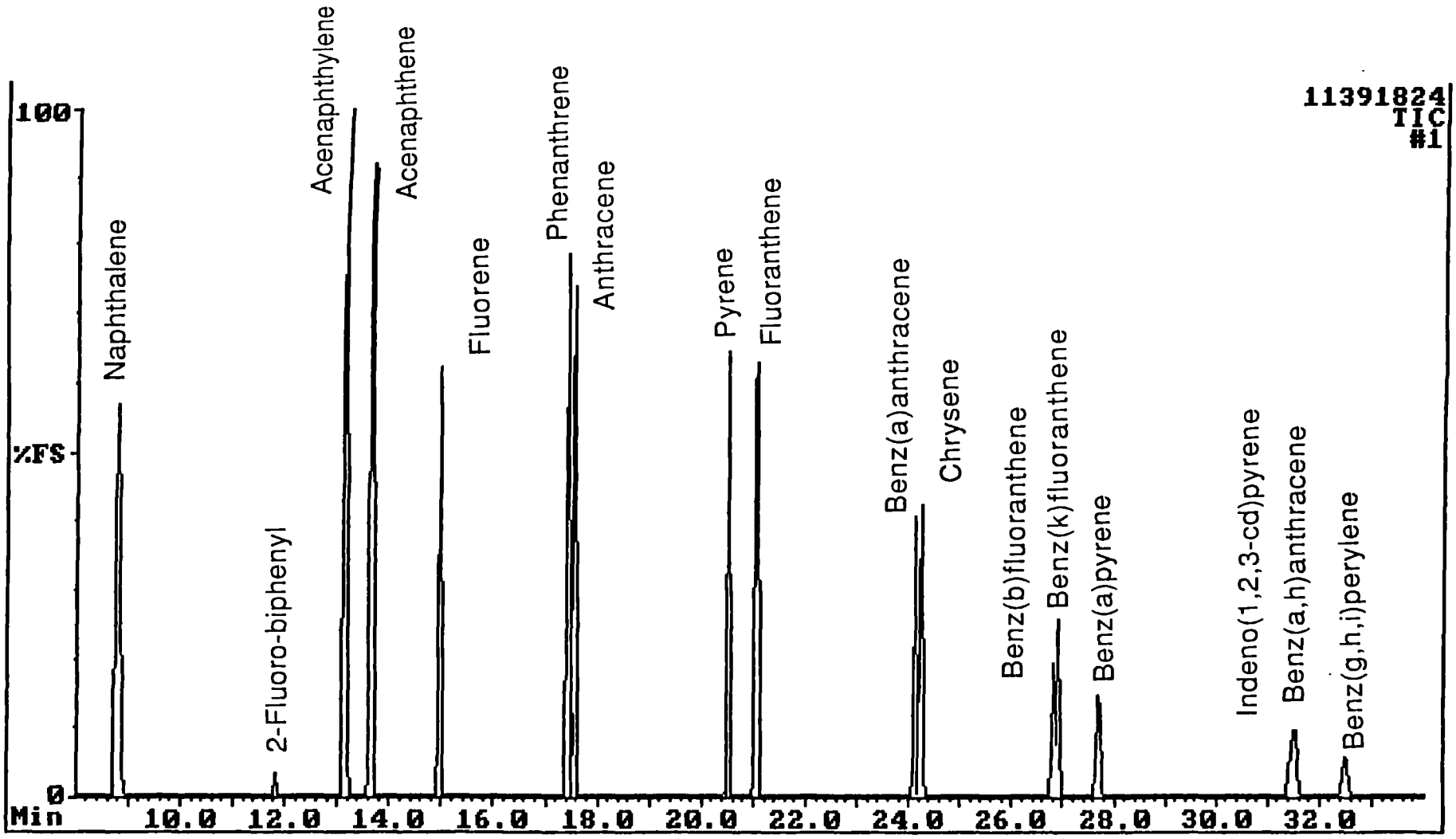


Figure : 10.3 A hardcopy output of an a chromatogram obtained from injecting 1000 ng standard with GC/MS in SIR Mode

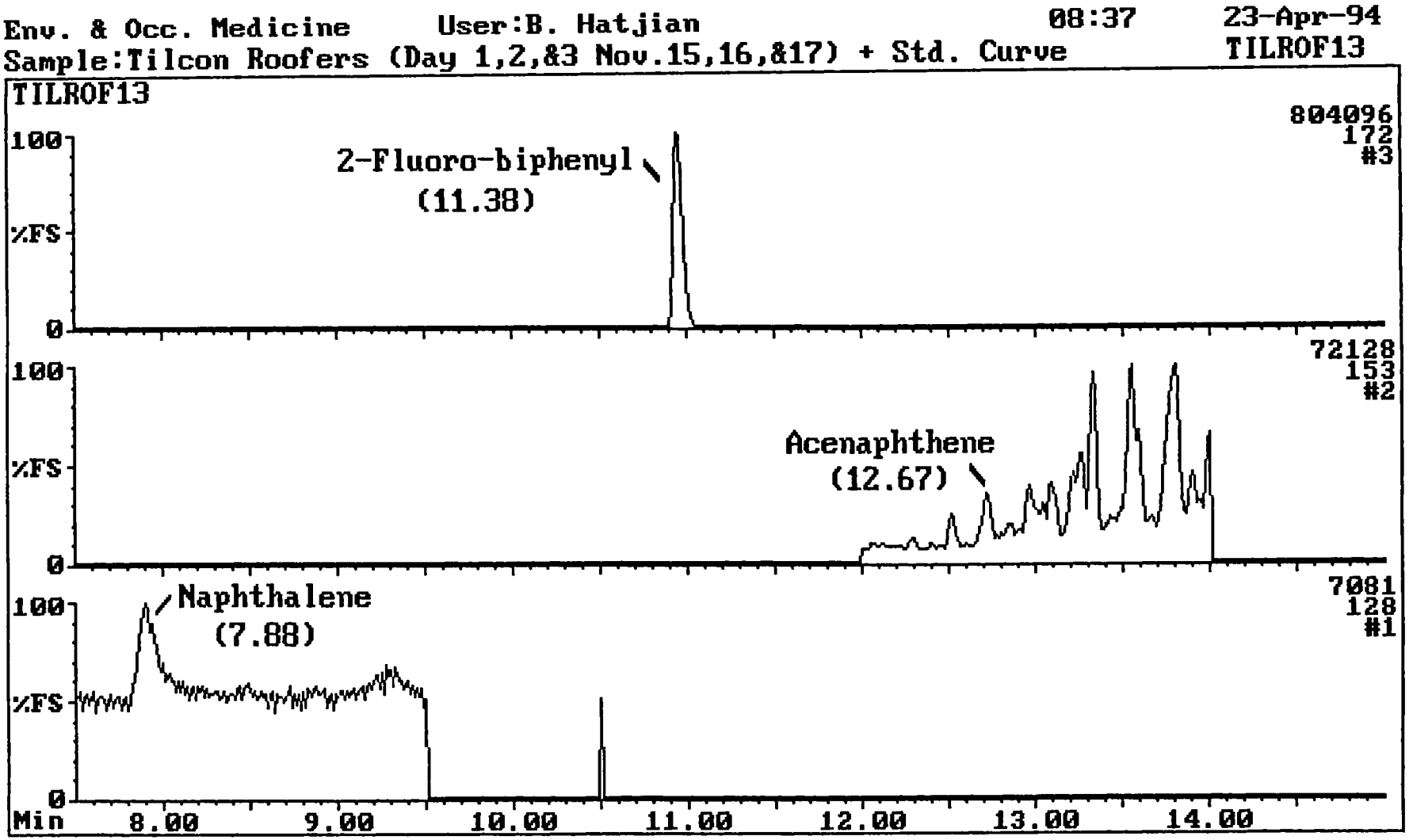


Figure : 10.4 a

A hardcopy output of ~~an~~ a chromatogram obtained from injecting the extract of a personal air sample with GC/MS in SIR Mode

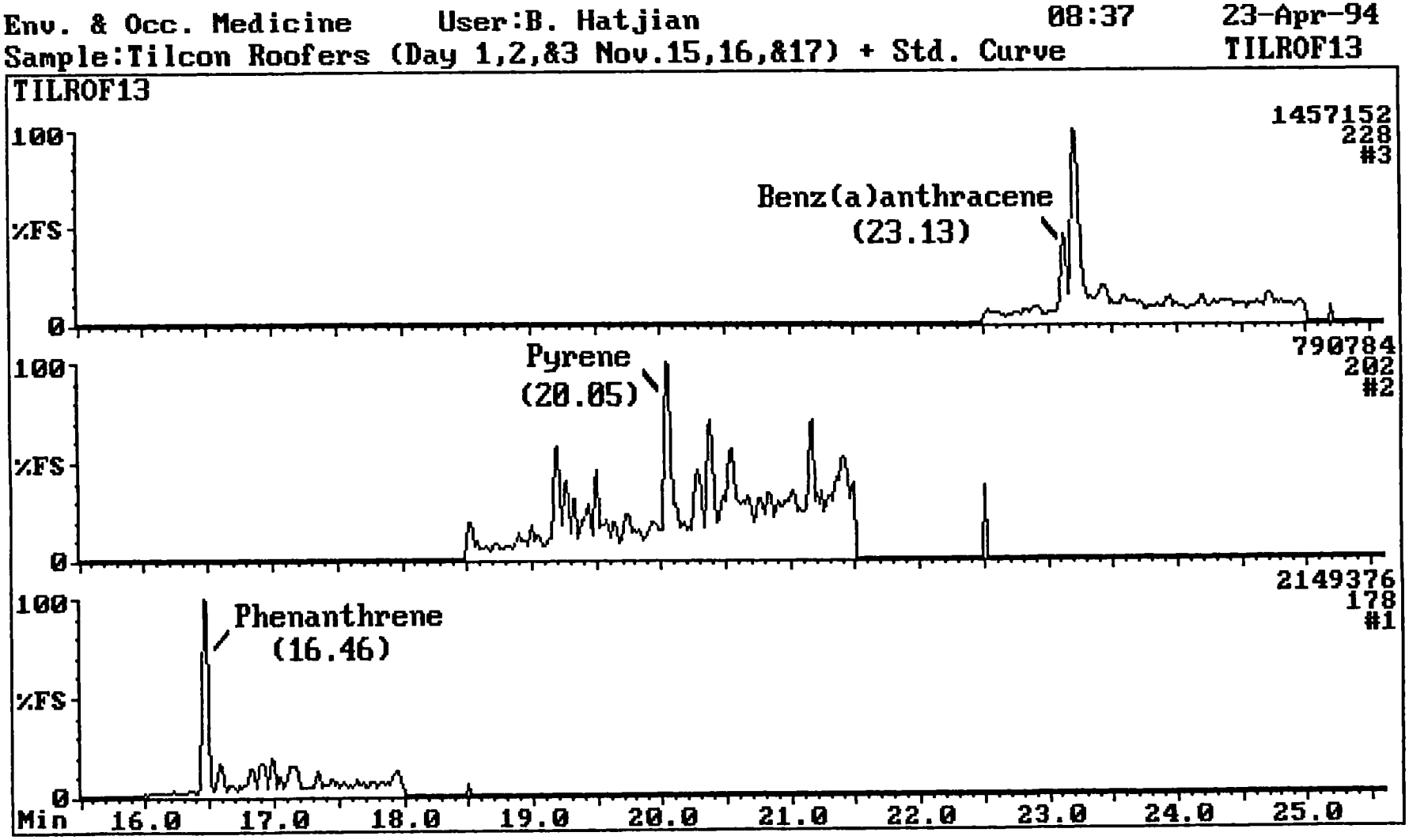


Figure : 10.4 b A hardcopy output of an a chromatogram obtained from injecting the extract of a personal air sample with GC/MS in SIR Mode

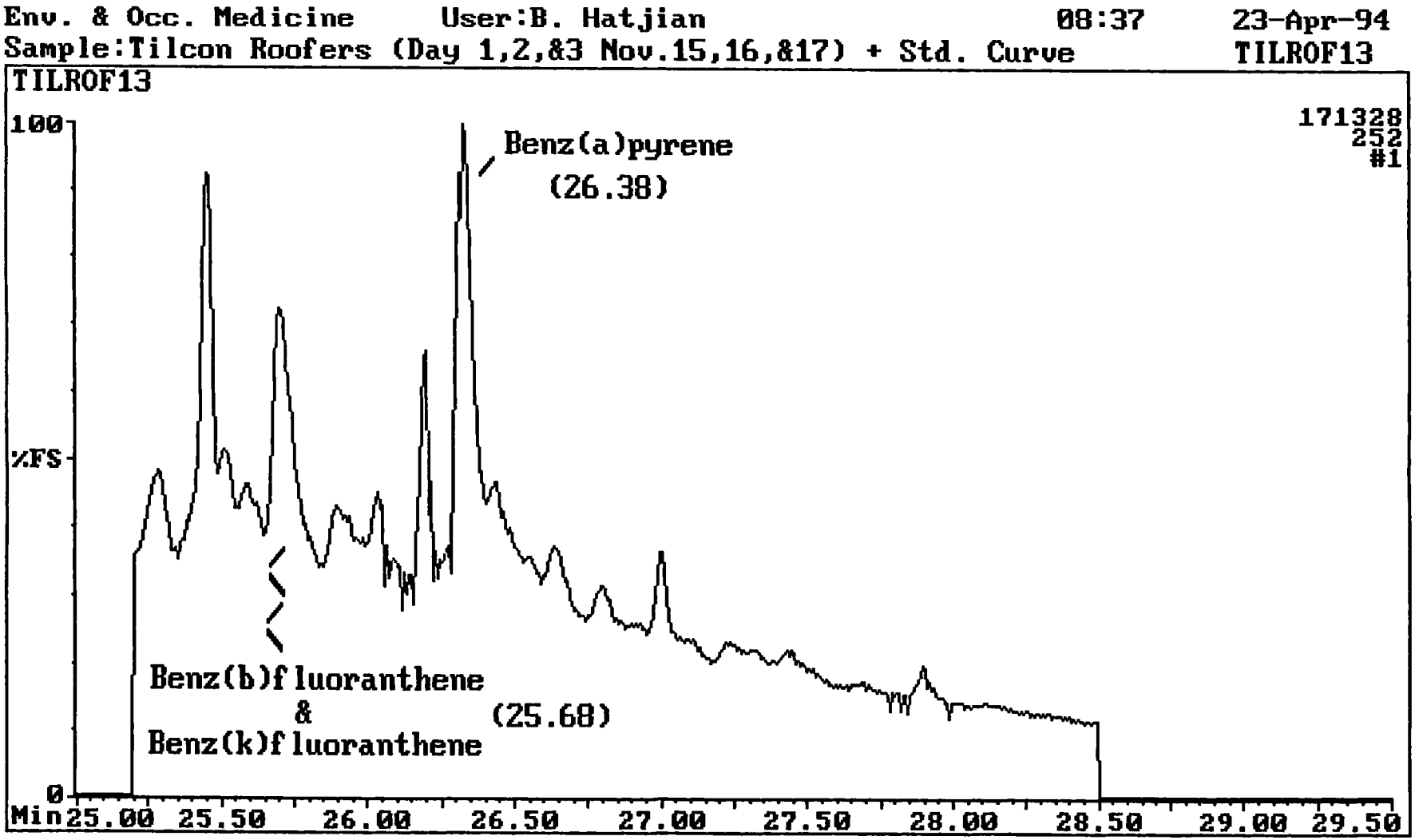


Figure : 10.4 c A hardcopy output of an a chromatogram obtained from injecting the extract of a personal air sample with GC/MS in SIR Mode

10.2 lists the 9 PAHs analysed for and quantified in this study with some of their specifications and the IARC evaluation of their carcinogenicity.

10.1.3 Evaporation, Partitioning and Recovery

An investigation of the efficiency of the recovery and evaporation procedure from the filter and the ORBO Tube was carried out. Also, partitioning of PAHs between DCM and the filter or the ORBO Tube was studied.

For studying the losses due to evaporation, standards were prepared in 5 ml DCM and spiked with 20 µl internal standard. They were then processed as described in the method. For the partitioning study standards were prepared as for evaporation and ORBO Tube beads were added to the glass tubes sonicated for 2 hr. and processed as described in the assay procedure. Partitioning was not studied with filters since they are not expected to have more affinity than DCM for the PAH-species. For recovery standards were spiked onto a set of filters and ORBO Tubes which were stored at - 70 °C for 2 hr. and then processed.

Tables 10.3a and 10.3b show that the percentage recovery from the ORBO tube was similar to that from the Teflon filter. For PAH with MWt < 202 the recovery ranged between 85.4 - 99.1 % and 88.5 - 99.2 % for the ORBO tube and Teflon filters respectively. For PAH MWt > 202 the recovery had a wider range for both ORBO tube and Teflon filter with recoveries as low as 70.6 % for the 50 ng standards in ORBO tubes and 69.0 % for 100 ng standard in Teflon filters. In general, for PAH with MWt > 202 the % recovery was lower at low concentrations, this however was not the case for PAH with MWt < 202 were % recovery was similar at either end of the standard curve and was highest for pyrene.

Table 10.3c illustrates that the range of % loss (the lowest is 0.3 % for pyrene at a concnetration of 0.05 µg and the highest is 16.8 % for naphthalene at a concentration of 0.01 µg) due to evaporation seem to contribute to the deviations from 100 % recovery values in both ORBO tube and Teflon filter trials at all concentrations for all PAH-species with MWt < 202. These observed values are consistent with the % loss reported by Thrane *et al.*

Table : 10.2 **Selected PAHs: Some of their physicochemical characteristics and the IARC evaluation of their carcinogenicity**

PAHs Selected	Synonyms	MWt.	Boiling Point	IARC Evaluation	Chem.Abstr.Service REG.No.	No. of Rings *
Naphthalene	Naphthalen	128	218	O	91-20-3	2
Phenanthrene	Phenanthren	178	338	+ -	85-01-8	3
Pyrene	Benzo(def)phenanthrene	202	393	-	129-00-0	4
Benz(a)anthracene	1,2-benzanthracene	228	425	+	56-55-3	4
Benz(b)fluoranthene	3,4-Benz(e)acephenanthrylene	252	481	+	205-99-2	5
Benz(k)fluoranthene	8,9-Benzfluoranthene	252	481	+	207-08-9	5
Benz(a)pyrene	3,4-benzpyrene	252	496	(+)	50-32-8	5
DiBenz(a,h)anthracene	1,2:5,6-Benzanthracene	278	524	+	53-70-3	5

+ : There is sufficient evidence that this PAH is carcinogenic in experimental animals.

- : The available data provide no evidence that pyrene per se is carcinogenic to experimental animals.

(+): There is sufficient evidence that this PAH is carcinogenic in experimental animals. Proven to be human carcinogen in more recent research. Harvey, (1991)

+-: The available data are inadequate to permit an evaluation of the carcinogenicity of phenanthrene in experimental animals

O : Has not been evaluated

* : No. of heterocyclic rings in the compound

Table : 10.3a Percentage recovery of PAH-species from ORBO tubes during analysis

	% RECOVERY at DIFFERENT CONCENTRATIONS				
PAH Species	**0.01 µg**	**0.05 µg**	**0.1 µg**	**1.0 µg**	**10 µg**
Naphthalene	96.8	***61.0***	98.6	95.1	96.6
Acenaphthene	95.1	99.1	94.4	89.5	85.4
Phenanthrene	94.3	83.5	98.1	97.3	88.5
Pyrene	98.0	93.0	91.0	96.1	90.1
Benz(a)anthracene	104.0	94.1	*78.7*	90.0	85.0
Benz(k)flouranthene	*	*73.6*	*79.8*	*78.6*	97.7
Benz(a)pyrene	*	*70.6*	86.3	*84.5*	98.5
Dibenz(a,h)anthracene	*	*73.1*	*69.3*	*83.5*	100.3

* Data not available
Note: Recovery < 85% (***bold italic script***)

Table : 10.3b Percentage recovery of PAH-species from Teflon filters during analysis

	% RECOVERY at DIFFERENT CONCENTRATIONS				
PAH Species	**0.01 µg**	**0.05 µg**	**0.1 µg**	**1.0 µg**	**10 µg**
Naphthalene	94.8	92.1	93.3	92.4	92.2
Acenaphthene	96.1	99.2	97.1	88.5	90.4
Phenanthrene	95.5	91.0	88.9	98.7	96.3
Pyrene	98.8	89.0	93.5	99.1	91.1
Benz(a)anthracene	88.0	96.2	88.2	***67.8***	95.2
Benz(k)flouranthene	*	***79.5***	***69.8***	***77.7***	93.5
Benz(a)pyrene	*	***74.6***	85.1	***80.2***	94.4
Dibenz(a,h)anthracene	*	***77.1***	***69.0***	***75.8***	90.7

* Data not available
Note: Recovery < 85% (***bold italic script***)

Table : 10.3c Percentage loss of PAH-species due to evaporation during analysis

	% LOSS at DIFFERENT CONCENTRATIONS				
PAH Species	**0.01 µg**	**0.05 µg**	**0.1 µg**	**1.0 µg**	**10 µg**
Naphthalene	***16.8***	10.5	10.5	13.3	7.4
Acenaphthene	9.5	4.8	11.9	9.8	8.5
Phenanthrene	10.0	6.7	0.5	1.6	2.8
Pyrene	4.7	0.3	7.6	7.8	5.7
Benz(a)anthracene	***22.3***	***19.3***	7.1	7.3	***22.5***
Benz(k)flouranthene	*	***15.3***	0.0	***25.3***	4.6
Benz(a)pyrene	*	2.5	***20.6***	***16.0***	14.0
Dibenz(a,h)anthracene	*	***45.2***	***32.9***	***36.5***	13.4

* Data not available
Note: Losses > 15% (***bold italic script***)

(1985) for these species. For the PAH-species (MWt. > 202) the % loss due to evaporation (table 10.3c) were higher than those reported by Thrane *et al.* (10 - 20 %), but were complementary with the % losses observed in the our recovery studies for both ORBO Tube and Teflon filter (tables 10.3a and 10.3b). Result in table 10.3d suggest that % loss recorded due to partitioning of PAH-species between DCM and ORBO tube seem to reflect those % losses observed due to evaporation.

This study can lead us to conclude that the % loss incurred during chemical analysis for PAH-species is mainly due the evaporation process which is manifested at higher degree in PAH with MWt > 202. Thrane *et al.* (1985) mentioned in their article that it is of extreme importance to monitor the evaporation process and that extreme losses can occur if sample are evaporated to dryness. The losses incurred for the low molecular weight PAHs could be explained by their volatility. The good recovery for pyrene could be due to its low volatility and reactivity. The low % recovery in those PAH (MWt. > 202) is difficult to explain. It could be due to either their high reactivity with possibility of decomposition during preparation or the slightly elevated CV of their standard curve which introduces variability in the quantification.

The evaporation loss observed for low MWt. PAHs ranged from 0.3 - 16.8% and 0 - 45.2%. for the high MWt. PAHs. This seemed to be the rate determining step in the % recovery for all PAH-species from both the filter and the adsorbent tube. Although appreciable losses were observed this will not affect the comparison of results between different samples since the inclusion of the internal standard (2-FB) will fully correct for these discrepancies assuming that 2-FB has got similar chemical and physical properties as the PAHs.

10.3 Characteristics of the Assay

The standard concentrations were 0.005, 0.01, 0.05, 0.1, 1, 10 and 100 ng for PAHs with MWt ≤ 228. For PAHs with MWt. ≥ 228 the the lowest standard was not detected. The coefficient of variation (CV) of the slope and the correlation coefficient (r^2) observed for the standard curves of all 9 PAHs ranged from 0.6 % to 9.7 % and 0.982 to 0.999, respectively. Table 10.1

Table : 10.3d Percentage Loss of PAH-Species due to their partitioning between ORBO tube's adsorbant material and DCM (assuming no losses occurring due to other sources)

	% LOSS at DIFFERENT CONCENTRATIONS				
PAH Species	**0.01 µg**	**0.05 µg**	**0.1 µg**	**1.0 µg**	**10 µg**
Naphthalene	0.9	2.1	2.0	7.6	3.3
Acenaphthene	11.4	5.6	6.1	5.6	2.7
Phenanthrene	***22.4***	8.0	12.9	1.3	4.1
Pyrene	10.8	0.0	12.7	2.1	6.4
Benz(a)anthracene	0.0	***26.8***	***20.9***	***18.4***	6.2
Benz(k)flouranthene	*	13.3	11.7	14.0	0.0
Benz(a)pyrene	*	0.0	***31.8***	***32.7***	3.9
Dibenz(a,h)anthracene	*	***36.7***	***35.0***	***20.1***	5.9

* Data not available
Note: Losses > 15% (***bold italic script***)

presents all the CVs of slopes and "r^2" of all the standard curves determined for all 9 PAHs and figure 10.5a and 10.5b show typical standard curves constructed over the range of standards for each of the PAHs. The detection limit differed for different PAHs. The low molecular weight PAHs (MWt. ≤ 228) with a boiling point ≤ 425 had a detection limit of 5 ng/m^3 when 0.96 m^3 is sampled. For the high molecular weight ≥ 252 with a boiling point ≥ 481 had a detection limit of 10 ng/m^3 when 0.96 m^3 is sampled.

10.4 Decontamination Process

The PAHs residues on all materials used was decontaminated before disposal or washing of glassware. The decontamination procedure was adopted from Castegnaro *et al.*, 1983. Contaminated glassware was soaked for 12 hr. in a solution of NaOH 2.5 M and $KMnO_4$ 1.5 M in a ratio (4:1). Glassware was then rinsed, washed or sent for incineration. Surfaces were evaluated using UV or "black light" to reveal contaminated areas. If applicable, decontaminated was by use of the solution described above followed by washing with detergent and finally rinsing.

Figure : 10.5 a Standard Curves of the Nine PAHs Analysed for in Static and Personal Air Samples

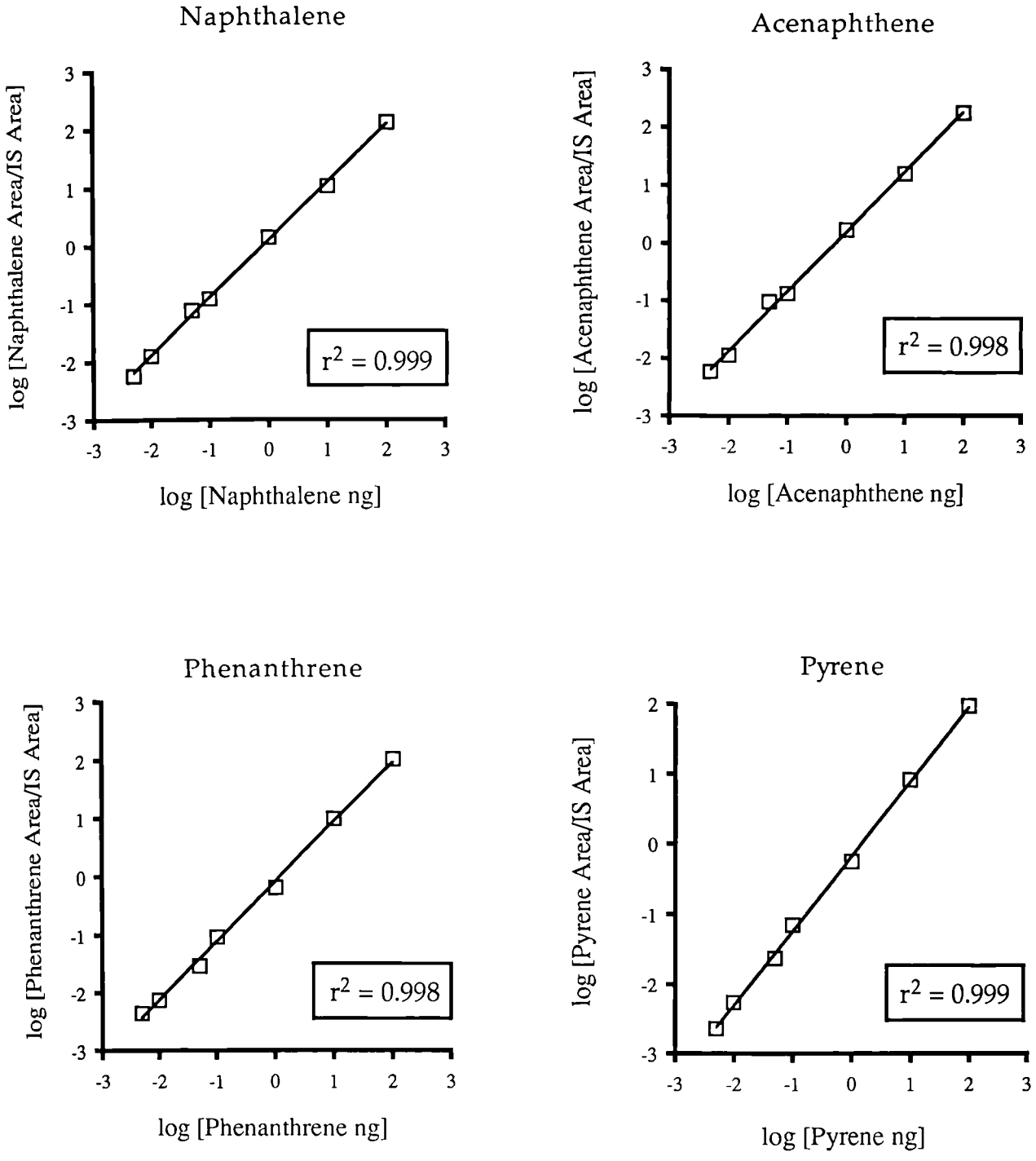
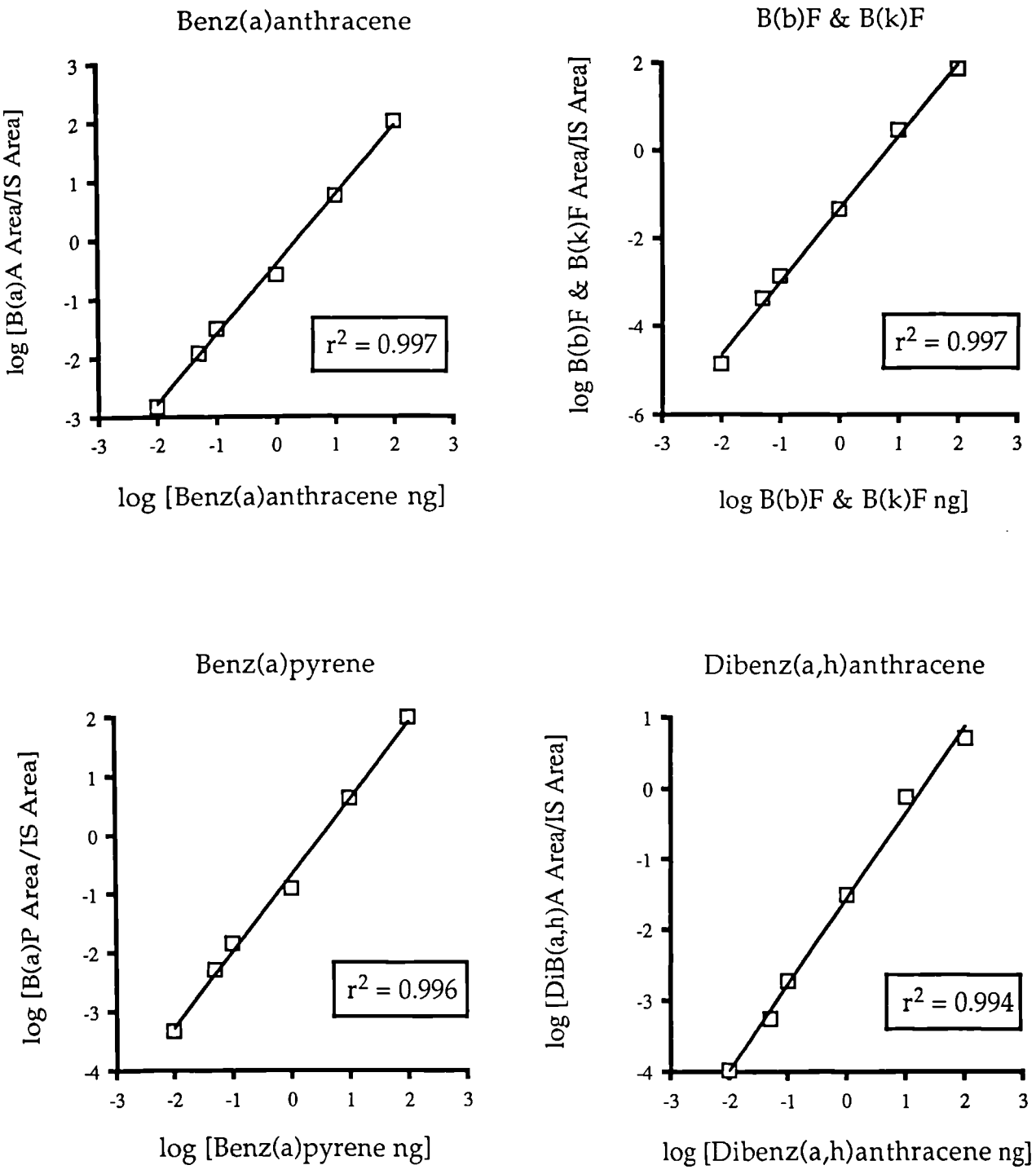


Figure : 10.5 b Standard Curves of the Nine PAHs Analysed for in Static and Personal Air Samples



10.2 MEASUREMENT OF URINARY THIOETHERS

10.2.1 List of Materials

1- Di-Sodium Hydrogen Phosphate $NaHPO_4$ (Fisons plc)
2- Ethylenediamine tetraaceticacid - EDTA - (Sigma Chemical Co.)
3- 5,5'-dithiobis(2-nitrobenzoic acid) -DTNB- (Sigma Chemical Co.)
4- N-acetylcystiene (Sigma Chemical Co.)
5- Hydrochloric Acid HCl (Fisons plc)
6- Sodium Hydroxide NaOH (Fisons plc)
7- Ethyl acetate (Fisons plc)
8- Nitrogen (BOC plc)

10.2.2 Procedure

Two ml aliquot of urine samples were acidified with 50 µl of HCl (4 M) and extracted twice by shaking with ethyl acetate (4 ml) for 15 minutes. The organic phase was separated by centrifugation at 1700 g and transferred, using glass Pasteur pipettes, to the same fresh glass tubes after each extraction. The ethyl acetate fraction were evaporated to dryness under a gentle stream of nitrogen at 50 ^{0}C. The extracts were then re-constituted in 2 ml of distilled water.

A solution of NaOH (4 M) was titrated against an HCl solution (4 M) to give a pH closest to 7.0 . The volume of titration were used to calculate the appropriate volume of NaOH and HCl to be added to the samples. This was necessary to keep the sample at a pH of approximately 7.0 which helps in avoiding colour development between free OH^- ions and DTNB/citrate reagent.

To 1 ml of urine extract in a glass tube approximately 0.5 ml NaOH (the exact volume depends on the results of the titration) was added. This sample tube was saturated with nitrogen then capped tightly. The glass tubes were left to incubate for 60 minutes in a hot plate set at 85 ^{0}C. After cooling the tube for 10 minutes on ice, approximately 0.5 ml HCl (the exact volume depends on the results of the titration) was added. The samples were left to stand for 5 minutes before adding 2 ml of phosphate/EDTA (pH 7.5) to 0.25 sample aliquot. This was followed by 0.3 ml reagent (DTNB/citrate). The

colour from the reaction developed within 5-10 minutes and absorbance of all samples and standards were read at 412 nm relative to distilled water within 30 to 40 minutes. A blank sample was included for all samples were the reagent was replaced by phosphate/EDTA buffer. The absorbance of the blank was subtracted from that of the samples. The absorbance of the reagent blank containing distilled water instead of urine extract was measured and subtracted from that corrected for the urine blank.

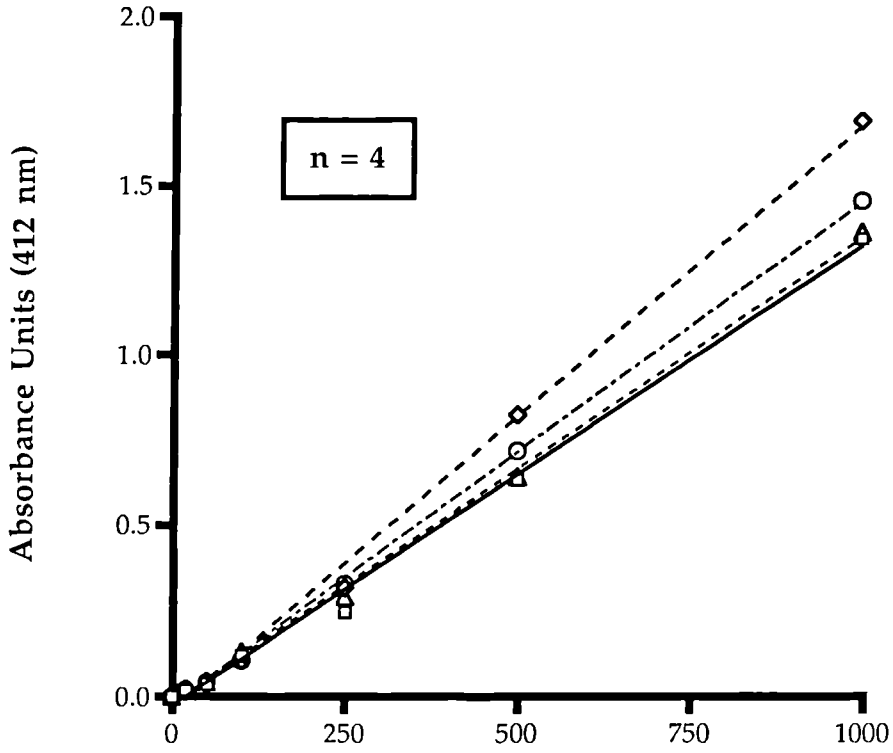
The absorbances were compared to those from standard solutions of N-acetylcysteine taken through exactly all the steps of the procedure except for the extraction steps. Concentrations of thioethers ([hydrolysed] - [non-hydrolysed]) were calculated by comparison to standard curves and were expressed as mmol thioethers /mol urinary creatinine.

10.2.3 Characteristics of the Assay

The CV of the standards (25, 50, 100, 250, 500 and 1000 mM), from the four standard curves shown in figure 10.6, was (10.3, 7.2, 10.4, 12.3, 12.6 and 11.0%), respectively. The r of the standard curve ranged from 0.998 to 0.999. The detection limit of the assay was 25 μM of thioethers.

Figure : 10.6

N-acetyl cysteine standard curves
constructed on four different days



10.3 MEASUREMENT OF URINARY 1-HYDROXYPYRENE

10.3.1 List of Material

1- 1-Hydroxypyrene Standard (Janssen Chemicals)
2- Methanol HPLC Grade (Fisons plc)
3- HCl and Acetic acid (BDH)
4- β-glucuronidase Type H-1 (Sigma Chemical Co.)
5- Vac-elut system (Jones Chromatography)
6- Nitrogen (BOC plc)
7- Iso-elute XL C18 - octadecyl cartridges (Jones Chromatography)
8- Hypersil BDS-C18 (5 µm, 25 cm x 4.6 cm i.d.) column (Shandon Scientific Ltd.)
9- HPLC/Fluorescence detector (Kontron plc)

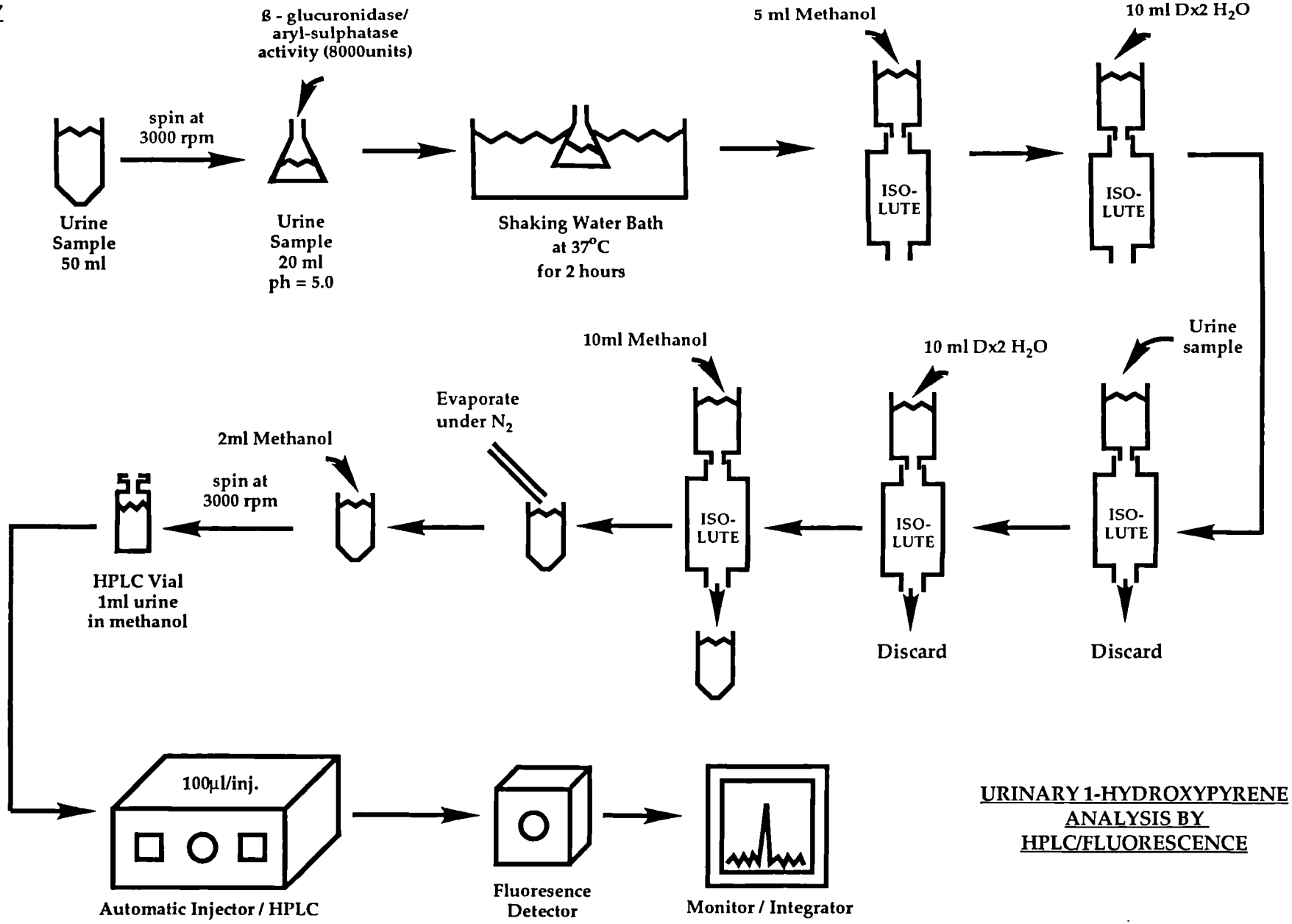
10.3.2 Procedure

The schematic diagram (fig. 10.7) shows the steps involved in the following procedure.

Twenty ml samples of urine, centrifuged at 1700 g, were adjusted to pH 5 using 1 M HCl and then buffered with 5 ml of 0.1 M acetate (pH 5). The samples were incubated for 2 hours with 8000 units of ß-glucuronidase Type H-1 at 37 °C in a shaking water bath. Iso-elute XL C18 - octadecyl cartridges were used for the purification of the samples and the separation of the pyrene metabolite. The cartridge was primed using a vac-elut system with 5 ml methanol, followed by 10 ml distilled water and the hydrolysed sample was passed through at a rate of < 3 ml/min. This flow was essential to achieve adequate recovery of analyte. Subsequently, the cartridge was washed with 8 ml of distilled water to elute the more polar contaminants. Finally, the retained 1-HP was eluted using 10 ml methanol. The solvent was evaporated to dryness at 60 °C under a gentle flow of nitrogen and was reconstituted in 2 ml methanol. Samples were centrifuged at 1700 g and 1.2 ml aliquots pipetted into 1.5 ml amber vials for HPLC analysis.

The analysis was done by reverse phase HPLC on a Kontron system. Hundred µl of each sample was automatically injected on a Hypersil BDS-C18 (5 µm, 25 cm x 4.6 cm i.d.) column. The flow rate was 0.7 ml/min. The solvent

Figure : 10.7



URINARY 1-HYDROXYPYRENE
ANALYSIS BY
HPLC/FLUORESCENCE

gradient was 10 min (methanol:H_2O; 40:60) on a linear gradient for 25 min. to (methanol:H_2O; 95:5) and then followed by a linear gradient for 5 min. to (methanol:H_2O; 40:60) and held for 7 min. The HPLC was equipped with a (Kontron) fluorescence spectrophotometer set at excitation and emission wavelength of 345 and 390 nm respectively. Slit width was adjusted to wide setting. The retention time for 1-HP was 30.3 min. Peak height were used for quantification. Results were expressed as µmol 1-HP / mol creatinine.

10.3.3 Elution Flow Rate and Recovery Study

The iso-elut XL C18-octadecyl cartridges had to be tested for efficiency of recovery. A urine sample with no free 1-HP was used to prepare spiked urine standards (2, 10, 25 nM). Three sets of standards were prepared. The three sets were eluted at flow rates of 3, 5, and 10 ml/min. The standard curves for the three standards was constructed and compared to that of a standard curve prepared in methanol. The recovery for the three tested concentrations was (91.5, 92.8 and 90.2 %), (80.5, 80.0 and 83.3 %) and (74.6, 66.2 and 74.3 %) at flow rates of 3, 5, and 10 ml/min., respectively. The CV of the slopes of the three standard curves was 9.8 % which is considered to be just acceptable. Figure 10.8 shows the standard curves constructed for the tested concentrations using the three chosen elution flow rates. Consequently, 3 ml/min was chosen as the optimum flow rate. It was difficult to achieve lower elution flow rates with the vac-elut system.

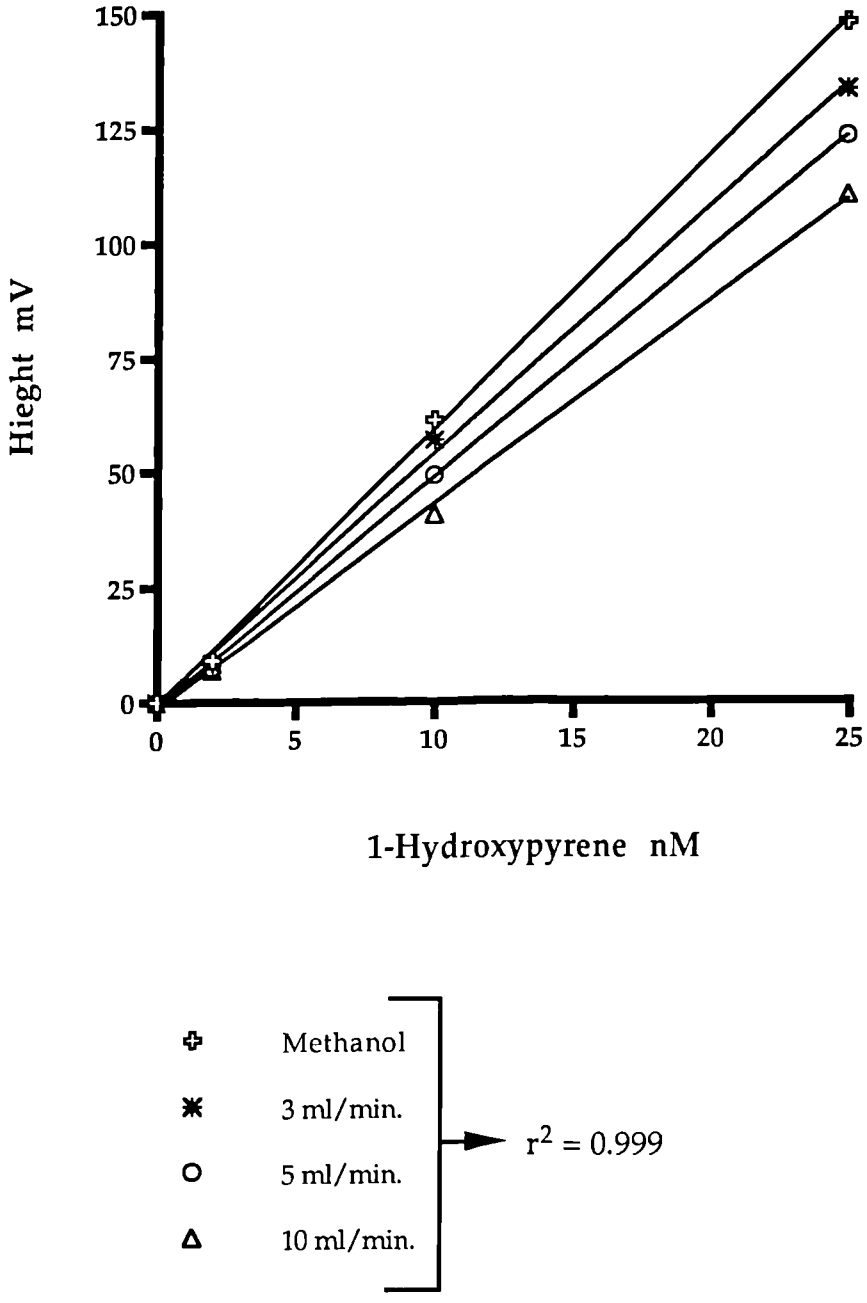
10.3.4 Enzyme Hydrolysis Study

The enzyme concentration of 8000 units was tested by Jongeneelen *et al.*, (1987[d]) to be in excess of the needed amounts to hydrolyse all the PAHs in urine's of dermatological patients treated with coal-tar. Consequently, we assumed that this concentration will be adequate to employ in our study knowing that the population we are sampling are less likely to have pyrene concentrations in their urine higher than the dermatological patients.

The ideal incubation period was determined from urine's of a non-smoker, a moderate smoker and a heavy smoker. Urine samples in triplicate were treated as described in the method but incubated for 2, 4 and 24 hours.

Figure : 10.8

Recovery of 1-Hydroxypyrene from Spiked Water Standards Using Three Different Elution Flow Rates Compared to Standards Prepared in Methanol



No free 1-HP was found in any of the samples (see chromatograms in fig. 10.9a and 10.9b). The results presented in table 7.4 shows clearly that the peak height of 1-HP after the 2 hrs incubation period were equivalent or higher than those of 4 and 24 hrs incubations. This suggested that the incubation time of 2 hrs. was sufficient to hydrolyse the 1-HP conjugates. Table 10.4 shows clearly that the heavy smoker gave the highest yield. The moderate smoker and the non-smoker gave more or less similar results. This similarity in the internal dose of 1-HP, in the non- and moderate smoker, could be due to dietary intake of PAHs.

10.3.5 Characteristics of the Assay

The 1-HP recovery (mean ± SD) from spiked urine standards (2, 5, 10, 25 and 100 nM) from three determinations when compared to a standards prepared in methanol was (92.0 ± 3.5, 93.6 ± 4.6, 89.9 ± 7.3, 89.0 ± 4.5, and 89.4 ± 3.0), respectively. Figure 10.10 shows one standard curve for 1-HP prepared in methanol and three standard curve of 1-HP in spiked urine samples using an elution flow rate of 3 ml/min. The CV of the standards (2, 5, 10, 25 and 100 nM) from three determinations was (3.9, 5.0, 8.1, 5.1 and 5.8%), respectively. The coefficient of variation of the slope of the standard curve over its full range (0, 2, 10, 25, and 100 nM) from three determinations in spiked urine samples was (5.8%). The correlation coefficient (r) for all the above four standard curves presented in figure 7.6 was 0.999. The y-intercept was not significantly different from zero at 95% confidence level. The detection limit was 1 nM with a signal to noise ratio of 1:3. The retention time for 1-HP under the conditions of the assay was 30.3 minutes. Figure 10.11 shows a trace 1-HP peak with a retention time 27.6 minutes from a sample from a PAHs-exposed worker following enzyme hydrolysis.

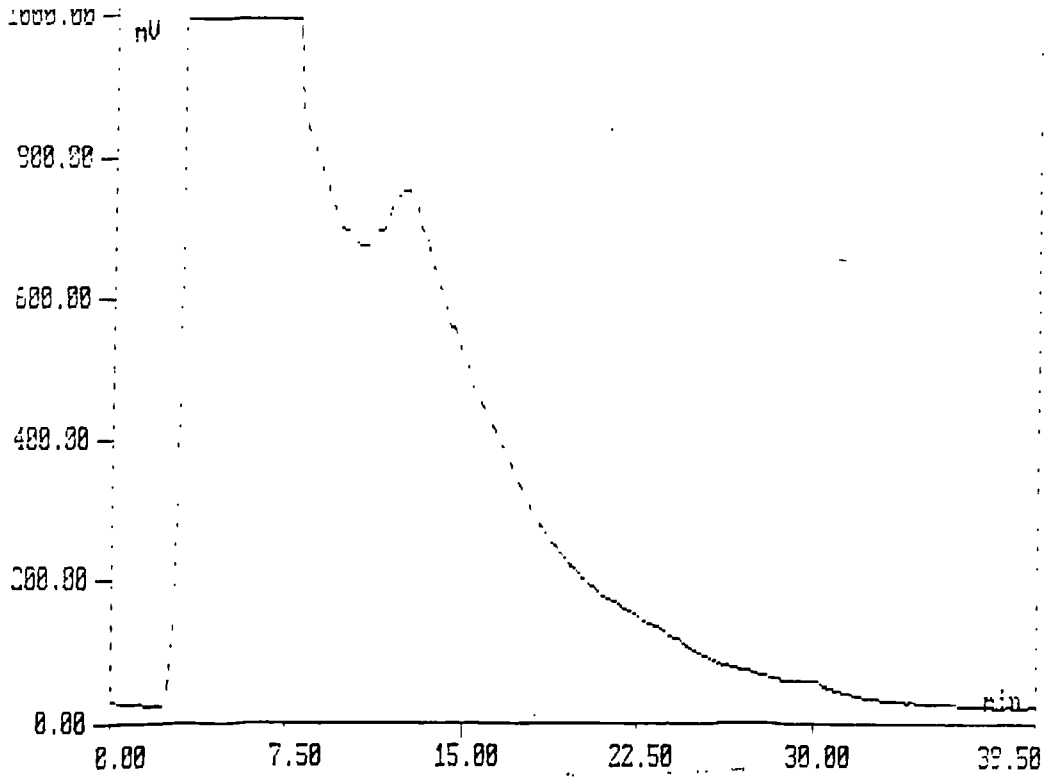
Table : 10.4 1-Hydroxypyrene yield versus time of incubation

Type of Smoker	2 Hours	4 Hours	24 Hours
Heavy	19.6 ± 1.4*	14.0 ± 0.75	11.3 ± 1.3
Moderate	7.71 ± 0.56*	7.06 ± 0.80	7.14 ± 1.5
None	7.24 ± 0.36*	7.02 ± 0.35	6.92 ± 0.85

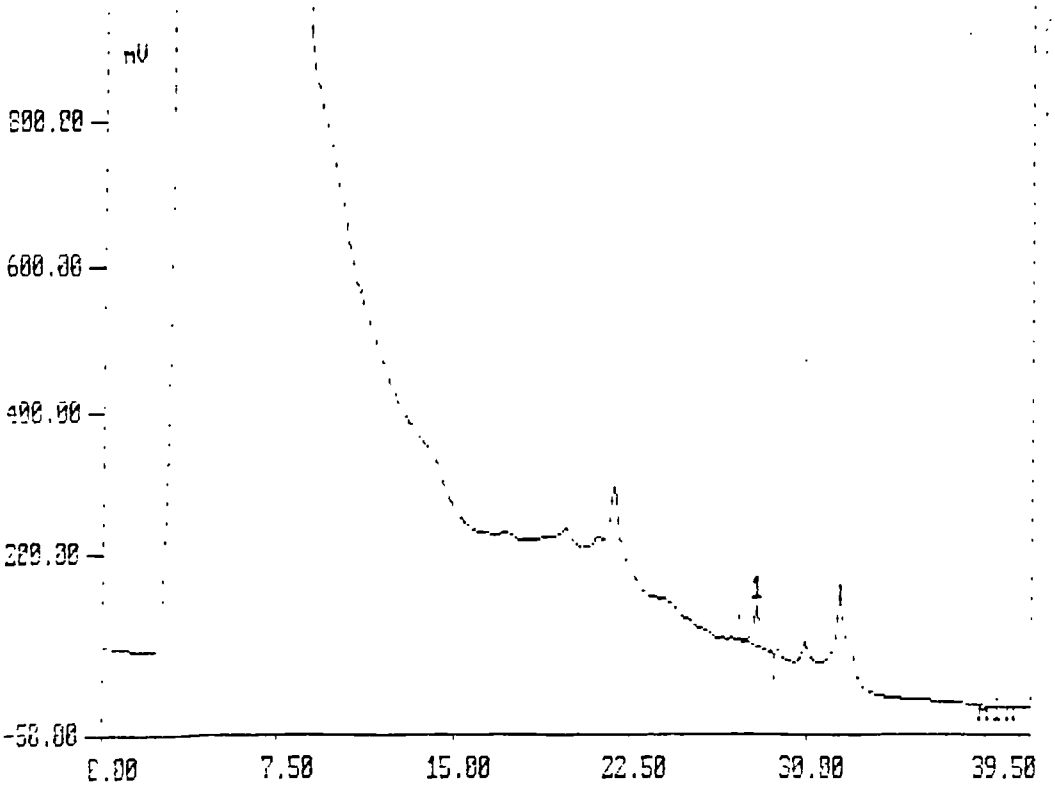
All results expressed in (Mean ± SD) mV

* The 2hours incubations had similar or higher yields than the 6 & 24 hours incubation times in all three subjects.

Figure : 10.9



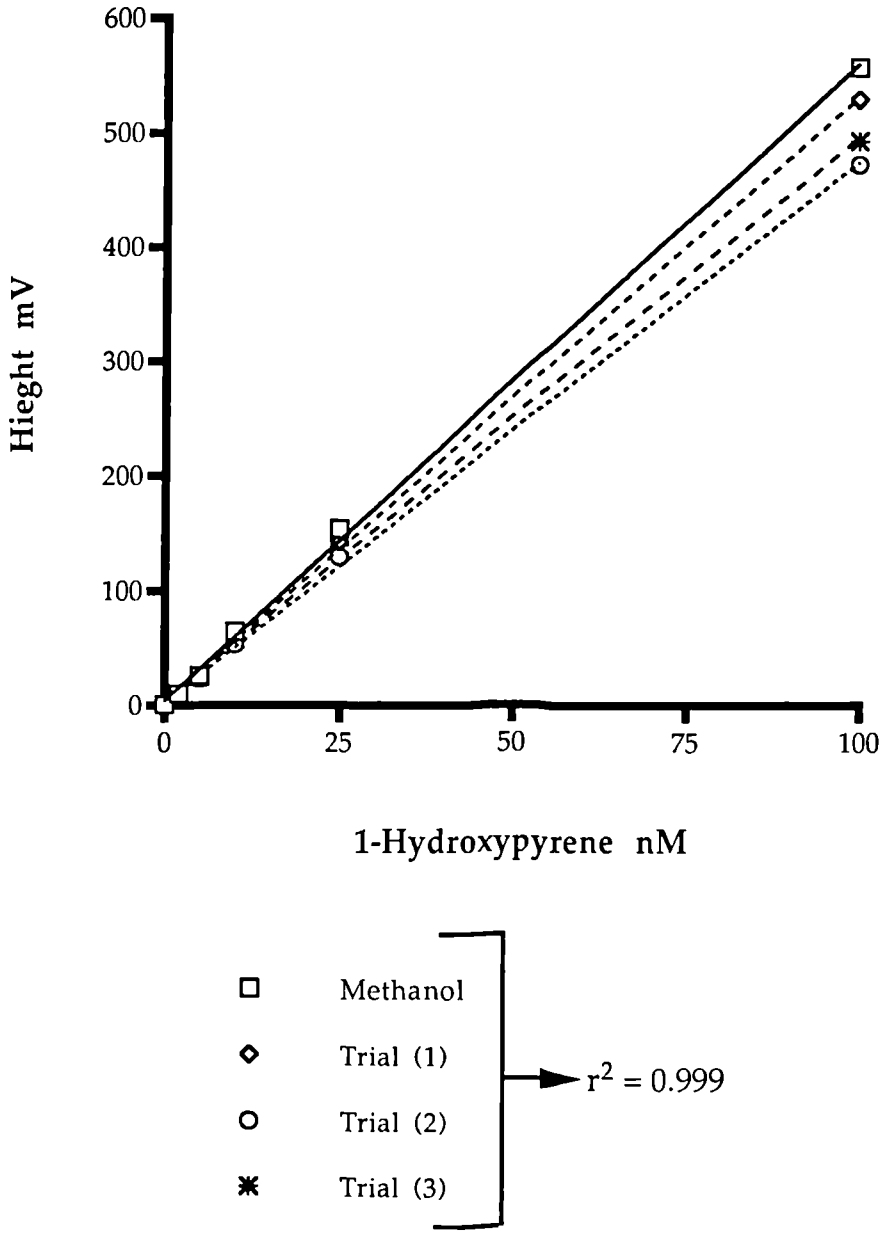
A) Chromatogram of non-conjugated urine sample of a heavy smoker (no free 1-hydroxypyrene)



(B) Chromatogram of a ß-glucuronidase conjugated urine sample of a heavy smoker (1-hydroxypyrene, peak)

Figure : 10.10

Recovery of 1-Hydroxypyrene from Spiked Urine Standards Compared to Standards Prepared in Methanol



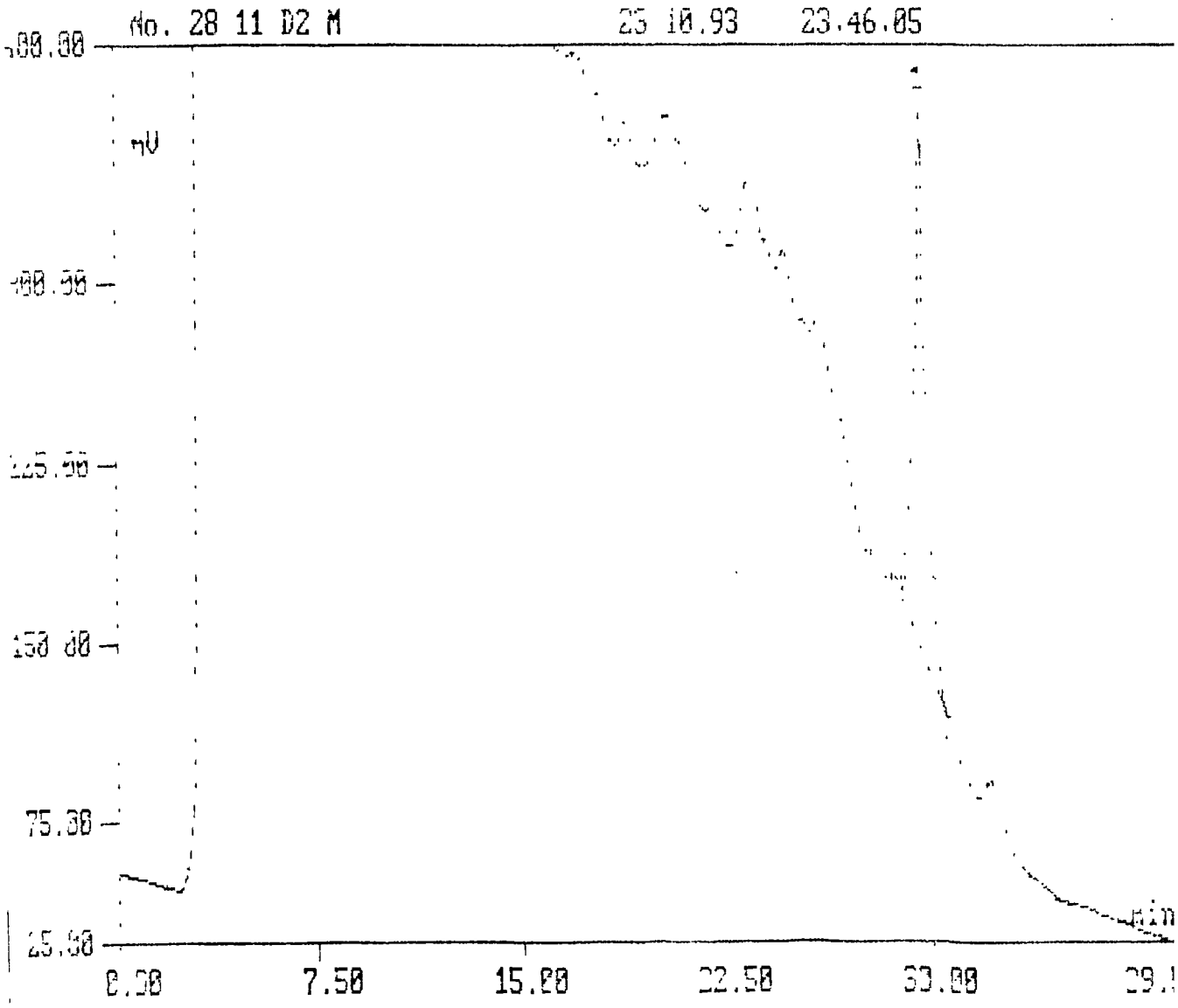


Figure : 10.11 Chromatogram of a ß-Glucuronidase conjugated urine sample of an exposed worker (1-hydroxypyrene peak # 1)

10.4 MEASUREMENT OF URINARY D-GLUCARIC ACID

The method described in detail below for the determination of UDGA is that of March (1963) as modified by March *et al.* (1974) and Latham (1974) as used by Edwards (1990).

10.4.1 List of Materials

1- Formic acid 90% (Fisons plc)
2- Trizma Hydrochloride (Sigma Chemical Co.)
3- Trizma Base (Sigma Chemical Co.)
4- Sodium Acetate Trihydrate (Fisons plc)
5- Acetate (Fisons plc)
6- Glycine (Sigma Chemical Co.)
7- D-glucaric acid (Sigma Chemical Co.)
8- Phenolphthalein Glucuronide (Sigma Chemical Co.)
9- ß-Glucuronidase (Sigma Chemical Co.)
10- Glass Tubes
11- Bunsen Burners
12- UV Spectrophotometer (Kontron)

10.4.2 Procedure

To one of a pair of screw-top glass tubes containing 2 ml of urine 0.4 ml of Tris buffer (1.75 M, pH 9.0) was added and the other 0.4 ml of formate buffer (2 M, pH 3.3). The tubes were capped and boiled for 60 minutes. While urine samples at room temperature Tris buffer and formate were added to the tubes in reverse order. 1.2 ml of acetate buffer (2 M, pH 4.8) was then added to both tubes. 1 ml aliquot of acid and alkali-hydrolysed samples were pipetted into fresh glass tubes containing 0.75 ml substrate - phenolphthalein glucuronide - (0.5 mM). To these were added 0.5 ml of enzyme - ß-glucuronidase - (500 IU/ml) and the mixture incubated at 37 ^{o}C for 30 minutes. Three ml of Glycine buffer (2 M, pH 12) was added to all tubes for stopping the reaction and colour development. Absorbance was read at 555 nm relative to water on a UV spectrophotometer. Sample blanks were carried through the incubation step in the presence of glycine prior to the addition of the enzyme.

Standards solution of d-glucaric acid were treated exactly as the sample; however they were not base treated. A blank of 3 ml distilled water was used to estimate uninhibited activity of the enzyme. From absorbance reading percentage inhibition was of the enzyme was determined by the formula:

$$\% \text{ Inhibition} = 100 \times \left\{ \frac{\text{Abs (Inhibited)}}{\text{Abs (Uninhibited)}} \right\}$$

whereby; Abs is the absorbance at 555 nm.

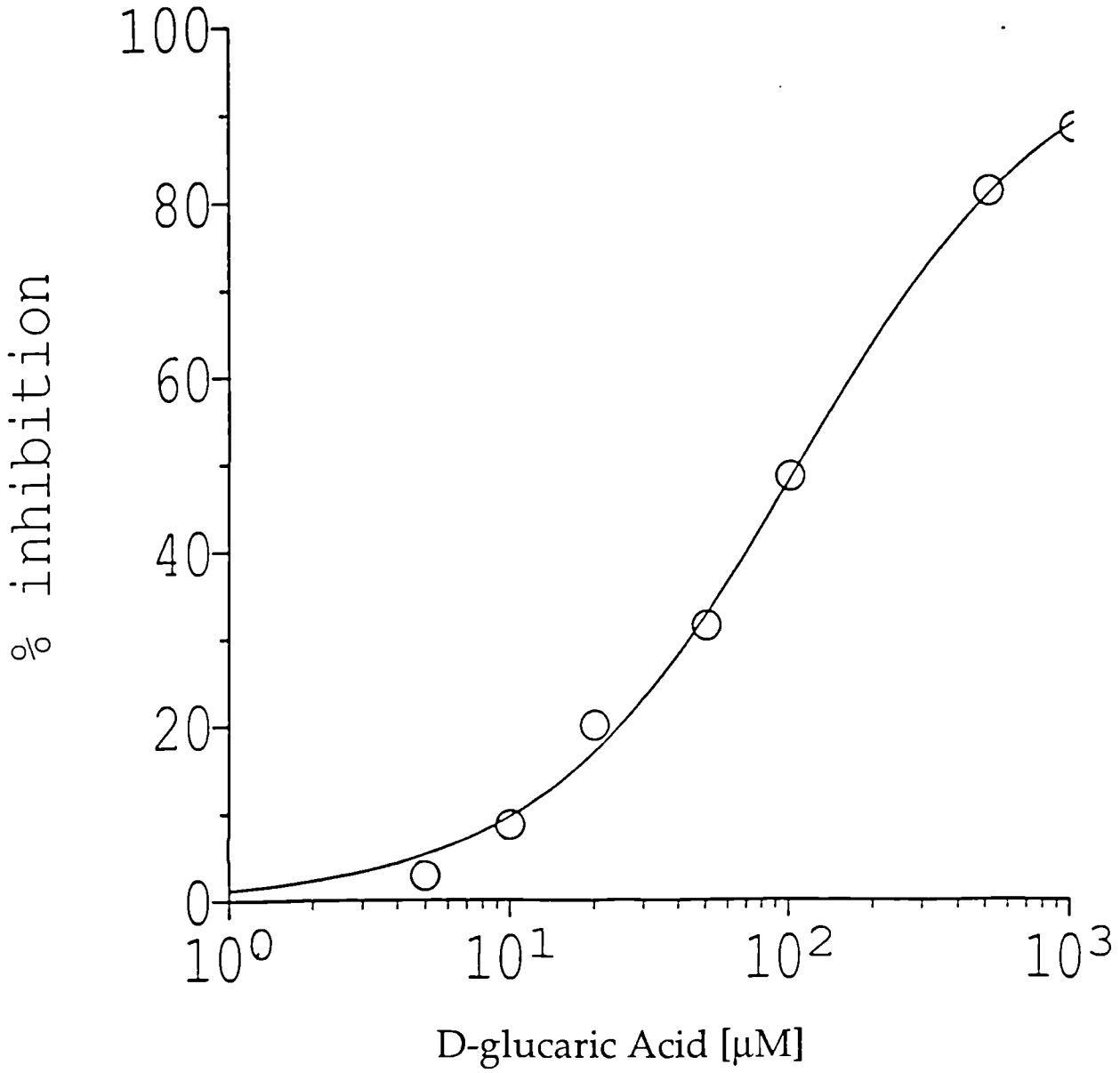
The sigmoid standard curve was constructed by plotting % inhibition versus the logarithm of the UDGA concentration. The curve was fitted to the data using GRAPHPAD, a computer graphics package (Motulski, 1987). D-glucaric acid concentrations in acid and base treated samples were estimated from the curve. The final UDGA concentration was determined from the difference, [acid hydrolysed] - [alkali hydrolysed] and expressed as mmol UDGA / mol creatinine.

10.4.3 Characteristics of the Assay

Figure 10.12 shows one standard curve constructed as described above. Estimates of the CV of the sigmoid standard curve, made at concentrations of 5, 20, 100, and 1000 mM of d-glucaric acid from five standard curves on different days was 3.8, 7.5, 8.5, and 13.3% respectively. The CV of UDGA in a urine sample analysed six times on the same day was 7.2%. The mean ± S.D. of the correlation coefficient (r) was at 0.999 ± 0.0006 for all five trials.

Figure : 10.12

D-Glucaric Acid Sigmoid Standard Curve



An output obtained from GRAPHPAD describing the characteristics of the above standard curve (r^2 = 0.999)

Sigmoid curve (log scale)
A=bottom, B=top, C=log(EC50), D='Hill' Slope

Final Results. Sum of Squares= 18.26711 (df= 5)
Goodness-of-fit assessed using actual distances; R squared=0.997

Parameter	Value	Approx. SE	%Error (C
A	0	(Constant)	
B	100	(Constant)	
C	2.030652	.0215401	1.1%
D	.9460025	.03894535	4.1%

10.5 MEASUREMENT OF MICRONUCLEI FREQUENCY IN EXFOLIATED EPITHELIAL CELLS

The method described below was adapted from Reali *et al.* (1987) with few modification.

10.5.1 List of Materials

1- Sodium Chloride (Fisons plc)
2- di-Sodium Hydrogen Phosphate (Fisons plc)
3- Potassium Chloride (Fisons plc)
4- Potassium di-Hydrogen Phosphate (Fisons plc)
5- Methanol - HPLC grade - (Fisons plc)
6- Acetic acid (Fisons plc)
7- Giemsa Stain (Sigma Chemical Co.)
8- May-Grunwald Stain (Sigma Chemical Co.)
9- Glass slides (BDH)
10- DePeX mounting medium (BDH)
11- Gurr - lenzol immersion oil - (BDH)
12- Light Microscope (x2500) (Carl Zeiss Jena)

10.5.2 Procedure

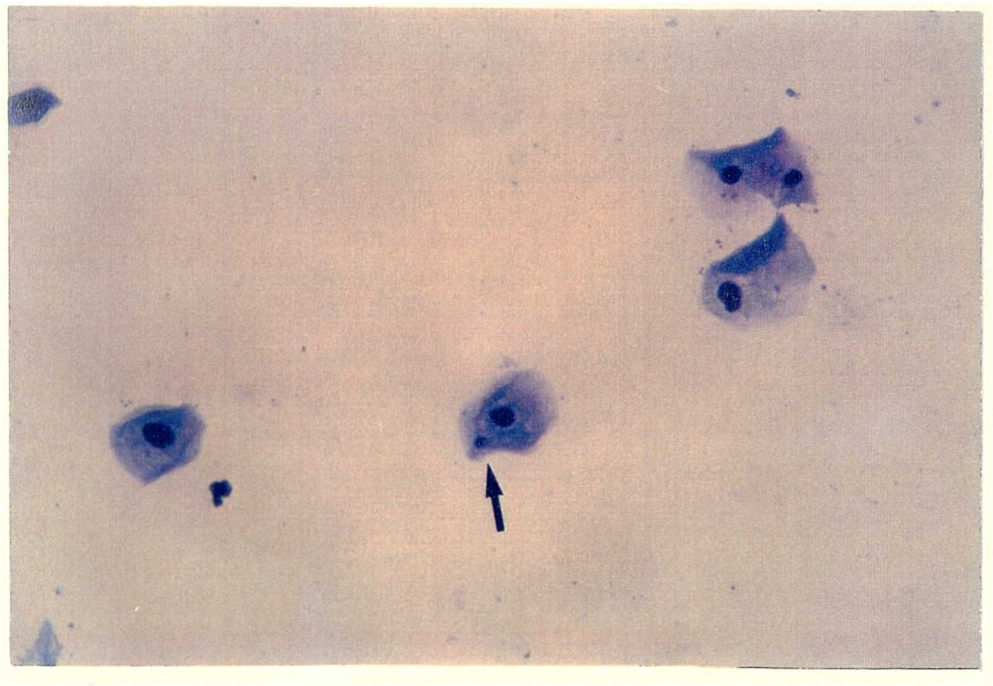
The sediment from 250 ml urine were collected by centrifugation at 1600 g for 10 minutes. The suspension was discarded and the sediment washed twice in PBS and recollected as above. Five ml of weak hypotonic solution (0.075 M KCl:PBS; 1:4) was added and the samples left to stand for 20 minutes. To the sediment 3 - 5 ml of fixative (MeOH:acetic acid; 3:1) was added dropwise. The samples were left to stand for 10 minutes before collecting the fixed pellet by centrifugation at 200 g for 10 minutes. The pellet was fixed twice with 5 ml of cold fixative and centrifuged at 200 g for 10 minutes. The pellet was resuspended in 2 ml of fresh cold fixative and dropped onto slides and air dried. The cells were fixed in methanol for 15 minutes then stained in a solution of (May-Grunwald stain : buffered water [Sorenson's buffer : water; 1:19]; 1:1) for 5 minutes. The slides were transferred to a solution of (Giemsa : buffered water; 9:1) for 15 minutes. They

were washed 3 times in buffered water and differentiated in distilled water for 2 minutes. Finally, slides were air dried and mounted using DePeX mounting medium (BDH). Micronuclei were scored under lenzol immersion oil using a light microscope with 2500X magnification. Figure 10.13a and 10.13b shows micronucleated and non-micronucleated exfoliated cells of a healthy male control. Micronuclei within the cytoplasm, 1/3 the size of the main nucleus and with the same intensity of stain were counted. Three thousand cells were counted for each sample. MN frequencies were expressed as MN/1000 cells.

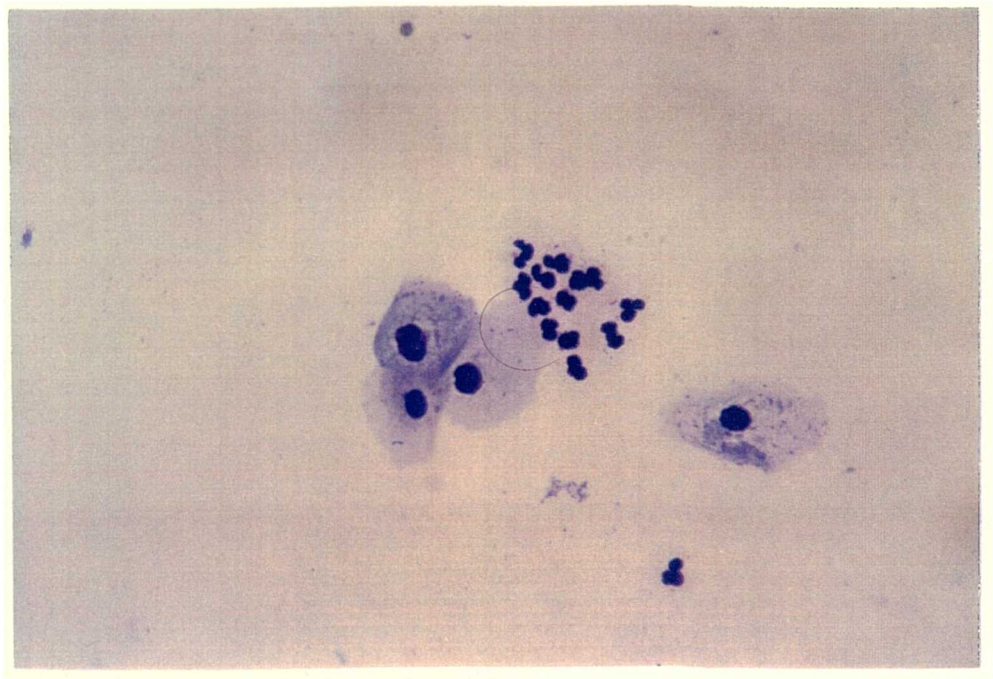
10.5.3 Characteristics of the Assay

The CV of the assay could not be determined in a reasonable number of subjects however it was calculated to be (< 5.0 %) from one individual sampled on three occasions one week apart. The intra-sample variability in the scoring procedure tested by one scorer was within acceptable limits. This is reflected in the (mean CV ± SD; 6.7 ± 2.2%) determined from scoring 9 slides 3 times which results are found in table 10.5.

Figure : 10.13 Micronuclei in exfoliated epithelial cells of the urinary system



(A) Five epithelial cells (one micronucleated) observed under light microscopy (magnification x 2500)



(B) Four epithelial non-micronucleated cells observed under light microscopy (magnification x 2500)

Table : 10.5 Results from which the coeffecient of variation of the MN Test was determined

	NUMBER OF MICRONUCLEI / 1000 CELLS								
	EXPERIMENT (I)			EXPERIMENT (II)			EXPERIMENT (III)		
	SLIDE CODE			SLIDE CODE			SLIDE CODE		
COUNT #	C	D	E	B	C	D	A	B	C
FIRST	28.3	47.6	51.6	45.3	51.3	45.3	45.1	42.6	48.8
SECOND	32.3	49.3	53.7	47.6	53.3	45.3	51.2	53.2	47.0
THIRD	44.5	50.2	46.8	50.3	47.6	51.3	45.1	42.5	49.1

10.6 MEASUREMENT OF SCE FREQUENCY IN HUMAN PBLS

Analysis for SCE frequency was conducted using a modified version of the method described by Edwards and Priestly (1993).

10.6.1 List of Materials

1- Sodium Chloride (Fisons plc)
2- di-Sodium Hydrogen Phosphate (Fisons plc)
3- Potassium Chloride (Fisons plc)
4- Potassium di-Hydrogen Phosphate (Fisons plc)
5- Polycarbonate Filter Holder SM 16510/11 (Sartorious)
6- Membrane Filters - 0.2 μm pore size, 47 mm dia. - (Whatman)
7- RPMI 1640 (HyClone Laboratories Inc.)
8- Sodium Hydrogen Carbonate (Fisons plc)
9- l-Glutamine (Sigma Chemical Co.)
10- Penicillin (Sigma Chemical Co.)
11- Streptomycin (Sigma Chemical Co.)
12- Phytohaemagglutinin M (Gibco)
13- 5-bromo-2'-deoxyuridine (Sigma Chemical Co.)
14- Demecolcine (Sigma Chemical Co.)
15- tri-Sodium Citrate (Fisons plc)
16- Sodium di-Hydrogen Phosphate (Fisons plc)
17- Methanol - HPLC grade - (Fisons plc)
18- Acetic acid (Fisons plc)
19- Giemsa Stain (Sigma Chemical Co.)
20- Glycerol (Sigma Chemical Co.)
21- Glass slides (BDH)
22- DePeX mounting medium (BDH)
23- Gurr - lenzol immersion oil - (BDH)
24- Light Microscope (x2500) (Carl Zeiss Jena)

10.6.2 Procedure

Blood samples were centrifuged at 1200 g for 5 min., the cell pellet was washed three times in phosphate buffered saline (PBS) and finally reconstituted to the original volume in PBS. Duplicate cultures were initiated

by adding 0.5 ml of washed blood to 4.5 ml of filtered RPMI 1640 medium with 2.05 mM l-glutamine and $NaHCO_4$ (2 g/L), penicillin (100 IU/ml), streptomycin sulphate (100 mg/ml) and phytohaemagglutinin M 50 μg/ml were added. Cultures were incubated in the dark at 37 °C under an atmosphere of 5% CO_2 in air for a total of 72 hours. At 24 hours, 5-bromo-2'-deoxyuridine was added at a final concentration of 20 μM. Finally, one drop of colcemid (demecolcine) (0.02% w/v) was added to all cell cultures at 70 hours. Duplicate cultures were pooled and cells were harvested by centrifugation at 700 g for 5 min. and 6 ml of KCl (0.075 mM) was added. Cells were left to incubate at 37 °C for 15 minutes and KCl was aspirated after centrifuging the samples at 200 g. Cell fixation was done three times using freshly prepared methanol/acetic acid (3/1 v/v) and they were then dropped on slides. The slides were exposed to ultraviolet light (254 nm and 366 nm) for 30 minutes under citrate buffer, rinsed with distilled water and then soaked for 15 minutes in 10X standard saline citrate at 60 °C. The slides were rinsed once more with distilled water and stained with 6% giemsa in Sorenson's buffer for 9 minutes. Finally, slides were rinsed again, dried and mounted using DePeX mounting medium (BDH). SCEs in 20 second division metaphase cells were scored blind by one observer, using light microscopy with 2500X magnification. Figure 10.14 shows a cell in the second metaphase with SCE on different chromosomes. Only cells containing 44 - 46 chromosomes were included in the analysis. Results were expressed as SCE/chromosome.

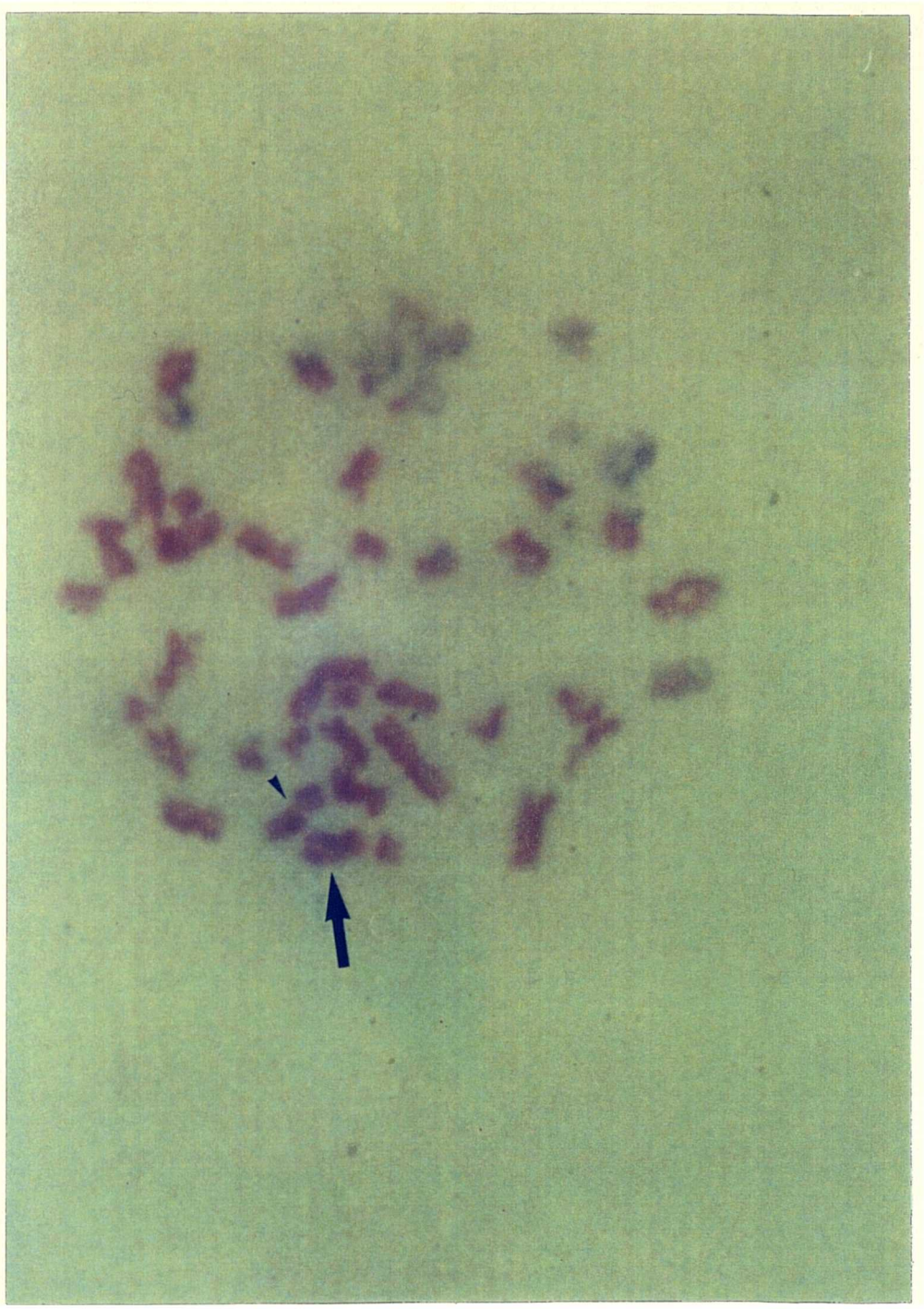
For evaluation of the replicative index was done in the first 100 metaphases, determining the portions of first (M_1), second (M_2) and third or more (M_3) mitotic divisions. The replicative index (RI) was given by the expression:

$$RI = \frac{(\#\text{of } M_1) + (2 \times \#\text{of } M_2) + (2 \times \#\text{of } M_3)}{M_1 + M_2 + M_3} \times 100$$

10.6.3 Characteristics of the Assay

The CV of the baseline SCE frequency of the assay was tested in group C1. Blood samples from the eight subjects in that group were obtained three times over a period of eight weeks at the development stages of the assay. The first two sets of samples were two weeks apart and the last set on

Figure : 10.14 Sister chromatid exchanges in human peripheral blood lymphocytes



Human chromosomes from a second division metaphase lymphocyte observed under light microscopy(magnification x 2500)

Note: SCEs arrowed

week number 8. The CV of the population's mean SCE frequency was < 5 %. Figure 10.15 shows the results obtained from this experiment. The CV of the population's mean RI replicative index was 6.0 %. Figure 10.16 shows the obtained from the three trials.

The variability in the SCE frequency scoring procedure was tested by one scorer scoring 1 slides 4 times. The CV was again < 5%.

Figure : 10.15

Individual and Mean ± SEM of SCE Frequency in PBLs of Departmental Staff Sampled Three Time in a Period of Eight Weeks

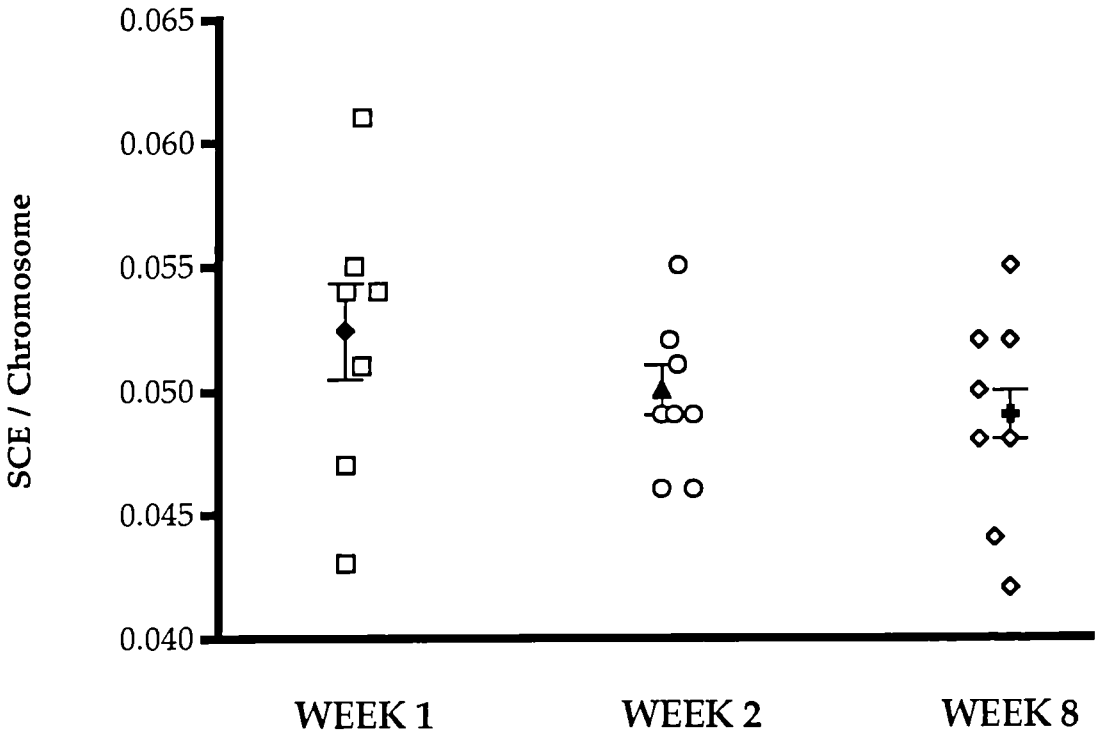
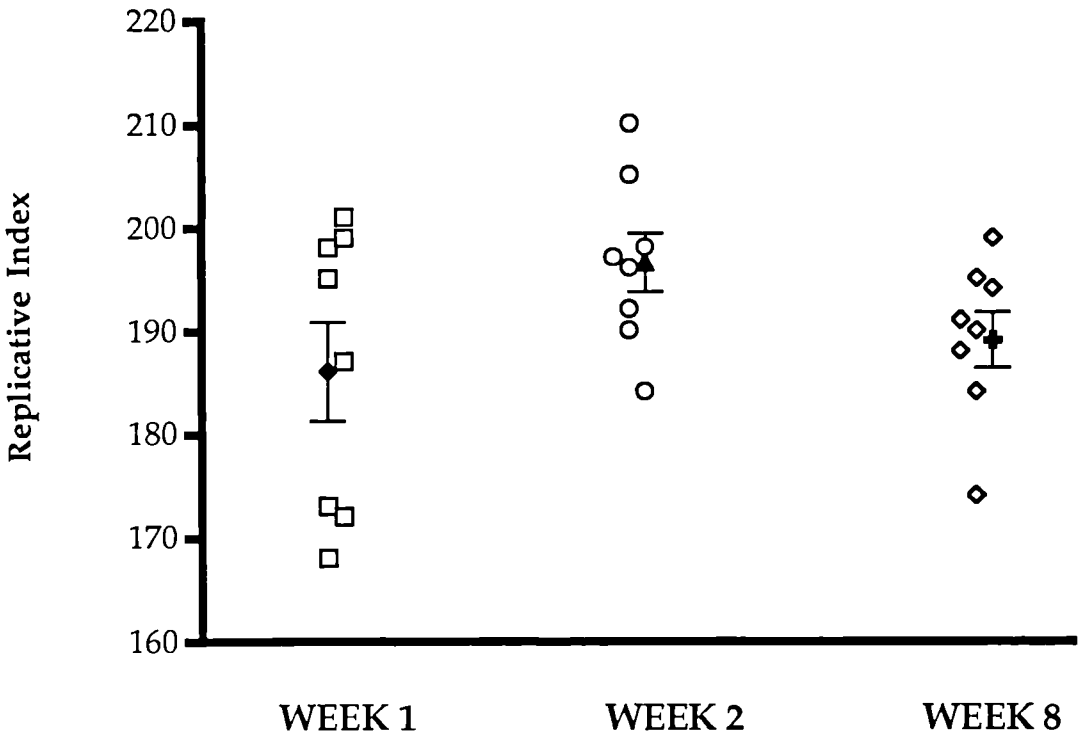


Figure : 10.16

Individual and Mean ± SEM of Replicative Index in PBLs of Departmental Staff Sampled Three Time in a Period of Eight Weeks



10.7 MEASUREMENT OF URINARY CREATININE

10.7.1 List of Material

1- Standard Creatinine (Sigma Chemical Co.)
2- Acetonitrile HPLC Grade (Fisons plc)
3- HCl (BDH)
4- $NH_4H_2PO_4$ (Fisons plc)
5- Column: Partisil 10 SCX (30 cm x 4.6 mm i.d.)
6- Guard Column: TG-P10SAX guard cartridge
7- HPLC/UV detector (Kontron plc)

10.7.2 Procedure

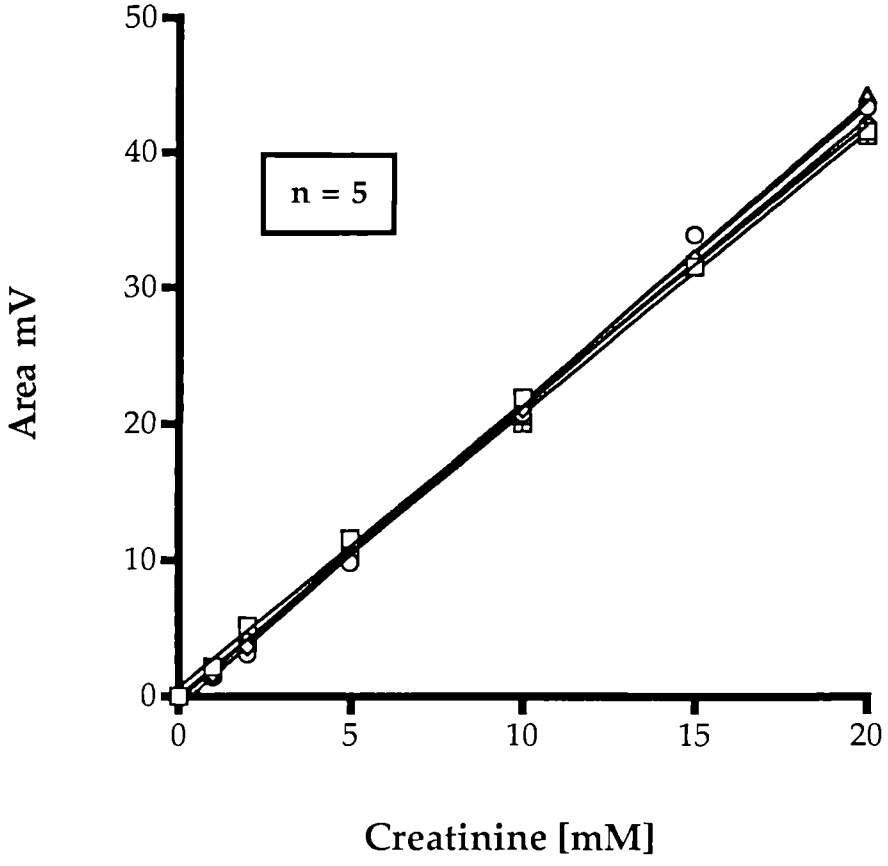
Creatinine concentrations were quantified using the reverse phase high performance liquid chromatography (HPLC) method of Huang and Chiou (1983), as modified by Muirhead *et al.* (1986). Ten µl of clear urine was diluted in 990µl of mobile phase ($NH_4H_2PO_4$ [20 mM] : acetonitrile, 9:1) and 20µl was automatically injected on to a Partisil SCX (25 cm x 4.6 mm i.d.) column fitted with a TG-P10SAX guard cartridge. The samples were eluted isocratically at a flow rate of 2 ml/min and detection was by UV absorbance at a wavelength of 254 nm. Peak areas were used to quantify creatinine concentrations in samples by comparison with standard curves.

10.7.3 Characteristics of the Assay

The CV of the assay from five standard at all concentrations (1, 2, 5, 10, 15 and 20 mM) was (18.5, 20.3, 6.5, 3.3, 3.1 and 3.0%) respectively. Figure 10.17 shows all five standard curve constructed. The CV of the slope of the curve was 3.3%. The correlation coefficient (r^2) of the standard curves ranged from 0.998 to 0.999. The y-intercept was not significantly different from zero at 95% confidence level. The detection limit of the assay was 1 mM of creatinine with a signal to noise ratio of 1:3. The retention time under the conditions of the assay was 7 minutes. Chromatogram is shown in see figure 10.18.

Figure : 10.17

Creatinine Assay Standard Curves
Constructed on Different Days



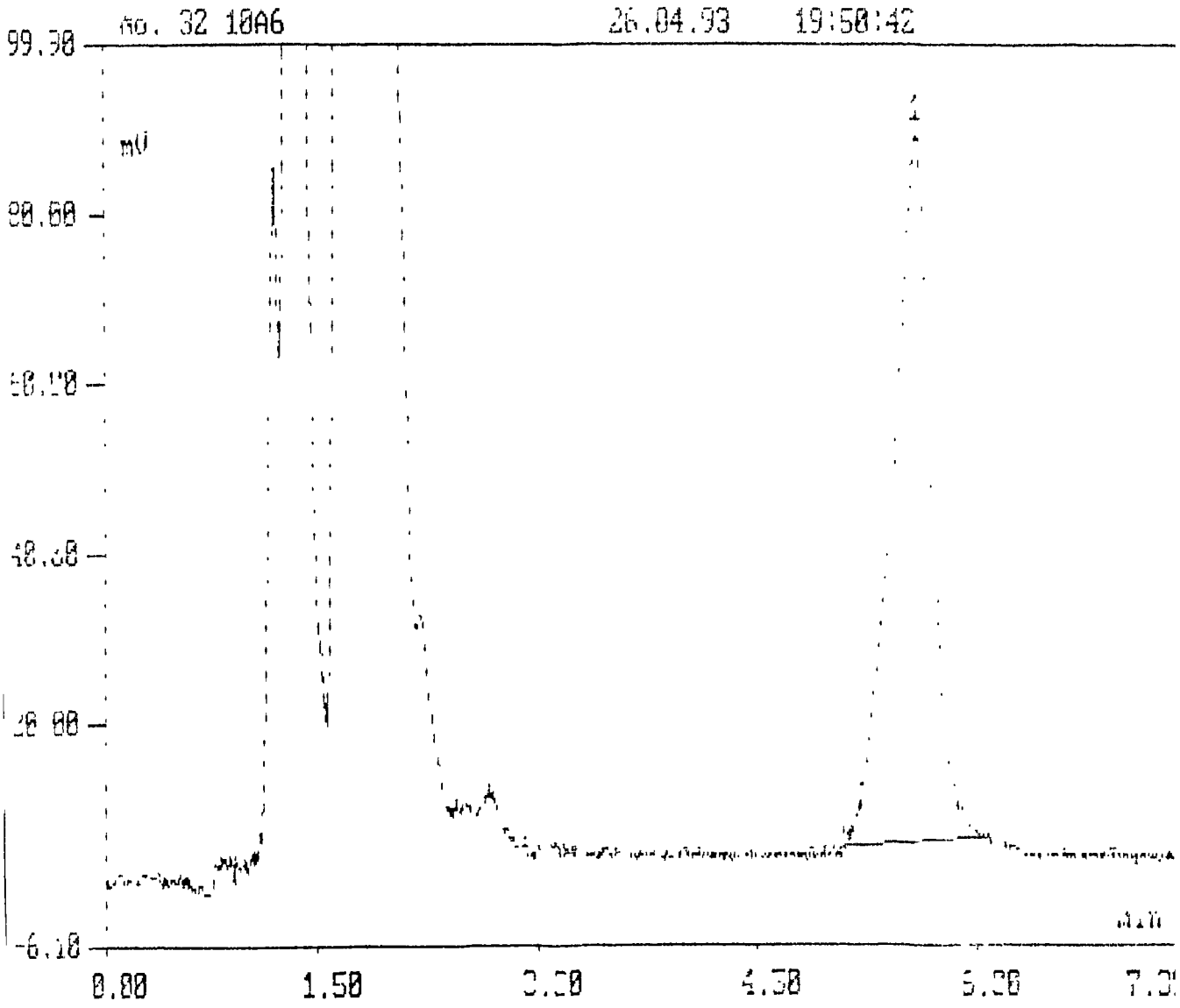


Figure : 10.18 HPLC chromatogram showing creatinine peak in a urine sample of a worker (creatinine peak # 1)

CHAPTER 11

STUDY DESIGN

11.1 STUDY POPULATION

The project proposal received approval from the local ethical committee prior to approaching any of the industries for volunteer recruitment. For this study 58 healthy male individuals were recruited. All participants were given a brief written description of the study. They all gave written informed consent which was also signed by a witness (Appendix I: study description and form). They were asked to complete a self-administered questionnaire (Appendix I: questionnaire). The information collected included demographic characteristics, smoking habits and history, alcohol and coffee consumption, diet, medication, alternative occupational exposure to chemicals or other hazards. Their job description was also defined from the questionnaire and this information was used to classify the population into exposed and control groups. None of the subjects used any respiratory protective equipment (RPE) or personal protective equipment (PPE).

The study was conducted in four different surveys A, B, C, and D described in detail below. The population groups recruited on each survey were subdivided into control and exposed groups as follows:

Group **C1**:	n=8;	university staff and students from our department.
Group **C2**:	n=6;	administrative office workers from an industry in the north east of England.
Group **M1**:	n=5;	manual workers non-occupationally exposed to PAHs
Group **M2**:	n=10;	manual workers non-occupationally exposed to PAHs
Group **P1**:	n=6;	pavers doing road maintenance by patching
Group **P2**:	n=10;	pavers resurfacing motorway
Group **R1**:	n=9;	roofers mastic asphalt roofing
Group **R2**:	n=4;	roofers mastic asphalt roofing

Groups **M1** and **M2** were pooled together and treated as one category of

workers called (M) for results analysis.

One volunteer was excluded from the study because of medication use. Eleven subjects mentioned that they do gardening at home, but did not specify any chemicals used (3 from each of groups P1, P2 and M and two from group R1). One individual of those who did gardening, from each of groups P1, P2 and M, was exposed to solvents from painting and the one from group P1 maintained his car in his spare time.

The manual workers (M), pavers (P1 & P2) and roofers (R1 & R2) started their work-shift around 7:00 am. and finished around 3:00 pm. while the departmental staff (C1) and office workers (C2) started at 9:00 am and finished at 5:00 pm.. All groups had a one hour lunch break.

11.2 SAMPLING SCHEME

11.2.1 Static and Personal Air Samples

Personal air monitoring was carried out for manual workers (M), pavers (P1 & P2) and roofers (R1 & R2) over the first three days of the surveys. Personal air monitoring was not carried out on three members of group M2 (2 laying insulation material and the warehouse keeper). Personal air sampling was carried out as described in heading 10.1.1 of chapter 10. Two ambient static samples were collected in the office building where group C2 worked and one sample in the department's main corridor on days 1, 2 and 3 of survey D to quantify background PAHs concentrations in the controls environment as described in part 10.1.1 of chapter 10.

11.2.2 Urine Sampling

All subjects except for group (C1) were asked to give pre- and post-shift urine samples over the first three days of the surveys. Samples in plastic bottles were coded and transported, within 1 hour to the laboratory in an opaque box. They were aliquoted into 30 ml plastic tubes and stored in four

batches at -70 °C pending analysis for UTh., 1-HP, UDGA, and creatinine. On day 4 of the survey the participants provided a morning urine sample (volume > 200 ml) in plastic bottles containing 10 ml glacial acetic acid. The samples were transported as others and stored at 4 -5 °C in a refrigerator for analysis of micronuclei in exfoliated cells within 24 hours.

All morning and afternoon urine samples of groups of manual workers both groups of pavers and roofers were voided between 6:00 and 9:00 am. and 2:00 and 3:00 pm. respectively. Those of the office workers were provided between 6:00 and 9:00 am and 3:00 and 4:30 pm..

11.2.3 Blood Sampling

On the morning of the fourth day of each of the surveys, blood samples were collected by venepuncture from subjects by a qualified phlebotomist. Two pavers from group P1 and two manual workers one from each of group M1 and M2 refused to give a blood sample. Cell culturing for SCE analysis was performed on the day of collection. For SCE analysis, on the fourth day, blood was drawn from an additional group of 8 male controls (departmental staff). This group was included in the study because the variability of their SCE frequency, from three determinations, had been previously determined to be acceptable (coefficient of variation <5%; see figure 8.8). All blood samples were stored in a cupboard at room temperature and re-cultured for SCE analysis the next day.

11.3 PROCESSES AND WORK CONDITIONS

The surveys studied in this thesis were all conducted in the district of Tyne & Wear in the north east of England between the months of March and November 1993. The prevailing weather conditions on each of the first 3 days on all the surveys are summarised in table 11.1. The surveys are summarised as follows:

Table : 11.1 Summary of the Weather Conditions on the First Three Days of Each Survey.

	Day Number (Date)	Air Temperature * °C	Relative Humidity * %	Wind Speed * m / sec	Sunshine Amount @ hr.
SURVEY A	1 (22 March 1993)	10.7 (2.9)	71.1 (10.3)	10.6 (3.7)	7.0
	2 (23 March 1993)	11.4 (2.0)	77.8 (4.4)	12.9 (2.0)	8.8
	3 (24 March 1993)	6.8 (2.1)	71.5 (4.5)	12.2 (3.2)	9.5
SURVEY B	1 (04 May 1993)	8.5 (2.3)	69.2 (9.9)	5.9 (1.7)	10.5
	2 (05 May 1993)	8.7 (0.8)	87.5 (11.7)	7.1 (1.0)	0.3
	3 (06 May 1993)	8.3 (0.9)	95.0 (5.8)	4.1 (3.0)	3.5
SURVEY C	1 (01 Nov. 1993)	8.9 (0.5)	74.6 (5.9)	6.6 (2.9)	nil
	2 (02 Nov. 1993)	8.8 (0.3)	75.8 (4.8)	8.5 (2.2)	nil
	3 (03 Nov. 1993)	9.0 (1.0)	85.5 (6.6)	7.4 (1.9)	nil
SURVEY D	1 (15 Nov. 1993)	4.9 (1.5)	78.8 (3.4)	6.3 (2.4)	3.7
	2 (16 Nov. 1993)	5.2 (0.8)	77.8 (10.3)	6.2 (1.8)	0.4
	3 (17 Nov. 1993)	3.7 (0.5)	88.0 (3.7)	10.2 (1.2)	5.2

* Data was obtained from Tynemouth Weather Centre (30 m Auxiliary Station)
Results of variables are mean (SEM) of the recordings every 3 hours between 00 and 24 GMT (see Appendix X for all individual recordings)

@ Data obtained from Newcastle Weather Centre (52 m Weather Centre Roof)

11.3.1 Survey A

Survey A was carried out on 4 days from March 22 to 25 (Monday to Thursday). Groups C1, M1 and P1 were recruited in this survey. Group C1 included 4 staff and 4 post-graduate students.

Group M1 included 5 manual workers 2 workers handling cement and 3 sewers maintenance workers. They were all not occupationally exposed to PAHs.

Group P1 containing 6 pavers were carrying out a road maintenance job by patching. The task was performed in couples. Each team of two went to a site. On day 1 and 2 Macadam (8 mm coarse base) was shovelled from the trucks into wheelbarrow and applied to patches on the road surface by shovels and spread by rakes. The trucks were fitted with covers. This reduced emission from asphalt. The same process was repeated on day 3 but Macadam (4 mm wear course) was applied. Pavers job description (e.g. raker, truck driver etc.) could not be specified because workers exchanged duties as needed.

11.3.2 Survey B

Survey B was carried out on 4 days from May 04 to 07 (Tuesday to Friday). Groups C1, M2 and R1 were recruited in this survey. Group C1 was exactly as in survey A.

Group M2 included 10 subjects with occupations as follows: 1 warehouse keeper, 3 site supervisors, 4 roofers doing work not involving bitumen (2 laying insulation material like rockwool, 1 slatter, 1 stripping lead) and 2 painting metal.

Group R1 included 9 roofers waterproofing a roof using felt (fibre rolled sheets) and hot oxidised grade bitumen. Each team of three roofers (1 kettleman/carrier, 1 mopman and 1 paperman) carried out the roofing operation on a site. The process involved laying felt and sealing it with hot bitumen. Briefly, the bitumen was heated in the kettles and the kettleman ladled hot bitumen in buckets which he then carried up the ladder onto the

roof and poured on the working area in front of the felt rolls. The mopman while kneeling down spread the bitumen using a trowel infront of the felt. The paperman bent and pushed hard and forward over the felt to unroll it and spread the bitumen underneath.

A direct subjective assessment of the process and working conditions was not possible due to the mobility of the working force and the lack of transportation facilities. The roofers shared the different tasks during the day consequently, it was not possible to define the job description of each worker. The operator had no means of controlling the temperature of the kettle; however, they reported to the author that the bitumen temperature was around 300 °C and the amount of fumes were considerable. Subjective assessment of the of the roofers hands on their return to the warehouse suggested that direct hand contact was minimal even without the use of protective gloves. The body surface seemed to be exposed mainly through atmospheric deposition (especially on day 1 of the survey when it was sunny and the roofers worked with light clothing on).

11.3.3 Survey C

Survey C was carried out on 4 days from November 01 to 04 (Monday to Thursday). Groups C1 and P2 were recruited in this survey. Group C1 was exactly as in survey A.

Group P2 was made up of 10 pavers executing a motorway resurfacing operation. The workforce included 1 foreman, 1 Paving Machine Driver "paver", 1 screwman, 1 tractor driver, 2 steel-tyre roller drivers, 4 rakers. The operation was a typical paving operation with 50 pen grade bitumen. A hot mixture of stone chips and bitumen (temperature ranging 160 - 180 °C) was delivered to the hopper of the paving machine (Blow-Knox) by the tractor. The paving machine was driven by the "paver" who was sitting 2 m above road level, by the tractor. The paving machine discharges the mix, with delivery temperature (150 - 160 °C), through the screw feed onto the road surface to a fixed width and level and partially compacted it to the required thickness of the pavement. The screwman operating the screw feed was located above the freshly laid asphalt. Two of the rakermen worked immediately behind the paving machine and manually corrected

imperfections and completed the edges on the newly laid asphalt layer (see fig. 11.1). The roller driver sitting at about 2 m above road level drove the a DC 13 roller at a distance of few meters behind the paving machine to fully compress the asphalt layer. Two rakers spread the mixture behind the roller. The foreman supervised all these operation.

On day 1 and 2 a coarse 50 pen bitumen base was applied with a mixture temperature of 160 - 170 °C and a delivery temperature of 150 °C. On day 3 a wear course 50 pen bitumen was applied with a mixture temperature of 170 - 180 °C and a delivery temperature of 160 - 170 °C. The author subjectively observed that the drivers of the tractor, roller and paver were exposed to bitumen and diesel fume. The screwman, rakers and foreman were mainly exposed to bitumen fume. Kerosine was used to stop the mixture from sticking to the paving machine hopper, consequently the workers could have been exposed to the kerosine fumes. Direct skin contact was limited. The traffic was not halted in the vicinity of the site which suggests exposure to a mixture of pollutants from car exhausts.

11.3.4 Survey D

Survey D was carried out on 4 days from November 15 to 18 (Monday to Thursday). Groups C1, C2 and R2 were recruited in this survey. Group C1 was exactly as in survey A.

Group C2 was a group of 6 administrative office workers who were not occupationally exposed to PAHs.

Group R2 was 4 roofers performing a mastic asphalt job on a roof of a 6 storey building (1 kettleman/mixer operator, 1 tipper, 2 trowellers). They used a mixture of 25 pen oxidised blown bitumen and fine mineral aggregate, in proportions that allows spreading the asphalt by hand when hot and which when cool formed a solid voidless impermeable layer. On the first half day of the survey the process involved applying a thin membrane of hot oxidised bitumen to the roof as a glass-fibre mats interply adhesive (see fig. 11.2a). The process was done as follows; the mixer operator heated bitumen in an open kettle from which he filled two buckets. The buckets were carried by the tipper who delivered the bitumen onto the roof to be spread by the trowellers

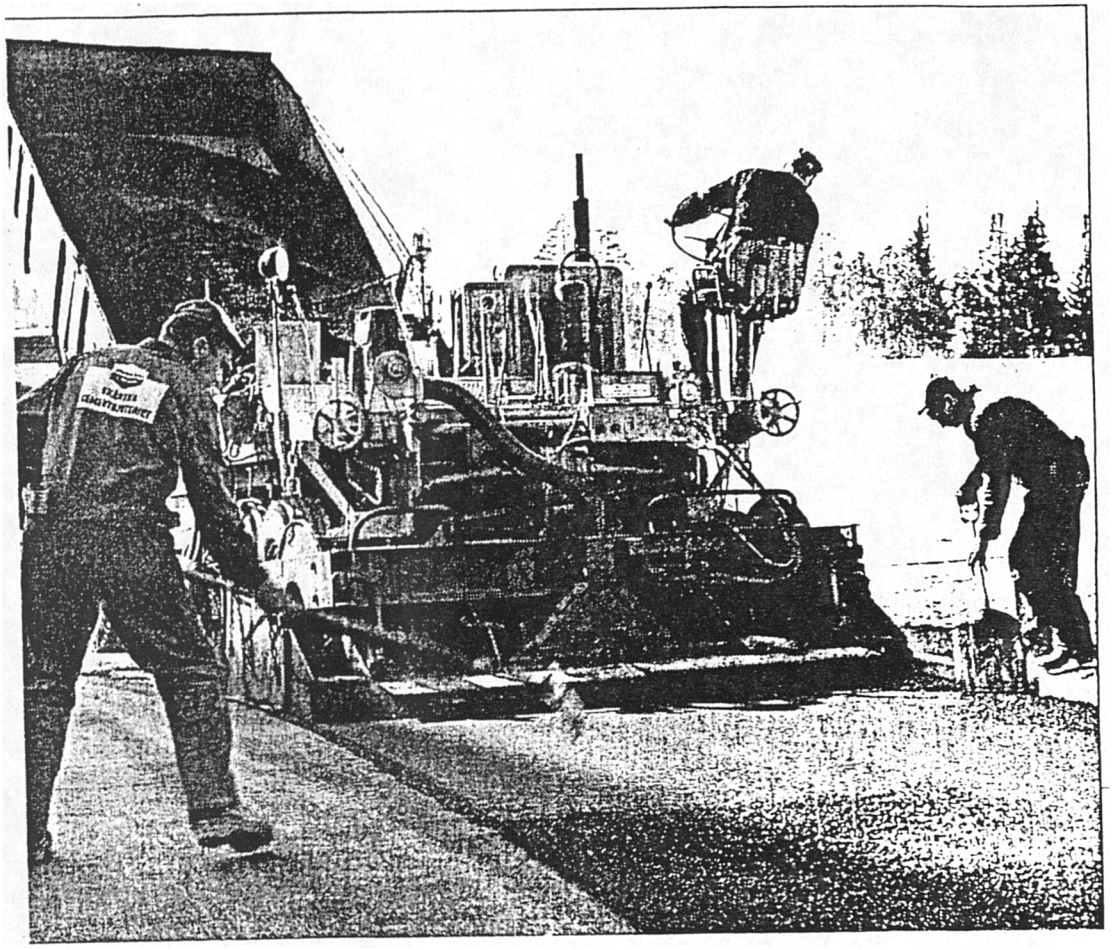


Figure : 11.1 Road Surfacing Process Similar to the One that Was Done in Survey C (One Paving Machine Driver and Two Rakers)

using brushes. The mastic asphalt process was carried out on days 2 and 3 as described. Mastic asphalt blocks were fed manually into a boiler (temp. 190 ^{o}C) by the mixer operator (see fig. 11.2b). He then emptied the hot material into two buckets which he had lined with cement dust to prevent sticking. He delivered the buckets to the tipper using a lift (see fig. 11.2c). The tipper emptied the buckets in a wheelbarrow and then poured the asphalt onto the insulation mats (see fig. 11.2d and 11.2e). The asphalt was then layered by trowellers by hand floating - a technique similar to that used for smoothing and levelling cement. Both trowellers while on their knees drew the freshly poured hot asphalt using wooden hand trowels (see fig. 11.2f). Finally, fine damp sand was thrown onto mastic layer as the bitumen was hardening by the trowellers.

The workers had specific job descriptions which they did on each day of the survey. The mixer operator was exposed to large amounts of fumes when feeding the boiler with asphalt blocks. This process was done three times per day. The tipper was exposed to bitumen fumes when pouring the hot material into the buckets and onto the roof. The trowellers had their heads over the freshly laid hot asphalt during the spreading process which left them virtually in a continuos plume of bitumen fumes (see fig. 11.2e and 11.2f). The workers did not wear protective gloves and direct skin contact with hot asphalt was higher than any of the other exposed groups monitored. Subjective assessment of exposure by observing the intensity of the emission and the position of the workers suggested the exposure of the roofers in this survey was higher than the pavers in survey C.

11.4 POPULATIONS GROUPING

For result analysis the groups were treated as follows: group C1 and C2 were handled separately althrough the statistical analysis of results. Groups M1 and M2 were treated as one group M and their results were dealt with as such althrough the statistical analysis. In the first phase of result analysis groups P1, R1, P2, and R2 were were dealt with separately. In the second phase, groups (P1 and P2) and (R1 and R2) were pooled into two main groups the "pavers (PV)" and "roofers (RF)", respectively. Again these were

dealt with separately. The population tree in figure 11.3 explains clearly how the population was divided into categories for result analysis and table 11.2 summarises the demographic variable, for each of the groups, collated from the questionnaires. Table 11.3 lists where available the specific job description of each volunteer in this study.

Figure : 11.2 a One of the Troweller Pointing at the Thin of Bitumen Film and the Glass-Fibre Mats Applied on Day One of Survey D

Figure : 11.2 b The Kettleman/mixer operator Feeding the Boiler With Mastic Asphalt Blocks

Figure : 11.2 c The Kettleman Has Emptied the Melted Mastic Asphalt in Buckets and Is Delivering Them Using the Lift Nearby

Figure : 11.2 d The Tipper is Delivering the Melted Mastic Asphalt to the Troweller Using the Wheelbarrow

Figure : 11.2 e The Tipper Emptying the Melted Mastic Asphalt From the Wheelbarrow on the Fibre-Glass Mats for the Trowellers to Spread

Note: See the Air Sampling Pump on the Back of Their Waist

Figure : 11.2 f The Two Troweller on Their Knees Spreading the Freshly Poured Hot Mastic Asphalt Using Wooden Hand Trowels

Table : 11.2 Summary of the Demographic Characteristics of the Sampled Populations

	AGE	SMOKING HABITS		COFFEE CONSUMPTION		ALCOHOL CONSUMPTION	
GROUP	Years mean ± SEM	Smokers # (~%)	# Cigarettes/day Median (Range)	Drinkers # (~%)	# Cups/Day Median (Range)	Drinkers # (~%)	Alc. Units/Week Median (Range)
Dept. Staff **C1** (n=8)	27.9 ± 1.9	2 (25)	10 (5 - 15)	8 (100)	3 (1 - 3)	7 (88)	20 (10 - 60)
Office Workers **C1** (n=6)	39.7 ± 4.9	3 (50)	15 (3 - 15)	5 (84)	6 (3 - 8)	4 (67)	35 (20 - 36)
Manual Workers **M** (n=15)	35.5 ± 3.2	4 (27)	15 (5 - 25)	10 (67)	4 (0.3 - 10)	12 (80)	28 (4 - 84)
Pavers (Patching) **P1** (n=6)	33.7 ± 3.1	2 (33)	15 (15 - 15)	4 (67)	2.5 (2 - 4)	4 (67)	35 (28 - 48)
Roofers **R1** (n=9)	32.8 ± 4.3	2 (22)	20 (15 - 25)	7 (78)	2 (1 - 8)	7 (78)	28 (14 - 84)
Pavers (Surfacing) **P2** (n=10)	35.0 ± 3.8	5 (50)	15 (8 - 25)	9 (90)	1 (1 - 5)	9 (90)	28 (14 - 70)
Roofers **R2** (n=4)	27.0 ± 3.2	3 (75)	15 (8 - 25)	4 (100)	3 (2 - 8)	4 (100)	24 (10 - 28)
Pavers (P1 & P2) **Pavers** (n=16)	35.1 ± 2.6	7 (44)	15 (8 - 25)	13 (81)	2 (1 - 5)	13 (81)	28 (14 - 70)
Roofers (R1 & R2) **Roofers** (n=13)	31.1 ± 3.1	5 (33)	15 (8 - 25)	11 (84)	2 (1 - 8)	11 (84)	28 (10 - 84)
Pavers & Roofers **Exposed** (n=29)	33.3 ± 2.0	12 (41)	15 (8 - 25)	24 (83)	2 (1 - 8)	24 (83)	28 (10 - 84)

Figure : 11.3

Population Tree

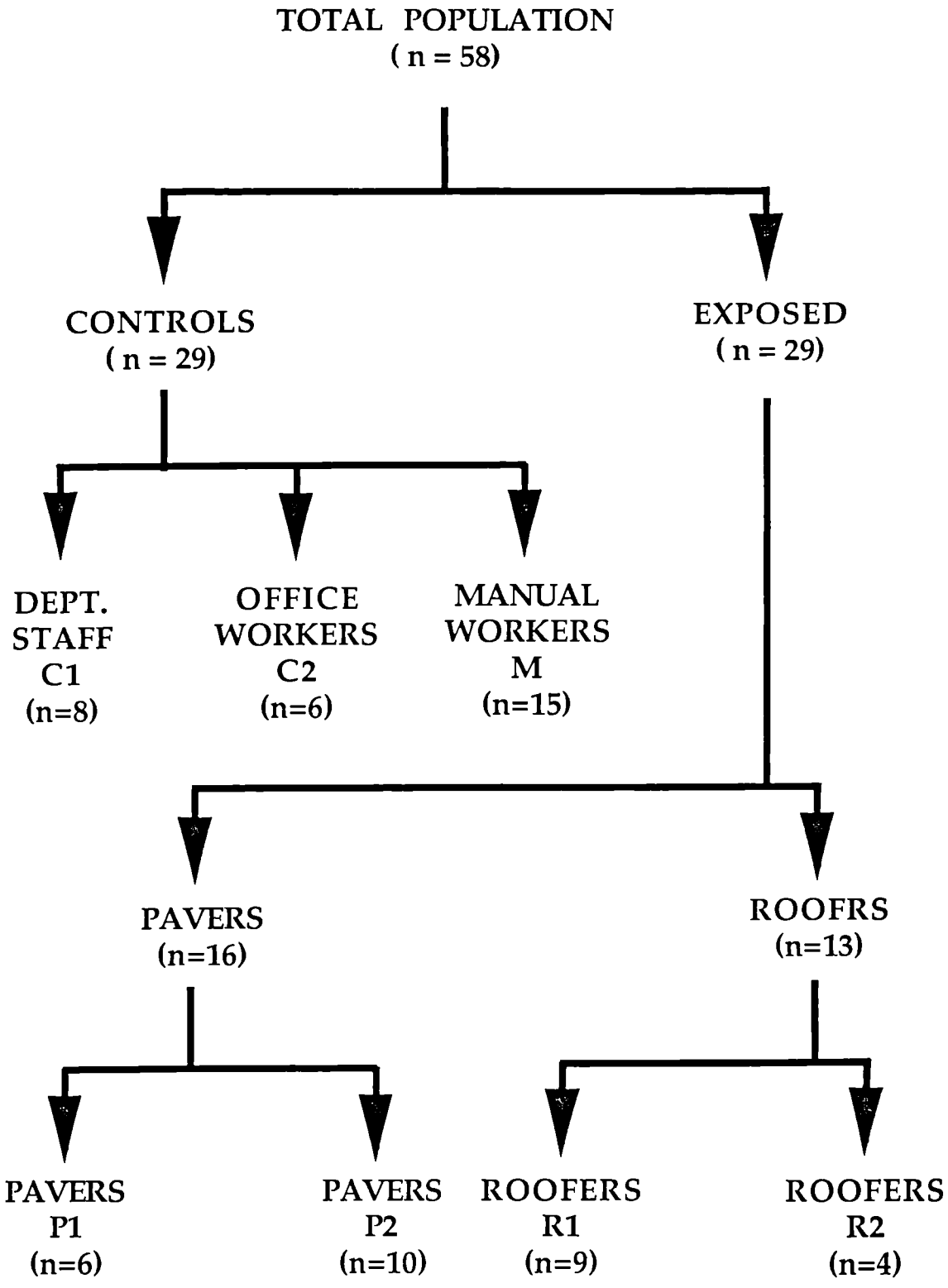


Table: 11.3 A list of all subjects and their job descriptions

Key:
Group M: Survey A &B
Group P1: Survey A
Group P2: Survey B
Group R1: Survey C
Group R2: Survey D

Manual Workers (Group M)	
Code	Job Description
70	Student
71	Student
72	Technician
73	Technician
74	Student
75	Lecturer
76	Student
77	Technician

Paver (Group P1)	
Code	Job Description
7	Paver *
8	Paver *
11	Paver *
13	Paver *
18	Paver *
19	Paver *

Roofer (Group R1)	
Code	Job Description
20	Roofer*
21	Roofer*
36	Roofer*
39	Roofer*
40	Roofer*
42	Roofer*
43	Roofer*
44	Roofer*
46	Roofer*

Roofer (Group R2)	
87	Mixer Operator
88	Troweller
89	Troweller
90	Tipper

Manual Workers (Group M)	
Code	Job Description
80	Office Work
81	Office Work
82	Office Work
83	Office Work
84	Sales Representative
86	Office Work

Manual Workers (Group M)	
Code	Job Description
10	Sewer Maintenance
14	Handling cement
15	Handling cement
16	Sewer Maintenance
17	Sewer Maintenance
30	Site supervisor
32	Sewer Maintenance
33	Site Supervisor
34	Site Supervisor
35	Slatter
37	Laying Insulation
38	Painting Metal
41	Painting Metal
45	Stripping Lead
48	Laying Insulation

Paver (Group P2)	
Code	Job Description
60	Raker
61	Raker
62	Raker
63	Tractor Driver
64	Screwman
65	Roller Driver
66	Foreman
67	Paver
68	Roller Driver
69	Raker

* Exact job description not specified

SECTION III

RESULTS AND DISCUSSION

CHAPTER 12

RESULTS OF

AMBIENT EXPOSURE TO PAHs

12.1 INTRODUCTION

The results of the ambient monitoring program in this study are presented in this chapter in three parts.

Part I: presents the results for the control air samples, the static air samples collected in the corridors of the departments where group C1 and C2 were working and the data collated from personal air samples collected in the breathing zone of the manual workers (groups M), groups of pavers (groups P1 and P2) and groups of roofers (groups R1 and R2).

The profile of 8-hour TWA concentrations (expressed in ng/m^3) of each PAH-species for each of the groups M, P1, P2, R1, and R2 will be presented separately in detail. The percentage of samples with detectable levels of all 9PAH-species will be given. Distribution of each PAH-species between the vapour phase and particulate matter was also determined. The median and range of the percentage contribution of each PAH-species to the sum of all nine PAHs are given calculated to the nearest 1 %.

The median and the range of airborne PAH-exposure indices (Σ9PAHs, Σ8PAHs or Σ4,5 ringed PAHs 8-hour TWA concentrations) will be reported for each group for all three days. A 3-day geometric mean of airborne PAH-exposure indices TWA concentrations will be calculated for each individual. They will be related to job classes and categories when possible. The differences in the means of the 3-day geometric mean data of TWA concentration of Σ9PAHs and Σ8PAHs will be compared between all groups using analysis of variance (ANOVA single factor). Then means of selected groups will be compared using the pooled standard deviation in an unpaired Student's t-test. In this thesis TWA concentration refer to an 8-hour TWA concentration.

Σ8PAHs refers to the sum of all PAHs excluding naphthalene while Σ4,5 ringed PAHs refers to those 4 & 5 ringed PAHs classified as animal or human carcinogens by IARC (see table 6.2).

Part II: presents the results from personal air samples of groups M, P1 and P2 pooled as "pavers" group (PV), and R1 and R2 pooled as

"roofers" group (RF). The data will be shown in the same way as in part I for the "pavers" and "roofers" group. This part addresses the effect of pooling the results of subgroups on the summary of the profile of PAHs as well as the airborns exposure to PAH-exposure.

Part III: presents the correlation between the logarithmic transformation TWA concentrations of the sum of both phases of naphthalene, phenanthrene, or pyrene with the sum of the TWA concentrations of the other Σ8PAHs (i.e. all PAHs less correlated PAH), other Σ7PAHs (i.e. all PAHs less naphthalene and correlated PAH) or Σ4,5 ringed PAHs in personal air samples collected for all groups on the three days of the survey. Similarly, correlation will be looked at in personal air samples of pooled groups.

The absolute data of the 9 PAHs analysed for, in both particulate and vapour form, in all personal air samples in this study are found in appendix II.

12.2 PART I:

12.2.1 Control and Static Air Samples

None of the control samples (filter or ORBO tube) analysed along with the personal air samples had detectable levels of any of the nine PAHs.

None of the static air samples collected on each of the first three days of survey D in the corridors of the buildings where groups C1 and C2 worked had detectable levels of any of the PAHs analysed for.

12.2.2 Manual Workers Group M

The manual workers were sampled on two occasions. Five workers (3 sewer maintenance workers and 2 handling cement) were

recruited in survey A and ten others (1 warehouse keeper, 2 site supervisors, 3 laying insulation material, 1 slatter, 1 stripping lead and 2 painting metal.) on survey B. Personal air samples were collected from the breathing zone of 12 workers on the first three days of the surveys. Three manual workers (M # 30, 32 and 48) in survey B were not included for personal ambient monitoring due to shortage in equipment availability. Thirty six samples were collected in total. Out of the total number of samples, 34 samples were analysed for PAHs content. Two samples (that of M # 10 and # 38 on days 2 and 3, respectively) were rejected due to pump failure. The filter head of the sample of M # 38 on day one was lost. PAHs content of this sample were analysed only on the ORBO tube and no distinction could be made between the vapour phase and particulate matter.

12.2.2.1 Profile of Two Ringed PAHs

Naphthalene was the most abundant PAH in the personal air samples on all days. It was detected in 91.2 % of the samples (table 12.1) and was found only in vapour form (table 12.2) except for sample of M # 34 on day 1 where it was < 1% in particulate form (appendix II). The median percentage of naphthalene in Σ9PAHs was at 90 % (fig. 12.1a) ranging from 6 to 100 %. If we consider that one sample (M # 41 on day 3) (Appendix II) with a percentage of 6 % is an outliers and exclude it from the analysis of the results then the range will be 55 to 100%. TWA concentrations of naphthalene ranged from n.d. to 9070 ng/m^3 with median of 207 ng/m^3 (fig. 12.2a), which was significantly lower than all other groups (Mann-Whitney U-test, non-adjusted for ties; $p < 0.05$) except R1 ($p > 0.05$).

12.2.2.2 Profile of Three Ringed PAHs

The three ringed PAHs were the second most abundant category of PAHs in personal air samples of group M. Acenaphthene and phenanthrene were detected in 70.6 % and 64.7 % of the samples, respectively (table 12.1). Acenaphthene was not detected in 6 samples out of 11 that had no detectable levels of phenanthrene. Acenaphthene was exclusively found in the vapour form trapped on the ORBO tube; while the median percentage distribution of phenanthrene was found to be 73 %

in vapour form and 27 % in particulate form ranging from 46 - 84 % and 16 - 54 %, respectively (table 12.2). The median percentage of acenaphthene in Σ9PAHs was 5 % ranging from < 1 - 34 %. Similarly, phenanthrene had a median percentage of 3 % ranging from < 1 - 44 % (fig. 12.1a). They had similar median TWA concentration profiles of {medain (range); 21.9 ng/m^3 (0 - 422)} for acenaphthene and {24.9 ng/m^3 (0 - 338)} for phenanthrene (fig. 12.2a). Median TWA concentrations for both 3 ringed PAHs in this group were significantly lower than those of groups P1, P2 and R2 but not R1 (Mann-Whitney U-test, non-adjusted for ties; $p < 0.05$).

12.2.2.3 Profile of Four Ringed PAHs

Four ringed PAHs were the third most common category of PAHs in personal air samples. Pyrene was detected in 20.6 % of the samples (7 out of 34) (table 12.1). Four of the 7 samples were those of M # 41 on days 1 and 3 and M # 45 on days 1 and 2. Both M # 41 and 45 worked on the roofs of houses performing jobs not including occupational exposure to PAHs (appendix II). The remaining three samples belonged to M # 16, 17 and 38 on day 1 who did sewer maintenance jobs (appendix II). The median percentage distribution of pyrene was found to be 24 % in vapour form and 76 % in particulate form ranging from < 1 - 66 % and 34 - 100 %, respectively (table 12.2). The median percentage of the TWA concentrations of pyrene in Σ9PAHs in all samples was < 1% ranging between < 1 % and 12 % (fig. 12.1a). TWA concentrations of pyrene ranged from n.d. - 164 ng/m^3 with a median of non-detectable levels (fig. 12.2a). Pyrene's TWA concentration for group M were significantly lower than that of group P1, P2, and R2, but not R1 (Mann-Whitney U-test, non-adjusted for ties; $p < 0.05$).

Benz(a)anthracene was detected in one sample (M # 41 on day 3). In that sample B(a)A was 37.0% in vapour form (table 12.2). The TWA concentration was 712 ng/m^3 contributing to 45.3 % of the total 9PAHs.

12.2.2.4 Profile of Five Ringed PAHs

B(b)F & B(k)F, B(a)P and DiB(a,h)A were detected in one sample belonging to M # 41 on day 3 (appendix II). In that sample, B(b)F & B(k)F

were found 28 % in vapour phase and 72 % in particulate matter (table 12.2). They contributed to 3 % of Σ9PAHs. Their TWA concentration was 45.0 ng/m^3. B(a)P was in particulate form only (table 12.2). It contributed to 8.3 % of the all 9PAHs. Its TWA concentration was 129.5 ng/m^3. DiB(a,h)A was found only as particulate matter (table 12.2), contributing to < 1 % of Σ9PAHs with a TWA concentration of 12.2 ng/m^3.

12.2.2.5 TWA Concentrations Of Airborne PAH-Exposure Indices

Σ9PAHs TWA concentrations: TWA concentrations of Σ9PAHs and in personal air samples were different on the three days of the survey. Day 1 had the highest median and range of TWA concentrations {1440 ng/m^3 (448 - 9190)}. Days 2 and 3 had TWA concentrations 13 and 23 fold lower than day 1 (table 12.3a). The CV of the median TWA concentrations was calculated at 140 %.

Σ8PAHs TWA concentrations: The median TWA concentration of Σ8PAHs was highest on day 1 (113 ng/m^3). This was 13.3 times higher than that on day 2 and 17.7 times that of day 3. The decreasing order in the Σ8PAHs TWA concentration between the first and last day was similar to that of Σ9PAHs. The variability of the external exposure to Σ8PAHs reflected by the CV (143%) of the median TWA concentration was similar to that of Σ9PAHs (table 12.3b).

Σ4,5 PAHs TWA concentrations: The median Σ4,5 PAHs TWA concentration was not detectable levels on all three days (table 12.3b).

12.2.2.6 PAHs External Exposure and Job Description (Group M)

All the individuals in this group are not occupationally exposed to PAHs; however they were exposed to measurable background levels.

Σ9PAHs TWA concentrations: Table 12.4a shows the job description of each manual worker and reports the Σ9PAHs TWA concentrations measured for each of them on the three days. The 3-day geometric mean (GM) for the Σ9PAHs TWA concentrations was highest

for the M # 45 925.4 ng/m^3. His external exposure was higher than two pavers in both groups P1 and P2 (table 12.4b) and eight roofers in group R1 (table 12.4c). The second highest Σ9PAHs TWA concentration GM of 795 ng/m^3 belonged to M # 41 who was painting metal on a roof. His external exposure was higher than one paver in groups P1 and one in group P2 (table 12.4b) and eight roofers in group R1 (table 12.4c). The site supervisors (M # 33 and # 34) had the lowest external exposure with 3-day GM of 147 ng/m^3 and 103 ng/m^3, respectively. The 3-day GM of all other workers ranged between 230 and 715 ng/m^3 which was very similar to range of 3-day GMs in group R1.

The average of the 3-day geometric means for the manual workers group (mean ± SEM; 448 ± 79.8) was lower than those of all other group except group R1 (table 12.4a, b and c).

Σ8PAHs TWA concentrations: Table 12.4a presents the 3-day GM of Σ8PAHs TWA concentration of individuals in group M with their job descriptions. The 3-day GM of Σ8PAHs TWA concentration was highest for the manual worker M # 41 (185 ng/m^3). His external exposure to Σ8PAHs was higher than 2 pavers in group P1, 4 pavers in group P2, 7 roofers in group R1 and 1 roofer in group R2 (tables 12.4b & 12.4c). Manual worker M # 15, # 16 and # 17, which had a 3-day GM for Σ9PAHs TWA concentration middle of the range in group M, had similar 3-day GMs which ranked fourth, third and second, respectively (table 12.4a). Their external exposure to Σ8PAHs was comparable to the low-exposed individuals in group P1, P2 and R2 (table 12.4b and c) but similar to most of those individuals in group R1 (table 12.4c). The 3-day GM for the external exposure of manual workers M # 33 and # 35 were not calculated since Σ8PAHs were only detectable in their personal air samples on day 1 (table 12.4a). The rest of the individuals in this group (M # 10, 14, 34, 37, 38 and 45) had relatively low exposure to Σ8PAHs with the highest GM at 45.0 ng/m^3 belonging to M # 38 (table 12.4a).

The average of the 3-day geometric means for the manual workers group (72.2 ± 16.1) was lower than those of all other group (table 12.4a, b and c).

Σ4,5 PAHs TWA concentrations: Only two samples had

detectable levels of Σ4,5 PAHs. That of manual worker number M # 16 on day 1 with a TWA concentration of 209 ng/m^3 and that of the M # 41 on day 3 (897 ng/m^3) (table 12.4a).

12.2.3 Pavers Group P1

This group was recruited on survey A. It included 6 pavers (P1 # 7, 8, 11, 13, 18 and 19) doing road maintenance by patching using Macadam. Job descriptions could not be defined due to the fact that each paver was carrying out more than one task. Eighteen personal air samples were collected and analysed for this group.

12.2.3.1 Profile of Two Ringed PAHs

As in group M, the two ringed PAHs represented by naphthalene was the most abundant compared to the other analysed PAHs. Naphthalene was detected only in vapour form on the ORBO tube (table 12.2) in all air samples collected on the three ambient monitoring exercises (table 12.1). The median percentage of naphthalene in the total of the 9PAHs was {84 % (40 - 93)} slightly lower than what was observed in group M however with a marginally wider range (fig. 12.1a). One sample - that of P1 # 11 on day 3 (appendix II) - with a median percentage of 19 % was considered an outlier and was excluded from the calculation of the median. This sample had the highest TWA concentration of phenanthrene and pyrene of all personal air samples in the above group. The median TWA concentration of naphthalene (1269 ng/m^3) was 6 fold higher in this group compared to group M (fig. 12.2a); while the range (69.1 - 4270 ng/m^3) was 1 fold lower.

12.2.3.2 Profile of Three Ringed PAHs

Three ringed PAHs, represented by acenaphthene and phenanthrene, were the second most abundant category of PAHs in the personal air samples collected for group P1. Both acenaphthene and phenanthrene were detected in 100 % of the samples (table 12.1). Acenaphthene was exclusively found in the vapour form trapped on the ORBO tube (table 12.2) except for one sample (P1 # 18 on day 1) where it

was found < 1 % in particulate form (appendix II). The median percentage distribution of phenanthrene was found to be 80 % in vapour form and 20 % in particulate form ranging from < 1 - 100 % for both phases (table 12.2). The median percentage of acenaphthene in Σ9PAHs was {8 % (1 - 42)} which was higher than in group M. Similarly, phenanthrene had a higher median percentage in this group {7 % (3 - 38)} (fig. 12.1a). The median TWA concentration of acenaphthene was {135 ng/m^3 (0 - 451)} which in this group was higher than that of phenanthrene {91.1 ng/m^3 (8.5 - 534)} (fig. 12.2a). The median TWA concentration for both acenaphthene and phenanthrene was 6 and 3.6 fold higher in this group in comparison to the manual workers group, respectively (fig. 12.2a).

12.2.3.3 Profile of Four Ringed PAHs

PAHs with four rings, represented in this study by pyrene and B(a)A were the third most common category of PAHs in personal air samples. Pyrene was detected in 50 % of the samples (table 12.1). Pyrene was detected in at least one of the personal air samples collected during the air monitoring sessions for each individuals. The median percentage of pyrene was found to be < 1 % in vapour form and 100 % in particulate form ranging from < 1 - 13 % and 87 - 100 %, respectively (table 12.2). The median percentage of the TWA levels of pyrene in Σ9PAHs in all samples was < 1% ranging between < 1 % and 3 % (fig. 12.1a). TWA concentrations of pyrene ranged from n.d. - 54.5 ng/m^3 with a median of 3.6 ng/m^3 (fig. 12.2a). This is an appreciable concentration when compared to non-detectable median level observed in group M.

Benz(a)anthracene, the other 4 ringed PAH analysed for, was detected in particulate form in one sample (that of P1 # 7 on day 1) (table 12.2) with a TWA concentration of 12.5 ng/m^3 (appendix II). It contributed to < 1 % of Σ9PAHs. That sample had the highest Σ9PAHs TWA concentration in group P1.

12.2.3.4 Profile of Five Ringed PAHs

Only one personal air sample (P1 # 7 on day 1) had detectable levels of B(b)F & B(k)F, B(a)P but not DiB(a,h)A (table 12.1). B(b)F & B(k)F and B(a)P were found trapped on the filter in particulate form (table 12.1)

and they separately contributed to < 1 % of Σ9PAHs. The TWA concentration for B(b)F & B(k)F and B(a)P was 12.1 and 12.9 ng/m^3, respectively (appendix II).

12.2.3.5 TWA Concentrations Of Airborne PAH-Exposure Indices

Σ9PAHs TWA concentrations: The CV of the median Σ9PAHs TWA concentrations in personal air samples for the three days of was 62.9 % (table 12.3a). Day 1 had the highest median and range of TWA concentrations {2720 ng/m^3 (271 - 5010)} of all three days. Days 2 and 3 had median Σ9PAHs TWA concentrations approximately 3 and 2.3 fold lower than day 1 (table 12.3a). The median TWA of group P1 was consistently higher than that of group M on all 3 days however with varying degrees. The highest difference recorded was on day 3 (19 fold) followed by (8 fold) on day 2 and (2 fold) on day 1 (table 12.3a).

Σ8PAHs TWA concentrations: The median Σ8PAHs TWA concentration was highest on day 1 {388 ng/m^3 (73.9 - 980)} (table 12.3). This was 2.1 and 1.7 fold higher than days 2 and 3, respectively. The CV (41.2 %) of the median Σ8PAHs TWA concentrations in personal air samples for the three days was smaller than what was calculated for Σ9PAHs (table 12.3b).

Σ4,5 PAHs TWA concentrations: The median Σ4,5 PAHs TWA concentration was not detectable levels on all three days (table 12.3c).

12.2.3.6 PAHs External Exposure and Job Description (Group P1)

For this group TWA concentrations of PAHs in personal air sample of pavers could only be related to each individual, but not to the task each of them handled. The reason for that is the variation in tasks performed during the working shift.

Σ9PAHs TWA concentrations: Table 12.4b reports the Σ9PAHs TWA concentrations measured for each of the pavers in group P1 on the three days of survey A. The 3-day geometric mean for the Σ9PAHs TWA concentration was highest for the paver P1 # 7 (3347 ng/m^3). His 3-day GM was 4.2 folds higher than that of paver P1 # 11 who worked with him at

the same sight (table 12.4b). The second highest 3-day GM was that of paver P1 # 13. His 3-day GM was ~ 9 folds higher than that of paver P1 # 19 who worked with him at the same sight (table 12.4b). The third highest 3-day GM was that of paver P1 # 8. His was ~ 1.6 folds higher than that of paver P1 # 18 who worked with him at the same sight (table 12.4b).

The average of the 3-day geometric means of group P1 (1584 ± 454) was higher than that of group M, R1 and RF (table 12.4a, b and c); however, the difference was not statistically significant (unpaired Student's t-test; $p<0.05$).

Σ8PAHs TWA concentrations: Table 12.4b presents the 3-day GM of Σ8PAHs TWA concentration of pavers in group P1. As in the case of Σ9PAHs the 3-day GM of Σ8PAHs TWA concentration was highest for the paver number P1 # 7 (623 ng/m^3). The second highest 3-day GM of Σ8PAHs TWA concentration (319 ng/m^3) was that of paver P1 # 11 who worked on the same sight as P1 # 7. Pavers P1 # 8 and # 18 who worked on the same sight had a 3-day GMs less than P1 # 7 and # 11, but higher than P1 # 13 and # 19 who also worked together (table 12.4b).

The average of the 3-day geometric means of group P1 (285 ± 79.0) was higher than that of group M, R1 and RF (table 12.4a and b); however, the difference was not statistically significant (unpaired Students T-test; $p<0.05$).

Σ4,5 PAHs TWA concentrations: Only one sample had detectable levels of Σ4,5 PAHs. That of paver number P1 # 7 on day 1 with a TWA concentration of 36.5 ng/m^3 (table 12.4b). This paver had the highest Σ9PAHs and Σ8PAHs TWA concentrations amongst personal air samples of all pavers in group P1 on all days.

12.2.4 Pavers Group P2

This group was recruited on survey C. It included 10 pavers (P2 # 60 to 69) resurfacing a motorway using 50 pen grade bitumen. Job descriptions could be defined in this group because each individual handled the same task during the whole period of the survey. The job

description is listed for all individuals in table 12.4b. For this group 30 personal air samples were collected and analysed for all 9PAHs.

12.2.4.1 Profile of Two Ringed PAHs

Once more the 2 ringed category of PAHs represented by naphthalene had the highest median TWA concentration compared to the other analysed PAHs (fig. 12.2a). Naphthalene was detected only in vapour form on the ORBO tube (table 12.2) in 96.7 % of the personal air samples collected on the three ambient monitoring sessions (table 12.1). The median percentage of naphthalene in the total of the 9PAHs was {88 % (43 - 99)} slightly higher than what was observed in group P1 (fig. 12.1a); however with an approximately equal spread. One sample - that of P2 # 65 on day 1 - with a median percentage of naphthalene in all 9PAHs (< 1 %) was considered an outlier and was excluded from the calculation of the median. This sample had the lowest TWA concentration of Σ9PAHs among all personal air samples in this group of pavers. The median TWA concentration of naphthalene (1450 ng/m^3) was slightly higher than that of group P1 (fig. 12.2a) while the range (n.d - 28600 ng/m^3) ~ 7 fold bigger. This wide range was 3.2 times bigger than group M.

12.2.4.2 Profile of Three Ringed PAHs

As in group P1, PAHs with three rings like acenaphthene and phenanthrene, in personal air samples of group P2 were the second most common category of PAHs. Acenaphthene and phenanthrene were detected in all the personal air samples (table 12.1). Acenaphthene was exclusively found in the vapour form trapped on the ORBO tube except for two sample (P2 # 67 and # 68 on day 3) where it was found at 5 and 23 % in particulate form, respectively (table 12.2). The two samples had TWA concentration for acenaphthene higher than its median TWA concentration in all samples reported below. Sample P2 # 67 had the highest recorded TWA concentration (701 ng/m^3); while the TWA concentration of sample P2 # 68 (194 ng/m^3) was 3 fold higher than the median. The median percentage distribution of phenanthrene was found to be 72 % in vapour form and 28 % in particulate from ranging from 19 - 100 % and < 1 - 81%, respectively (table 12.2). The median percentage of acenaphthene in Σ9PAHs was 4 % ranging from 0 - 49 % (fig. 12.1a).

Phenanthrene had a higher median percentage of 7 % ranging from 1 - 51 % (fig. 12.1a). The median TWA concentration of acenaphthene was {60.4 ng/m^3 (12.0 - 701)} which in this group was lower than that of phenanthrene {73.2 ng/m^3 (17.6 - 2590)} (fig. 12.2a).

12.2.4.3 Profile of Four Ringed PAHs

Again, the four ringed PAHs were the third most common category of PAHs in personal air samples. Pyrene was detected in 70 % of the samples (table 12.1). None of the individuals had no detectable level of pyrene in his personal air sample on all of the air monitoring sessions. Pyrene was found to be almost exclusively in particulate matter form (table 12.2). In only, two samples (that of P2 # 66 foreman and # 68 steel-wheel roller on days 1 and 2, respectively) pyrene was found solely in vapour form and in "abnormally" high TWA concentration of 841 and 822 ng/m^3, respectively. These two samples were classified as "abnormal" because they had TWA concentrations of Σ9PAHs comparable to the median of the group. The median percentage of the TWA concentration of pyrene in Σ9PAHs in all samples was < 1% ranging between < 1 % and 58 % (fig. 12.1a). If the "abnormally" high samples were excluded from the analysis the range will be < 1 to 2 % which is similar to that which was observed in group P1. TWA concentrations of pyrene ranged from n.d. - 840 ng/m^3 with a median of 7.0 ng/m^3. If the "abnormally" high samples were considered as outliers and ruled out from the analysis of the data then the range for pyrene's TWA average will be from n.d. to 302.2 ng/m^3 with a median of 6.6 ng/m^3. Although; 302 ng/m^3 pyrene's TWA concentration in sample P2 # 67 on day 3 is 46 fold higher than pyrene's median TWA concentration in all samples it was not considered abnormal since the TWA concentration of Σ9PAHs in this sample (32339.8 ng/m^3) was 18 fold higher than median of the group on day 3 (table 12.3a). The median TWA concentration of pyrene observed in this group of pavers is roughly twice as high as that calculated for group P1 (fig. 12.2a).

Benz(a)anthracene, was detected in 3 samples (that of P2 # 62, 63, and 67 on days 3, 1 and 2, respectively) with a TWA concentration of 12.5 ng/m^3 for the former two and 69.7 ng/m^3 for the latter. The sample with highest TWA concentration of B(a)A had the maximum recorded TWA concentration of the Σ9PAHs in the whole group (appendix II).

12.2.4.4 Profile of Five Ringed PAHs

B(b)F & B(k)F and B(a)P were detected in the personal air samples of the driver of the paving machine (P2 # 67) on day 3 (appendix II). That sample had the highest TWA concentration of Σ9PAHs of 32339.8 ng/m^3. DiB(a,h)A levels were lower than the detection limit of the assay in all samples.

12.2.4.5 TWA Concentrations Of Airborne PAH-Exposure Indices

Σ9PAHs TWA concentrations: It was evident that the variability in the TWA concentrations of Σ9PAHs in personal air samples on the three days of the survey in this group of pavers (P2) was similar to that of group P1 but not M. This was reflected in a similar CV of 59.5 % (table 12.3a). Day 3 had the highest median and range of Σ9PAHs TWA concentrations {2230 ng/m^3 (394 - 32300)} of all three days. Days 1 and 2 had a median Σ9PAHs TWA concentrations approximately 4.5 and 1.1 fold lower than day 3 (table 12.3a). Table 12.3a shows that the range of the median TWA of group P2 observed over the three day period was larger than those observed in group P1.

Σ8PAHs TWA concentrations: Day 3 had the highest median and range of Σ8PAHs TWA concentrations {370 ng/m^3 (39.4 - 3730)} followed by day 2 and day 3 (table 12.3b). The CV (78.2 %) of the median Σ8PAHs TWA concentration was similar to what was calculated for Σ9PAHs. It was nearly half the CV of group M.

Σ4,5 PAHs TWA concentrations: The median of Σ4,5 PAHs TWA concentration was at not detectable levels on all three days (table 12.3c).

12.2.4.6 PAHs External Exposure and Job Description (Group P2)

Σ9PAHs TWA concentrations: Table 12.4b shows the job description of each paver and reports the Σ9PAHs TWA concentrations measured for each of them on the three days. The 3-day geometric mean for the Σ9PAHs TWA concentrations was highest for the paver driving the Blow Knox machine 5740 ng/m^3. The screwman had the third highest

3-day GM of Σ9PAHs TWA concentration 3050 ng/m^3. The tractor driver, the foreman and one of the steel-tyre roller drivers (P2 # 68) had comparable 3-day GMs. Roller driver (P2 # 65) had the lowest 3-day GM among all of group P2 461 ng/m^3. It must be noted that his TWA concentration for Σ9PAHs on day 1 was lowest among all the samples (see range in table 12.3a). It was 8 fold lower than the second lowest recorded TWA concentration belonging to raker (P2 # 61) on day 1 reported in table 12.4b. Moreover, it was ~ 11 fold lower than the median of TWA concentration in all samples reported in table 12.3a. If this sample was considered as an artefact of sampling or analysis and excluded from the results, then the 3-day GM of TWA concentration for the roller driver will be 1460 ng/m^3 which is similar to the other roller driver (P2 #s 68). Three (P2 #s 60, 61 and 62) out of four rakers had similar Σ9PAHs TWA concentration profiles were day 1 < day 2 < day 3. The 3-day GM of Σ9PAHs TWA concentration for their air samples were 1830, 907 and 1300 ng/m^3, respectively. They all worked at a distance from the paving machine and had exposures similar to that of the foreman, tractor driver and steel-tyre roller driver number (P2 # 68). The GM of Σ9PAHs TWA concentrations (3570 ng/m^3) for raker number P2 # 69, who worked just behind the machine, was higher than the other rakers and similar to those recorded for the screwman.

The average of the 3-day geometric means of group P2 (2100 ± 502) was the highest among all groups. It was only significantly different from that of group M and R1 (unpaired Student's t-test; $p<0.05$) (table 12.4a, b and c).

<u>Σ8PAHs TWA concentrations</u>: Table 12.4b presents the 3-day GM of Σ8PAHs TWA concentration of individuals in group P2 with their job descriptions. As in the case of Σ9PAHs the 3-day GM of Σ8PAHs TWA concentration was highest for the driver of the paving machine (576 ng/m^3). The screwman's 3-day GM of Σ8PAHs TWA concentration was 268 ng/m^3 which was almost identical to the driver of the steel-tyre roller (P2 # 68) and the foreman. The other roller driver (P2 # 66) had the lowest 3-day GM of the TWA concentration of Σ8PAHs 102 ng/m^3. The 3-day GM of Σ8PAHs TWA concentration was 211 ng/m^3 for the tractor driver. All four rakers had more or less similar 3-day GMs for their Σ8PAHs TWA

concentrations with the lowest being 115 ng/m^3 and the highest 187 ng/m^3.

The average of the 3-day geometric means of group P2 (223 ± 44.6) was significantly higher than group M and R1 (unpaired Student's t-test; $p<0.05$) (table 12.4a and b). All those of other groups were higher than that of group P2.

Σ4,5 PAHs TWA concentrations: Only three samples had detectable levels of Σ4,5 PAHs. That of raker number P2 # 62 on day 3 and the tractor driver on day 1 with a TWA concentration of 12.5 ng/m^3 and the highest observed for the driver of paving machine on day 3 (126.8 ng/m^3) (table 12.4b).

12.2.5 Roofers Group R1

This group of roofers was monitored on survey B. It included 9 roofers (R2 # 20, 21, 36, 39, 40, 42-44 and 46) water proofing a roof using fibre rolled sheets and oxidised grade bitumen. Job descriptions could not be defined in this group because each individual carried out different task on and between different days of the survey. Roofers number (R1 # 20 and 21) had no personal air samples on day 1 of the survey due to shortage in available equipment. A total of 25 personal air samples were taken and analysed for this group.

12.2.5.1 Profile of Two Ringed PAHs

Naphthalene, in personal air samples of group R1, had the highest median TWA concentrations compared to the other analysed PAHs (fig. 12.2a). As in the previously described groups it was detected only in vapour form on the ORBO tube (table 12.2) in all personal air samples collected on the three ambient monitoring sessions (table 12.1). Naphthalene was not detected in two personal samples those of (R1 # 43 and 46 on day 3). These two samples had a distinctly different distribution of PAHs in comparison to all other samples collected in all surveys (appendix II). The PAHs with the highest TWA concentration in both samples were the four ringed PAHs (pyrene and B(a)A) contributing 17

and 48 % to the Σ9PAHs in the former sample and 14 and 43 % in the latter sample. The sum of the percentages of both 4 ringed PAHs was the highest among all air samples collected in all the surveys. The median percentage of naphthalene in the total of the 9PAHs {80 % (0 - 100)} was slightly lower than what was observed in both groups of pavers (fig. 12.1a). The median TWA concentration of naphthalene (258 ng/m^3) was similar to that of group M (fig. 12.2a); however with a range (n.d. - 3130 ng/m^3) 3 fold smaller than group M.

12.2.5.2 Profile of Three Ringed PAHs

Phenanthrene and acenaphthene were respectively the second and third most abundant PAHs in air samples collected in this survey. Acenaphthene was not detected in 3 samples out of 25 while phenanthrene level was below the detection limit in two samples (table 12.1). Acenaphthene was exclusively found in the vapour form trapped on the ORBO tube (table 12.2). The median percentage distribution of phenanthrene was found to be 83 % in vapour phase and 17 % in particulate form ranging from < 1 - 100 % for both forms (table 12.2). The median percentage of acenaphthene in Σ9PAHs was 5 % ranging from < 1 - 13 % (fig. 12.1a). As in group P1 and P2 phenanthrene had a higher median percentage of 9 % ranging from < 1 - 32 % (fig. 12.1a). The median TWA concentration of acenaphthene was {10.3 ng/m^3 (n.d. - 355)} which was lower than group M (fig. 12.2a); however with a similar range. On the other hand, phenanthrene's median TWA concentration {(22.0 ng/m^3 (0 - 588)} was quite similar to that of group M (fig. 12.2a) with a slightly wider spread.

12.2.5.3 Profile of Four Ringed PAHs

Once more we observed that the four ringed PAHs were the third most common category of PAHs in personal air samples. Pyrene was detected in 56 % of the samples (table 12.1). Not all of the individuals had detectable levels of pyrene in their personal air sample. Roofers (R2 # 20 and 39) exposure to pyrene was below detectable levels on all three days. Pyrene was found exclusively in the form of particulate matter (table 12.2). The median percentage of the TWA concentration of pyrene in Σ9PAHs in all samples was 1% ranging between < 1 % and 17 % (fig. 12.1). TWA

concentrations of pyrene ranged from n.d. - 292 ng/m^3 with a median of 8.8 ng/m^3 (fig. 12.2a). Pyrene's median TWA concentration and range were very similar to that of group P2.

Benz(a)anthracene, was detected in 48 % of the samples which was high when compared to those of group M, P1 and P2 (table 12.1). It was detected in only those samples with detectable levels of pyrene (appendix II). It was never found in the vapour form (table 12.2). Its median percentage distribution is < 1 % ranging from < 1 to 48 % (fig. 12.1a). Two samples that of (R1 # 43 and 46 on day 3) had 43 and 48 %, respectively. These are the "abnormal" two samples which had no detectable levels of naphthalene. The median TWA concentration was n.d. (fig. 12.2a) with a range of n.d - 50.7 ng/m^3.

12.2.5.4 Profile of Five Ringed PAHs

B(b)F & B(k)F were detected in 16 % of the personal air samples (table 12.1). More specifically, they were detected in those samples belonging to roofers number R1 # 40, 42, 43 and 44 on day 1. All samples with detectable B(b)F & B(k)F had B(a)P present in them. B(a)P was detected in 28 % of the samples (table 12.1). DiB(a,h)A was higher than the detection limit in only one sample (R1 # 40 on day 1) (table 12.1). That was one of the only two sample in all the surveys with detectable quantities of this PAH. Five ringed PAHs were only found in the particulate form (table 12.2). They all had a median percentage distribution in Σ9PAHs of < 1% (fig. 12.1a) with ranges of 0 - 14, 0 - 13 and 0 - 45 for B(b)F & B(k)F, B(a)P and DiB(a,h)A, respectively. All those samples with measurable amounts of 5 ringed PAHs had detectable levels of both 4 ringed PAHs.

12.2.5.5 TWA Concentrations Of Airborne PAH-Exposure Indices

<u>Σ9PAHs TWA concentrations</u>: There was marked inter-day variability reflected in the CV (127 %) of the median of Σ9PAHs TWA concentrations of PAHs in personal air samples in this group of roofers (table 12.3a). This variability was only comparable to group M. As in groups M, day 1 had the highest median and range of TWA concentrations {1500 ng/m^3, (815 - 2340)}. Days 2 and 3 had Σ9PAHs TWA concentrations

approximately 6 and 19 fold lower than day 1 (table 12.3a). Median Σ9PAHs TWA concentrations for this group of roofers was lower than those of both groups of pavers; however, they were comparable with those of group M (table 12.3a). The ranges of Σ9PAHs TWA concentrations were much smaller than group P1 and at least 4 fold smaller than those of group P2 but comparable to group M (table 12.3a).

Σ8PAHs TWA concentrations: The Σ8PAHs TWA concentrations on day 1 had the highest recorded median of 271 ng/m^3. It was ~ 12 fold higher than day 2 and 4.7 fold than that of day 3 (table 12.3b). A CV of 114 % was calculated for the median TWA concentration of Σ8PAHs from the three days. This CV reflected variability in the external exposure of this group similar to group M but more than both groups P1 and P2.

Σ4,5 PAHs TWA concentrations: The median TWA concentration of Σ8PAHs for day 1 and 2 was n.d. Only day 3 had a median of {25.0 ng/m^3 (n.d. - 48.6)} (table 12.3c).

12.2.5.6 PAHs External Exposure and Job Description (Group R1)

For this group TWA concentrations of PAHs in personal air sample of roofers could only be related between individuals, but not to the job description of individuals. The reason for that is the variation in tasks performed during the working shift.

Σ9PAHs TWA concentrations: Table 12.4c reports the Σ9PAHs TWA concentrations measured for each of the roofers in group R1 on the three days of survey B. The 3-day geometric mean for the Σ9PAHs TWA concentrations was highest for the roofer R1 # 21 (1100 ng/m^3). His 3-day GM was 10 folds higher than paver R1 # 20 who worked with him at the same sight (table 12.4c). The R1 # 20 had the lowest GM in group R1. The second highest 3-day GM (789 ng/m^3) was that of roofer R1 # 36. All other roofers in this group had GMs ranging from (220 - 497 ng/m^3) which were similar to those of the manual workers in group M.

The average of the 3-day geometric means of group R1 (442 ± 109) was the lowest among all groups including group M (table 12.4a, b and c).

Σ8PAHs TWA concentrations: Table 12.4c presents the 3-day GM of Σ8PAHs TWA concentration of all personal air samples of roofers in group R1. Roofer number R1 # 36, who had the second highest 3-day GM for Σ9PAHs TWA concentration had the highest 3-day GM (225 ng/m^3) for Σ8PAHs TWA concentration. On the other hand, roofer number R1 # 21, who had the highest GM for Σ9PAHs TWA concentration had the second highest GM (197 ng/m^3) for Σ8PAHs (table 12.4c). The 3-day GMs of roofer # 40, 42 and 46 were in the middle range of group R1 which was similar to the middle range of group M (table 12.4c). The roofers (R1 # 20, 39, 43 and 44) with the lowest exposure to Σ8PAHs had 3-day GMs ranging between 18.4 - 65.5 ng/m^3.

The average of the 3-day geometric means of group R1 (107 ± 24.7) was the lowest among all groups excluding group M (table 12.4a, b and c).

Σ4,5 PAHs TWA concentrations: Although the profile of the external exposure of group R1, reflected in the 3-day GMs of TWA concentrations of Σ9PAHs and Σ8PAHs, was similar to that of group M; they were exposed to higher levels of Σ4,5PAHs. Roofer (R1 # 36, 40, 43 and 46) had 3-day GMs for Σ4,5PAHs ranging from 23.2 - 43.1 ng/m^3 (table 12.4c). Roofer (R1 # 21, 42 and 44) had one personal air sample with detectable levels of Σ4,5PAHs (table 12.4c). Only roofers (R1 # 20 and # 39) had no detectable levels of Σ4,5PAHs in any of their personal air samples. The GMs of Σ4,5PAHs TWA concentration in this group was higher than that of group M, P1, and P2 but not R2.

12.2.6 Roofers Group R2

This group of roofers was monitored on survey D. It included 4 roofers (R2 # 87, 88, 89 and 90) water proofing a roof using glass-fibre mats and mastic asphalt. Job descriptions were defined in this group because each roofer performed the same task during the whole period of the survey. The job description are listed for all individuals in table 12.4c. Personal air samples (n=12) were collected and analysed for 9PAHs for this group.

12.2.6.1 Profile of Two Ringed PAHs

Similar to all other groups naphthalene had the highest median percentage distribution among 9PAHs (fig. 12.1a) and the highest median TWA concentration of all other analysed PAHs (fig. 12.2a). Again, it was detected in all personal air samples (table 12.1) only in vapour form on the ORBO tube (table 12.2). The median percentage and range of naphthalene in Σ9PAHs {61 %, (27 -95)} was the lowest of all groups (fig. 12.1a) with the narrowest range of 27 - 95 %. The median TWA concentration of naphthalene (938 ng/m^3) was higher than in group R1 but lower than both group of pavers (fig. 12.2a). The range was 69.1 - 4270 ng/m^3.

12.2.6.2 Profile of Three Ringed PAHs

As in group R1 phenanthrene and acenaphthene were the second and third most abundant PAHs in all the personal air samples collected. They were both detected in all samples (table 12.1). Acenaphthene was found in particulate form in 3 samples out of 12. The particulate matter portion represented 28, 10, and 8 % of the total in samples of roofers R2 # 87 on day 3, R2 # 89 on day 2 and R2 # 90 on day 1, respectively (appendix II). The median percentage distribution of phenanthrene was found to be 35 % in vapour from and 65 % in particulate from ranging from 18 - 91 % and 9 - 82 %, respectively (table 12.2). This was the only group with a higher percentage of phenanthrene in particulate form rather than vapour form. The median percentage of acenaphthene in Σ9PAHs was 4 % ranging from 2 - 7 % (fig. 12.1a). The median percentage of phenanthrene in Σ9PAHs {20 %, (3 - 40 %)} was higher than all other group. Its median percentage contribution among Σ9PAHs was 2 fold higher than in group R1 and ~ 3 fold higher than in both groups of pavers (fig. 12.1a). The median TWA concentration of acenaphthene (80.1 ng/m^3) was higher than all groups except group P1 (fig. 12.2a); while the range (15.6 - 530 ng/m^3) was similar to those of all other groups. The median TWA concentration for phenanthrene {(412 ng/m^3, (30.3 - 1610)} was significantly higher than all groups (fig. 12.2a). The largest p-value (p = 0.018) was obtained when testing for a significant difference between median TWA concentration of phenanthrene in groups R2 and P1 using the Mann-Whitney U-test, non-adjusted for ties.

12.2.6.3 Profile of Four Ringed PAHs

Both four ringed PAHs were in detectable levels in all personal air samples except that of roofer number (R2 # 88) on day 2 (91.7 % of the samples with detectable levels pyrene or B(a)A) (table 12.1). Pyrene was found in vapour form in 5 out of 12 samples. The median percentage of pyrene in the vapour phase was < 1% with a range of < 1 - 20 % (table 12.2). It was evident from the distribution that pyrene was mainly in the form of particulate matter which had a median of 100 % with a range 80 - 100 % (table 12.2). Pyrene's median TWA concentration contributed to 7 % of the total of 9PAHs (fig. 12.1a) ranging between < 1 and 14 %. TWA concentrations of pyrene ranged from n.d. - 480 ng/m^3 with a median of 121 ng/m^3 (fig. 12.2a). Pyrene's median TWA concentration in group R2 was significantly higher than all other group (Mann-Whitney U-test, non-adjusted for ties; $p < 0.05$). The range was also bigger.

Benz(a)anthracene, was detected as particulate matter in all the personal air samples (table 12.2) except for that of roofer R2 # 89 on day 2 were it was present 9 % in vapour phase (appendix II). It was detected in a considerably larger proportion of personal air samples in this group compared to all other groups (table 12.1). Its median percentage distribution among all 9PAHs was calculated to be 2 % (fig. 12.1a) ranging from < 1 to 12 %. The median TWA concentration was 40.6 ng/m^3 (fig. 12.2a) with a range of n.d - 550 ng/m^3. The median TWA concentration was significantly higher than in all other groups (Mann-Whitney U-test, non-adjusted for ties; $p < 0.05$).

12.2.6.4 Profile of Five Ringed PAHs

B(b)F & B(k)F were detected in 50 % of the personal air samples as particulate matter (table 12.2). The proportion of detectable samples was considerably higher than all other groups (table 12.1). Only roofer number R2 # 88 had no measurable levels of 5 ringed PAHs in any of his air samples. B(a)P was detected in 3 samples (25%) (table 12.1) one belonging to each of the roofers R2 # 87, 89 and 90 (appendix II). B(a)P was present at ~ 58 % in vapour form in one of the three samples (that of R2 # 89 on day 2, appendix II). DiB(a,h)A was not detected in any of the samples. B(b)F & B(k)F and B(a)P had a median percentage distribution of < 1% with ranges

of < 1 - 1% and < 1 - 5, respectively (fig. 12.1a). All those samples with measurable amounts of 5 ringed PAHs had detectable of both 4 ringed PAHs.

12.2.6.5 TWA Concentrations Of Airborne PAH-Exposure Indices

Σ9PAHs TWA concentrations: Table 12.3a shows the results described in this paragraph. Inter-day variability in the TWA concentrations of Σ9PAHs in personal air samples was lesser than groups M and R1 but comparable to group P1 and P2. This was reflected in the relatively low CV of 52.5 %. As in groups M, P1 and R1 day 1 had the highest median and widest range of Σ9PAHs TWA concentrations {3040 ng/m^3, (991 - 7460)}. Days 2 had a median TWA concentrations {2600 ng/m^3, (997 -4310)} nearly matching that of day 1. The median TWA concentration on day 3 was ~ 3.5 fold smaller than day 1 (table 12.3a).

Σ8PAHs TWA concentrations: Table 12.3b shows the results described in this paragraph. The median Σ8PAHs TWA concentration (1220 ng/m^3) was highest on day 2 followed by day 1 and day 3. This group had the highest median Σ8PAHs TWA concentration on days 1 and 2 and marginally lower than the group P2 which had the highest median on day 3. The variability reflected by the CV (56.4%) of the median TWA concentration of Σ8PAHs on the three days was less than groups M, P2 and R1, but not P1 which had a CV of 41.3%.

Σ4,5 PAHs TWA concentrations: The median of Σ4,5 PAHs TWA concentration was 47.4, 229 and 16.6 ng/m^3 for days 1, 2, and 3, respectively. The CV for these medians is 106 %. This was the only group with medians higher than n.d. on all three days of the survey (table 12.3c).

12.2.6.6 PAHs External Exposure and Job Description

Σ9PAHs TWA concentrations: Table 12.4a shows the job description of each roofer and reports the daily and 3-day geometric mean of Σ9PAHs TWA concentrations measured for each individual. The geometric mean for the TWA concentrations of Σ9PAHs was highest for the kettleman/mixer operator (4030 ng/m^3). His external exposure to Σ9PAHs was highest on day 1 (7460 ng/m^3) when he was working on the

kettle. On the other hand his exposure to Σ8PAHs (1740 ng/m^3) was highest on day 2 when he was on the mixer. The second highest exposed roofer was the tipper with a 3-day GM for Σ9PAHs TWA concentration of 2060 ng/m^3. His exposure was 2.5 and 4.3 fold higher on day 1 when compared to days 2 and 3, respectively. The external exposure of troweller number (R2 # 88) was 2 fold and 3.5 fold lower than that of troweller (R2 # 89) on days 1 and 2, respectively while on day 3 their external doses were equivalent. The 3-day GM for Σ9PAHs TWA concentration of the former was 884 ng/m^3 and the latter 1500 ng/m^3.

The average of the 3-day geometric means of group R2 (2120 ± 680) was the highest among all groups (table 12.4a, b and c). It was significantly higher than group M and R1 (unpaired Student's t-test; $p<0.05$).

Σ8PAHs TWA concentrations: As in the case of TWA concentration of Σ9PAHs, the kettleman/mixer operator had the highest 3-day GM for Σ8PAHs TWA concentration in this group (1318 ng/m^3) (table 12.4b). His external exposure was highest on day 2 (1740 ng/m^3) when he was working on the mixer. The tipper had the second highest 3-day GM for Σ8PAHs TWA concentration of 947 ng/m^3. Troweller R2 # 89 had a 3-day GM for Σ8PAHs of 643 ng/m^3 which was ~ 5.7 times higher than troweller R2 # 88. As in the case of Σ9PAHs, their Σ8PAHs TWA concentration on day 3 were virtually equal (table 12.4b).

The average of the 3-day geometric means of group R2 (755 ± 254) was the highest among all groups (table 12.4a, b and c). It was significantly higher than group M and R1 (unpaired Student's t-test; $p<0.05$).

Σ4,5 PAHs TWA concentrations: The 3-day GM of Σ4,5 PAHs TWA concentration was highest for the tipper (130 ng/m^3) which was 1.7 times higher than the kettleman/mixer operator (table 12.4c). Once more, the troweller R2 # 89 had a 3-day GM for Σ4,5 PAHs TWA concentration (56.9 ng/m^3) which was 4.4 times higher than that of troweller R2 # 88 (12.9 ng/m^3). The Σ4,5 PAHs TWA concentration profile was similar to that of Σ9PAHs and Σ8PAHs in personal air samples of the tipper and troweller R2 # 89; whereby, the external exposure was highest on day 2 and lowest on day 3 for the former and highest on day 1 and lowest on day 3 for the latter. This was not the case for the kettleman/mixer operator and

troweller R2 # 88 (table 12.4c)

12.3 PART II:

This second part of the chapter presents the ambient monitoring results for groups P1 and P2 treated as one group which was named "pavers" and groups R1 and R2 which was named "roofers".

12.3.1 Pavers Group

This group included 16 pavers. The total number of personal air samples was 48 (i.e. those from group P1 and P2 pooled).

12.3.1.1 Profile of Two Ringed PAHs

The profile of naphthalene in personal air samples of the pavers group did not differ significantly from the subgroups with respect to number of samples with detectable levels (86.5 %) (table 12.1). It was found in the vapour form only (table 12.2). Its median TWA concentration of 1320 ng/m^3 was very close to both groups P1 and P2 (fig. 12.2a and b). The effect of combining the two group was on the range of TWA concentration. The observed range was that of group P2 which encompassed the range of TWA concentration observed in group P1.

12.3.1.2 Profile of Three Ringed PAHs

A similar effect as on naphthalene was observed for the 3 ringed PAHs. The percentage of samples with detectable levels of either acenaphthene and phenanthrene in the pooled group were similar to either of the individual groups (table 12.1). Acenaphthene was almost exclusively found in vapour form as in both groups P1 and P2. The percentage distribution of phenanthrene between vapour phase and particulate matter was 72 and 28 %, respectively (table 12.2). This was like what was observed in group P2 but slightly different to P1. The median TWA concentration for both 3 ringed PAHs in the pavers group was very

much the same as group P2 but not group P1 (fig. 12.2a and b). Acenaphthene's median TWA concentration in the pavers group (67.4 ng/m^3) was half that of group P1 while for phenanthrene (82.2 ng/m^3) it was slightly lower. Like in the case of naphthalene, the range of the TWA concentration of either of the 3 ringed PAHs in the pooled group was that of group P2 and contained that of group P1.

12.3.1.3 Profile of Four Ringed PAHs

The characteristics of the profile of pyrene in the pavers group did not differ a lot from either of the individual group and particularly group P2. For instance the percentage of detectable samples was 60 % when it was 50 and 70 % for group P1 and P2, respectively (table 12.1). It was found chiefly in particulate form (table 12.2). The median percentage of pyrene in Σ9PAHs was <1 % which was the same as group P1 and P2 (fig. 12.1a and b); while the range was that of group P2. The median and range of the TWA concentration {7.0 ng/m^3 (n.d. - 840) was like what was calculated for group P2. Group P1 had a median half that of the pooled group. Again, the range of group P1 was within the limits of that of the pavers group.

Similar differences were seen in the profile of benz(a)anthracene as in the case of pyrene between the pooled group and its subgroups. The percentage of detectable samples was 8.3 % when it was 5.6 and 10 % for group P1 and P2, respectively (table 12.1). As in both separate groups it was detected in the particulate form in all samples except that detected in group P1. Its median percentage distribution among Σ9PAHs {< 1 % (< 1 - 1%)} was the same as both groups (fig. 12.1a and b). The TWA concentration was n.d. as in groups P1 and P2 (fig. 12.2a and b) while the range that of P2 and contained that of group P1.

12.3.1.4 Profile of Five Ringed PAHs

The 5 ringed PAHs in the pavers group had a profile very similar to that of group P1 and P2. The only difference for any of the 5 ringed PAHs was that the range TWA concentrations for each of the 5 ringed PAHs was that of group P2 and comprised that of P1.

12.3.1.5 TWA Concentrations Of Airborne PAH-Exposure Indices

Σ9PAHs TWA concentrations: The CV of 44.7 % for the median of Σ9PAHs TWA concentration calculated from the three days in pavers group was lower than both subgroups (table 12.3a). The median Σ9PAHs TWA concentration on day 1 was closer to that of group P2 rather than P1 (table 12.3a). On day 3 the range was that of group P2 encompassing that of group P1. On day 2 the range of pavers group had the minimum of group P1 and maximum of group of P2 (table 12.3a).

Σ8PAHs TWA concentrations: The CV of 54.6 % for the median Σ8PAHs TWA concentration calculated from the three days in pavers group was midway between the CV of group P1 and P2 (table 12.3b). In the pavers group the median TWA concentration on day 1 was half that of group P1 and double that of group P2. On day 2 the median was exactly that of group P2. On day 3 it was nearly the same as group P2 (table 12.3b). As for the range, on day 1 the composite group's range was comparable to both subgroup. On day 2 the minimum and maximum were those of group P1 and P2, respectively. On day 3, the range was that of group P2 (table 12.3b).

Σ4,5 PAHs TWA concentrations: The CV of the median Σ4,5PAHs TWA concentration was not calculated since the median on day all three days was calculated at n.d. levels (table 12.3c). The medians on all three days for both subgroups was n.d. as well. On day 1 the range was that of group P1, on day 2 it was the same as both subgroups and day 3 it was that of group P2 (table 12.3c).

12.3.1.6 PAHs External Exposure and Job Description

The ambient PAH-exposure of all individual in this group have been described above under groups of pavers P1 and P2.

The average 3-day geometric mean of TWA concentrations of Σ9PAHs was (1900 ± 482). It was higher than that of group P1 but lower than group P2. It was significantly different from that of group M and R1 (unpaired Students T-test; $p<0.05$). As for the average 3-day geometric mean of TWA concentrations of Σ8PAHs it was (235 ± 56.5). The reverse of

the 3-day GM of TWA concentration of Σ9PAHs was observed; whereby the 3-day GM of TWA concentration of Σ8PAHs was higher than that of group P2 but lower than group P1.

12.3.2 Roofers Group

12.3.2.1 Profile of Two Ringed PAHs

The profile of naphthalene in personal air samples of the roofers group did not differ significantly with respect to number of samples with detectable levels (table 12.1) and the form it is present in most samples (table 12.2). On the other hand the median TWA concentration 590.4 ng/m^3 for the pooled group was higher than groups R1 and lower than group R2 (fig. 12.2a and b). The effect of combining the two group was on the range of TWA concentration (n.d. - 6170 ng/m^3) as well; where it did not represent the range of either of group R1 (n.d. - 3130 ng/m^3) or R2 (515 - 6173 ng/m^3)

12.3.2.2 Profile of Three Ringed PAHs

The 3 ringed PAHs had profile similar across the pooled and individual groups. The median percentage distribution of acenaphthene among Σ9PAHs in the combined group was {5 %, (< 1 - 13 %)} which was similar to the subgroups R1 and R2 (fig. 12.1a and b). The median TWA concentration of phenanthrene was {12 %, (< 1 - 48 %)} which was midway between group R1 and R2. The roofers group differed from group R2 but not R1 in the median TWA concentrations of both acenaphthene and phenanthrene. For acenaphthene it was 15.6 ng/m^3. This was close to that of the group R1 but 8 fold lower group R2 (fig. 12.2a and b). As for phenanthrene it was 93.4 ng/m^3 which was ~ 4 times bigger than group R1 but ~ 4 smaller than group R2 (fig. 12.2a and b). The range of the acenaphthene's TWA concentration in the composite group (n.d. - 530 ng/m^3) was nearly representative of group R1 (n.d. - 355 ng/m^3) and R2 (15.6 - 530 ng/m^3). For phenanthrene the range (n.d. - 1610 ng/m^3) was marginally bigger than R2 and encompassed that of R1 (n.d. - 585).

12.3.2.3 Profile of Four Ringed PAHs

The characteristics of the profile of the 4 ringed PAHs in the composite group of roofers were as follows. The percentage of samples with measurable concentrations of pyrene was 67.6 %. This is higher than the percentage of samples with detectable levels of pyrene in group R1 by 12.6 % however lesser than that of group R2 by 24.1 % (table 12.1). Pyrene was predominantly found in the form of particulate matter across the personal air samples of the whole group (table 12.2). The median percentage of pyrene within the Σ9PAHs was {4 % (< 1 - 17 %)}. This was approximately half way between what was observed in group R1 and R2 (fig. 12.2a and b). Pyrene's median TWA concentration was 12.2 ng/m^3 with a range of < 1 - 480. This was in the same order as that of group R1 but ~ 11 times less than that of group R2 (fig. 12.2a and b).

Similar difference were seen in the profile of benz(a)anthracene as in the case of pyrene between the pooled group and its subgroups. The percentage of detectable samples was 62.2 % while it was 52 and 91.7 % for group R1 and R2, respectively (table 12.1). It was found in particulate form in all samples except that observed in group R2 (heading 12.2.5.3). Its median and range of percentage distribution among Σ9PAHs was {1 % (< 1 - 48%)} (fig. 12.1b). The median was half that of group R2. The TWA concentration was 12.6 ng/m^3. This median TWA concentration was obviously higher than the n.d. median of group R1 and 3.2 fold less than group R2 (fig. 35a & 35b). The range of the TWA concentration (< 1 - 550 ng/m^3) was exactly that of group R2 which encompassed that of group R1.

12.3.2.4 Profile of Five Ringed PAHs

Twenty seven % of the personal air samples had detectable levels of either B(b)F & B(k)F or B(a)P. For B(b)F & B(k)F this was greater than what was observed in group R1 but approximately half of what was detectable in group R2; while for B(a)P it was close to the observed % in both subgroups (table 12.1). Only one sample (R1 # 40 on day 1) had measurable levels of DiB(a,h)A. Each of the 5 ringed PAHs had a median percentage distribution among all 9PAHs of < 1 % which was the same for the both individual groups (fig. 12.1a and b). Similarly, the median TWA

concentration for each of the 5 ringed PAHs was n.d. as in both groups R1 and R2 (fig. 12.2a and b).

12.3.2.5 TWA Concentrations Of PAHs

Σ9PAHs TWA concentrations: The variability in the median Σ9PAHs TWA concentrations quantified in a CV of 106 % which reflected that of group R1 but not R2 (table 12.3a). On day 1 the median TWA concentration was {1520 ng/m^3 (815 - 7460)}. This median was 1.5 times bigger than group R1 and 2 folds smaller than group R2. The range had the minimum of group R1 and the maximum of group R2. On day 2 and 3 the median was in the same order of magnitude as group R1 but much lower than group R2 and the range had the minimum of group R1 and the maximum of group R2 (table 12.3a).

Σ8PAHs TWA concentrations: Table 12.3b shows that the CV of the daily median Σ8PAHs TWA concentration in the roofers group was close to that of group R1 and about twice that of group R2. All the daily medians were very close to those of group R1 and considerably different from group R2. All the daily ranges of Σ8PAHs in the roofers group had the minimum of group R1 and the maximum of group R2.

Σ4,5 PAHs TWA concentrations: The CV of the daily median Σ4,5PAHs TWA concentration was not calculated for the roofers group since day 2 had a median of n.d. levels (table 12.3c). On day 1 the median was 25.5 ng/m^3. This was approximately half that of group R2; while not comparable to the group R1 which had a median of n.d. levels (table 12.3c). On day 3 the median of the roofers group was very close to the medians of both subgroups (table 12.3c). The range of Σ4,5PAHs TWA concentrations on all 3 days had the minimum of group R1 and the maximum of group R2 (table 12.3c).

12.3.1.6 PAHs External Exposure and Job Description

The ambient PAH-exposure of all individual in this group have been described above under groups of pavers R1 and R2.

The average 3-day geometric mean of TWA concentrations of

Σ9PAHs was (958 ± 358). It was higher than that of group R1 but lower than group R2. It was significantly different from that of group M (unpaired Student's t-test; $p<0.05$). As for the average 3-day geometric mean of TWA concentrations of Σ8PAHs it was (306 ± 102). Again, it was higher than that of group R1 but lower than group R2.

12.4 PART III:

This part of this chapter reports the correlation existing between the TWA concentrations of specific PAH-species and that of the sum of a selection from other quantified PAHs in personal air samples of subgroups, combined groups and all groups pooled together.

12.4.1 Correlation of Naphthalene With PAHs

Naphthalene vs. Σ8PAHs: The TWA concentration of naphthalene correlated highly significantly ($p < 0.0001$) with that of Σ8PAHs in personal air sample of subjects in subgroups M, P1, P2, R1 but not R2 (table 12.5). When grouped R1 and R2 were pooled as one group "roofers" the correlation between the above two variables was strong and very significant. Combing the roofers subgroups masked the borderline significant correlation ($p = 0.06$) in the personal air samples of group R2.

Naphthalene vs. Σ4,5PAHs: The TWA concentration of naphthalene correlated weakly ($0.400 \leq r \leq 0.445$) and non-significantly with that of Σ4,5PAHs in personal air samples with detectable levels of 4 and 5 ringed PAHs in subgroups R1, R2 and the combined roofers group (table 12.5). The correlation seen in the personal air samples of all groups between the above variables was weak but with borderline significance ($p = 0.06$) which slightly masked the no significance in the roofers group (table 12.5).

12.4.2 Correlation of Phenanthrene With PAHs

Phenanthrene vs. ∑8PAHs: The TWA concentration of phenanthrene correlated highly significantly ($p < 0.0001$) with that of ∑8PAHs in all subgroups except R2 (table 12.6). The correlation coefficient "r" ranged between 0.666 in group M and 0.823 in group P1 (table 12.6). The pooling of the pavers or roofers subgroups did not have much effect on the correlation existing between the above variables. The only effect was the masking the relatively weak significance ($p = 0.017$) in group R2 (table 12.6).

Phenanthrene vs. ∑8PAHs - [naphthalene]: Table 12.6 shows that the exclusion of naphthalene's TWA concentration from that of ∑8PAHs, the correlations of TWA concentration of phenanthrene with that of ∑8PAHs - [naphthalene] were stronger ($0.715 < r < 0.942$) and highly significant ($p < 0.0001$) in all the groups including group R2 (table 12.6).

Phenanthrene vs. ∑4,5PAHs: TWA concentrations of phenanthrene were significantly related to those of ∑4,5PAHs in personal air samples of group R2 ($p = 0.0004$) but not R1 ($p = 0.183$) . In the combined roofers group the linear correlation between phenanthrene and ∑4,5PAHs was significant ($p = 0.0010$) but did not reflect any of its subgroups. The correlation coefficients existing between phenanthrene and ∑4,5PAHs (table 12.6) were stronger and more significant than those of naphthalene with ∑4,5PAHs (table 12.5).

12.4.2 Correlation of Pyrene With PAHs

Pyrene vs. ∑8PAHs: The correlation between TWA concentrations of pyrene and that of ∑8PAHs in personal air samples of groups M and P2 were weak ($r = 0.005$ and 0.089, respectively and not significant ($p = 0.9068$ and 0.7002, respectively) (table 12.7). In groups P1, R1 and R2 the correlation coefficients were similar to those of naphthalene with ∑8PAHs (table 12.5) but weaker than phenanthrene (table 12.6). The weak and non-significant ($r = 0.176$; $p = 0.3522$) correlation in the combined groups of pavers masked the relatively strong and significant correlation

in subgroup P1 (table 12.7). As for the pooled group of roofers the relationship between pyrene and Σ8PAHs was stronger (r = 0.784) and more significant ($p < 0.0001$) than either of the subgroups R1 and R2 (table 12.7).

Pyrene vs. Σ8PAHs - [naphthalene]: The TWA concentration of pyrene correlated significantly with Σ8PAHs - [naphthalene] in personal air samples of groups P1 ($p = 0.0289$) and R2 ($p < 0.0001$) but not in those of groups M, P2 and R1 (table 12.7). The strength and significance of these correlations measured by the correlation coefficient "r" and the p-value (table 12.7) were much weaker and less significant than those of phenanthrene with Σ8PAHs - [naphthalene] (table 12.6).

Pyrene vs. Σ4,5PAHs: Highly significant ($0.0001 > p = 0.0084$) and strong ($0.706 \leq r \leq 0.932$) correlations were observed between the TWA concentration of pyrene and that of Σ4,5PAHs in personal air samples of subgroups, combined groups and all groups (table 12.7). These correlation were much stronger and more significant than those of either naphthalene or phenanthrene with Σ4,5PAHs.

Table : 12.1 Number and percentage of samples with detectable levels of specific PAH-species in personal air samples of groups M, P1, P2, R1, R2, pavers (PV) and roofers (RF)

Group	Total # of Samples	Samples with detectable levels of PAH-species; number (≈%)							
		Naphthalene	Acenaphthene	Phenanthrene	Pyrene	B(a)A	B(b)F & B(k)F	B(a)P	DiB(a,h)A
M	34	31 (91.2)	24 (70.6)	23 (67.6)	7 (20.6)	1 (2.9)	2 (5.9)	2 (5.9)	1 (2.9)
P1	18	18 (100)	18 (100)	18 (100)	9 (50)	1 (5.6)	1 (5.6)	1 (5.6)	n.d.
P2	30	29 (96.7)	30 (100)	30 (100)	21 (70)	3 (10)	1 (3.3)	1 (3.3)	n.d.
R1	25	23 (92)	22 (88)	23 (92)	14 (56)	12 (48)	4 (16)	7 (28)	1 (4)
R2	12	12 (100)	12 (100)	12 (100)	11 (91.7)	11 (91.7)	6 (50)	3 (25)	n.d.
Pavers	48	47 (97.9)	46 (95.8)	48 (100)	30 (62.5)	4 (8.3)	2 (4.2)	2 (4.2)	n.d.
Roofers	37	35 (94.6)	34 (91.9)	35 (94.6)	25 (67.6)	23 (62.2)	10 (27)	10 (27)	1 (2.7)

Table : 12.2 The median percentage distribution of individual PAH-species between vapour and particulate matter phase in personal air samples with detectable levels in all groups

	Median (range) percentage distribution of PAH-species between vapour and particulate Matter %															
	Napthalene		Acenaphthene		Phenanthrene		Pyrene		B(a)A		B(b)F & B(k)F		B(a)P		DiB(a,h)A	
Group	Vap. %	Part. %	Vap. %	Part. %	Vap. %	Part. %	Vap. %	Part. %	Vap. %	Part. %	Vap. %	Part. %	Vap. %	Part. %	Vap. %	Part. %
M	100 (<1-100)	< 1	100**	< 1	73 (46-84)	27 (16 - 54)	24 (<1 66)	76 (34 - 100)	37	63*	72	28*	< 1	100*	< 1	100*
P1	100**	< 1	100**	< 1	80 (<1-100)	20 (<1 - 100)	<1 (<1 - 13)	100 (87 - 100)	< 1	100*	< 1	100*	< 1	100*	n.d.	n.d.
P2	100**	< 1	100**	< 1	72 (19-100)	28 (<1 - 81)	<1 (<1 - 100)	100 (<1 - 100)	< 1	100**	< 1	100*	< 1	100*	n.d.	n.d.
R1	100**	< 1	100**	< 1 (<1 - 95)	83 (<1-100)	17 (<1 - 100)	<1 (<1 - 100)	100 (<1 - 100)	< 1	100**	< 1	100**	< 1	100**	< 1	100*
R2	100**	< 1	100	<1 (<1 - 28)	35 (18-91)	65 (9 - 82)	<1(<1 - 20)	100 (80 - 100)	< 1	100 (9 - 100)	< 1	100**	< 1 (<1 - 42)	100 (58 - 100)	n.d.	n.d.
Pavers	100**	< 1	100**	< 1	72 (<1 - 100)	28 (<1 - 100)	<1(<1-100)	100 (<1 - 100)	< 1	100**	< 1	100**	< 1	100**	n.d.	n.d.
Roofers	100**	< 1	100**	<1 (<1 - 95)	76 (<1 - 100)	24 (<1 - 100)	<1(<1-100)	100 (<1 - 100)	< 1	100 (9 - 100)	< 1	100**	< 1	100 (34 - 100)	< 1	100*

Vap.: Vapour form

Part.: Particulate matter

*: One sample with detectable levels, no median and range

**: all detectable samples with same distribution

n.d.: Not detectable

Table : 12.3 Median and range of daily time weighted average concentrations of Σ9PAHs, Σ8PAHs and Σ4,5PAHs in Personal Air Samples of Group M, P1, P2, R1, R2, pavers and roofers on Days 1, 2 and 3 and the CV of the median between days

(A)

	TWA Concentration of Σ9PAHs in ng/m³			
	Median (range)			
Group	Day 1	Day 2	Day 3	CV (%)
M	1440 (448 - 9190)	111 (23.7 - 3700)	98.2 (n.d. - 1570)	140
P1	2720 (271 - 5010)	861 (84.0 - 4960)	1170 (794 - 3920)	62.9
P2	500 (45.9 - 11100)	1970 (955 - 12500)	2230 (394 - 32300)	59.5
R1	1500 (815 - 2340)	253 (146 - 3730)	77 (5.4 - 836)	127
R2	3040 (991 - 7460)	2600 (997 - 4310)	880 (658 - 2030)	52.5
Pavers	723 (45.9 - 11100)	1770 (84.0 - 12500)	1950 (395 - 32300)	44.7
Roofers	1520 (815 - 7460)	369 (146 - 4310)	176 (5.4 - 2030)	106

(B)

	TWA Concentration of Σ8PAHs in ng/m³			
	Median (range)			
Group	Day 1	Day 2	Day 3	CV (%)
M	112 (36.2 - 446)	8.4 (n.d. - 624)	6.3 (n.d. - 1470)	143
P1	388 (73.9 - 980)	181 (15.0 - 848)	225 (100 - 899)	41.2
P2	60.0 (36.2 - 905)	172 (99.7 - 946)	370 (39.4 - 3730)	78.2
R1	271 (143 - 494)	22.8 (10.7 - 850)	58.0 (59.3 - 284)	114.0
R2	1020 (221 - 2530)	1220 (45.9 - 2470)	308.7 (143 - 1020)	56.4
Pavers	116 (36.2 - 980)	172 (15.0 - 946)	334 (39.4 - 3730)	54.6
Roofers	279 (143 - 2530)	45.9 (10.7 - 2470)	80.2 (59.3 - 1020)	93.2

(C)

	TWA Concentration of Σ4,5PAHs in ng/m³			
	Median (range)			
Group	Day 1	Day 2	Day 3	CV (%)
M	n.d. (n.d. - 209)	n.d. (n.d. - n.d.)	n.d. (n.d. - 897)	n.c.
P1	n.d. (n.d. - 36.5)	n.d. (n.d. - n.d.)	n.d. (n.d. - n.d.)	n.c.
P2	n.d. (n.d. - 12.5)	n.d. (n.d. - n.d.)	n.d. (n.d. - 127)	n.c.
R1	n.d. (n.d. - 82.3)	n.d. (n.d. - 21.4)	25.0 (n.d. - 48.6)	n.c.
R2	47.5 (16.4 - 847)	229 (n.d. - 255)	16.6 (10.2 - 76.2)	106
Pavers	n.d. (n.d. - 36.5)	n.d. (n.d. - n.d.)	n.d. (n.d. - 127)	n.c.
Roofers	25.5 (n.d. - 847)	n.d. (n.d. - 255)	25.0 (n.d. - 76.2)	n.c.

n.c.: Not calculated n.d: Not detectable

CV: Coefficient of variation

Table : 12.4a

Daily, 3-day geometric means and confidence intervals of time weighted average concentrations of Σ9PAHs, Σ8PAHs and Σ4,5PAHs in personal air samples of manual workers (group M)

		Daily, Geometric Mean, and Confidence Intervals of Time Weighted Average Concentration (ng/m3)														
Manual Workers		Σ 9 PAHs					Σ 8 PAHs					Σ 4,5 PAHs				
Code	Job Description	Day 1	Day 2	Day 3	GM	95% CI	Day 1	Day 2	Day 3	GM	95% CI	Day 1	Day 2	Day 3	GM	95% CI
10	Sewer Maintenance	1420	P.F.	124	420	38.3 - 4580	81.5	n.d.	17.9	38.2	8.7 - 167	n.d.	n.d.	n.d.	n.d.	n.d.
14	Handling cement	448	118	n.d.	230	62.2 - 849	36.2	7.2	n.d.	16.1	62.2 - 849	n.d.	n.d.	n.d.	n.d.	n.d.
15	Handling cement	951	875	440	715	443 - 1160	109	137	197	143	102 - 201	n.d.	n.d.	n.d.	n.d.	n.d.
16	Sewer Maintenance	811	3700	59.3	562	52.8 - 5990	115	624	25.9	123	20.3 - 745	n.d.	n.d.	n.d.	n.d.	n.d.
17	Sewer Maintenance	3030	170	399	590	113 - 2960	598	36.7	70.7	116	25.1 - 404	209	n.d.	n.d.	*209*	***
33	Site Supervisor	1050	42.3	72.3	148	21.1 - 1030	56.4	n.d.	n.d.	56.4	***	n.d.	n.d.	n.d.	n.d.	n.d.
34	Site Supervisor	816	23.7	56.2	103	12.8 - 830	47.7	n.d.	6.0	16.9	2.2 - 130	n.d.	n.d.	n.d.	n.d.	n.d.
35	Slatter	1450	59.2	n.s.	293	12.8 - 6710	63.4	n.d.	n.d.	63.4	***	n.d.	n.d.	n.d.	n.d.	n.d.
37	Laying Insulation	1800	179	63.8	274	39.6 - 1890	165	7.1	6.5	19.7	2.5 - 158	n.d.	n.d.	n.d.	n.d.	n.d.
38	Painting Metal	1870	101	P.F.	435	24.7 - 7620	184	11.0	n.d.	45.0	2.9 - 709	n.d.	n.d.	n.d.	n.d.	n.d.
41	Painting Metal	2880	111.0	1570	795	96.5 - 3910	446	9.6	1470	185	10.0 - 1930	n.d.	n.d.	897	*897*	***
45	Stripping Lead	9190	71.6	n.d.	811	7.0 - 94000	122	16.2	n.d.	44.5	6.2 - 321	n.d.	n.d.	n.d.	n.d.	n.d.
Mean					448					72.2					N.C.	
SEM					71.2					16.1					N.C.	

n.d.: Not detectable; N.C.: Not calculated; n.s.: No sample; P.F.: Pump failure

GM: 3-day geometric mean

95% CI: 95 % Confidence interval

***: One sample is detectable no GM and 95% CI calculated

Table : 12.4b

Daily, 3-day geometric means and confidence intervals of time weighted average concentrations of Σ9PAHs, Σ8PAHs and Σ4,5PAHs in personal air samples of pavers (groups P1 & P2)

		Daily, Geometric Mean, and Confidence Intervals of Time Weighted Average Concentration (ng/m3)														
Paver		Σ 9 PAHs					Σ 8 PAHs					Σ 4,5 PAHs				
Code	Job Description	Day 1	Day 2	Day 3	GM	95 % CI	Day 1	Day 2	Day 3	GM	95 % CI	Day 1	Day 2	Day 3	GM	95 % CI
7	Paver *	5010	1910	3920	**3347**	1890 - 5906	980	275	899	**623**	279 - 1390	36.5	n.d.	n.d.	**36.5**	***
8	Paver *	2950	1490	1200	**1741**	1020 - 2960	461	242	204	**284**	174 - 461	n.d.	n.d.	n.d.	**n.d.**	n.d.
11	Paver *	2490	198	1060	**806**	188 - 3470	315	119	859	**319**	104 - 973	n.d.	n.d.	n.d.	**n.d.**	n.d.
13	Paver *	1550	4960	1500	**2259**	1020 - 4400	113	848	217	**275**	62.8 - 344	n.d.	n.d.	n.d.	**n.d.**	n.d.
18	Paver *	4850	232	1140	**1086**	194 - 6070	576	29.5	246	**161**	28.5 - 910	n.d.	n.d.	n.d.	**n.d.**	n.d.
19	Paver *	271	84.0	794	**262**	73.6 - 935	73.9	15.0	100	**48.1**	15.1 - 153	n.d.	n.d.	n.d.	**n.d.**	n.d.
Mean					1584					285					N.C.	
SEM					454					79.0					N.C.	
60	Raker	720	2190	3860	**1830**	695 - 4800	68.4	148	646	**187**	51.4 - 680	n.d.	n.d.	n.d.	**n.d.**	n.d.
61	Raker	369	955	2120	**907**	337 - 2430	50.4	99.7	306	**115**	41.2 - 323	n.d.	n.d.	n.d.	**n.d.**	n.d.
62	Raker	533	1780	2340	**1300**	535 - 3180	36.2	126	379	**120**	31.8 - 453	n.d.	n.d.	12.5	**12.5**	***
63	Tractor Driver	725	1760	2700	**1510**	722 - 3220	124	197	385	**211**	121 - 382	12.5	n.d.	n.d.	**12.5**	***
64	Screwman	460	7530	8220	**3050**	478 - 19600	43.2	444	999	**268**	42.2 - 1680	n.d.	n.d.	n.d.	**n.d.**	n.d.
65	Roller Driver	45.9	1470	1450	**461**	48.0 - 4410	45.9	122	188	**102**	44.9 - 231	n.d.	n.d.	n.d.	**n.d.**	n.d.
66	Foreman	1590	1070	1780	**1450**	1070 - 1950	905	102	212	**270**	76.8 - 948	n.d.	n.d.	n.d.	**n.d.**	n.d.
67	Paver	468	12500	32300	**5740**	463 - 70700	126	406	3730	**576**	70.8 - 3800	n.d.	n.d.	127	**127**	***
68	Roller Driver	395	2160	2110	**1220**	403 - 3670	51.5	946	362	**260**	48.6 - 1390	n.d.	n.d.	n.d.	**n.d.**	n.d.
69	Raker	11100	10400	395	**3570**	413 - 31200	120	386	39.4	**122**	33.6 - 444	n.d.	n.d.	n.d.	**n.d.**	n.d.
Mean					2100					223					N.C.	
SEM					502					44.6					N.C.	

* Exact job description not specified n.d.: Not detectable; N.C.: Not calculated GM: 3-day geometric mean 95% CI: 95 % Confidence interval

***: One sample is detectable no GM and 95% CI calculated

Table : 12.4c

Daily, 3-day geometric means and confidence intervals of time weighted average concentrations of Σ9PAHs, Σ8PAHs and Σ4,5PAHs in personal air samples of roofers (groups R1 & R2)

Roofer		Daily, Geometric Mean, and Confidence Intervals of Time Weighted Average Concentration (ng/m3)														
		Σ 9 PAHs					Σ 8 PAHs					Σ 4,5 PAHs				
Code	Job Description	Day 1	Day 2	Day 3	GM	95 % CI	Day 1	Day 2	Day 3	GM	95 % CI	Day 1	Day 2	Day 3	GM	95 % CI
20	Roofer*	n.s.	146	77	**106**	56.4 - 199	n.s.	19.4	17.4	**18.4**	16.5 - 20.4	n.d.	n.d.	n.d.	**n.d.**	n.d.
21	Roofer*	n.s.	3730	324	**1100**	100 - 12000	n.s.	590	65.9	**197**	23.0 - 1690	n.d.	n.d.	13.8	**13.8**	***
36	Roofer*	815	3420	176	**789**	147 - 4230	143	850	94.4	**225**	60.1 - 844	n.d.	21.4	25.2	**23.2**	19.7 - 27.3
39	Roofer*	2270	253	71.9	**345**	47.9 - 2480	194	16.1	9.7	**31.2**	5.1 - 191	n.d.	n.d.	n.d.	**n.d.**	n.d.
40	Roofer*	983	165	836	**514**	169 - 1590	271	61.1	285	**168**	62.3 - 452	82.3	20.0	48.6	**43.1**	19.2 - 96.7
42	Roofer*	1960	369	5.4	**157**	5.1 - 4910	494	22.2	n.d.	**105**	5.0 - 2191	72.1	n.d.	n.d.	**72.1**	n.d.
43	Roofer*	1010	174	60.6	**220**	44.0 - 1100	279	10.7	60.6	**56.5**	9.0 - 358	36.0	n.d.	29.4	**32.5**	26.6 - 39.7
44	Roofer*	2340	361	145	**497**	99.9 - 2470	222	22.8	55.6	**65.5**	17.9 - 240	n.d.	n.d.	29.4	**29.4**	n.d.
46	Roofer*	1500	181	58.0	**250**	38.7 - 1620	484	35.0	58.0	**99.4**	20.5 - 482	41.4	n.d.	25.0	**32.2**	19.6 - 52.7
Mean					442					107					35.2	
SEM					109					24.7					7.0	
87	Mixer Operator	7460	4310	2030	**4030**	1930 - 8430	1290	1740	1020	**1318**	971 - 1782	25.5	229	76.2	**76.3**	22.1 - 264
88	Troweller	991	997	699	**884**	702 - 1110	221	45.9	144	**113**	45.3 - 284	16.4	n.d.	10.2	**12.9**	5.0 - 24.1
89	Troweller	1520	3390	658	**1500**	594 - 3800	753	2470	143	**643**	127 - 3240	69.4	255	10.4	**56.9**	6.1 - 396
90	Tipper	4550	1810	1060	**2060**	898 - 4730	2530	709	474	**947**	352 - 2540	847	115	22.7	**130**	16.7 - 1010
Mean					2120					755					69.1	
SEM					680					255					24.3	

* Exact job description not specified n.d.: Not detectable GM: 3-day geometric mean

***: One sample is detectable no GM and 95% CI calculated 95% CI: 95 % Confidence interval

Table : 12.5 Correlations between logarithmic transformation (log base 10) TWA concentration of naphthalene and Σ8PAHs or Σ4,5PAHs in personal air samples of group M, P1, P2, R1, R2, pavers (PV), roofers (RF) and all groups

	Naphthalene vs Σ8PAHs			Naphthalene vs Σ4,5PAHs		
Group	**n**	**r**	**p-value**	**n**	**r**	**p-value**
M	27	0.703	<0.0001	**	**	**
P1	18	0.682	0.0002	**	**	**
P2	30	0.698	<0.0001	**	**	**
R1	24	0.816	<0.0001	10	0.445	0.20
R2	12	0.558	0.06	11	0.436	0.21
Pavers	38	0.674	<0.0001	**	**	**
Roofers	36	0.799	<0.0001	21	0.400	0.21
All groups	101	0.727	<0.0001	28	0.378	0.06

**: Not enough data points to describe a correlation

Table : 12.6 Correlations between the logarithmic transformation (log base 10) of TWA concentration of phenanthrene and Σ8PAHs, Σ8PAHs - [naphthalene] or Σ4,5PAHs in personal air samples of group M, P1, P2, R1, R2, pavers (PV), roofers (RF) and all groups

	Phenanthrene vs Σ8PAHs			Phenanthrene vs Σ8PAHs - [Naph.]			Phenanthrene vs Σ4,5PAHs		
Group	**n**	**r**	**p-value**	**n**	**r**	**p-value**	**n**	**r**	**p-value**
M	22	0.666	<0.0001	22	0.942	<0.0001	**	**	**
P1	18	0.823	<0.0001	16	0.896	<0.0001	**	**	**
P2	30	0.746	<0.0001	30	0.715	<0.0001	**	**	**
R1	23	0.764	<0.0001	22	0.899	<0.0001	12	0.412	0.183
R2	12	0.669	0.017	11	0.972	<0.0001	11	0.873	0.0004
Pavers	48	0.759	<0.0001	46	0.781	<0.0001	**	**	**
Roofers	35	0.804	<0.0001	33	0.933	<0.0001	27	0.601	0.0010
All groups	105	0.734	<0.0001	101	0.877	<0.0001	29	0.573	0.0012

**: Not enough data points to describe a correlation

[Naph.]: Naphthalene TWA concentration

Table : 12.7 Correlations between the logarithmic transformation (log base 10) of TWA concentration of pyrene and Σ8PAHs, Σ8PAHs - [naphthalene] or Σ4,5PAHs in personal air samples of group M, P1, P2, R1, R2, pavers (PV), roofers (RF) and all groups

	Pyrene vs Σ8PAHs			Pyrene vs Σ8PAHs - [Naph.]			Pyrene vs Σ4,5PAHs		
Group	**n**	**r**	**p-value**	**n**	**r**	**p-value**	**n**	**r**	**p-value**
M	7	0.005	0.9068	7	0.544	0.1034	**	**	**
P1	9	0.744	0.0142	9	0.650	0.0289	**	**	**
P2	21	0.089	0.7002	21	0.214	0.3506	**	**	**
R1	13	0.688	0.0094	13	0.430	0.1424	11	0.746	0.0084
R2	11	0.766	0.006	11	0.949	<0.0001	11	0.932	<0.0001
Pavers	30	0.176	0.3522	30	0.247	0.1882	**	**	**
Roofers	24	0.784	<0.0001	24	0.794	<0.0001	22	0.730	0.0002
All groups	61	0.276	0.0316	61	0.501	<0.0001	28	0.706	<0.0001

**: Not enough data points to describe a correlation

[Naph.]: Naphthalene TWA concentration

Figure : 12.1a Profile of median percentage distribution of nine PAH-species in personal air samples of groups M, P1, P2, R1 and R2

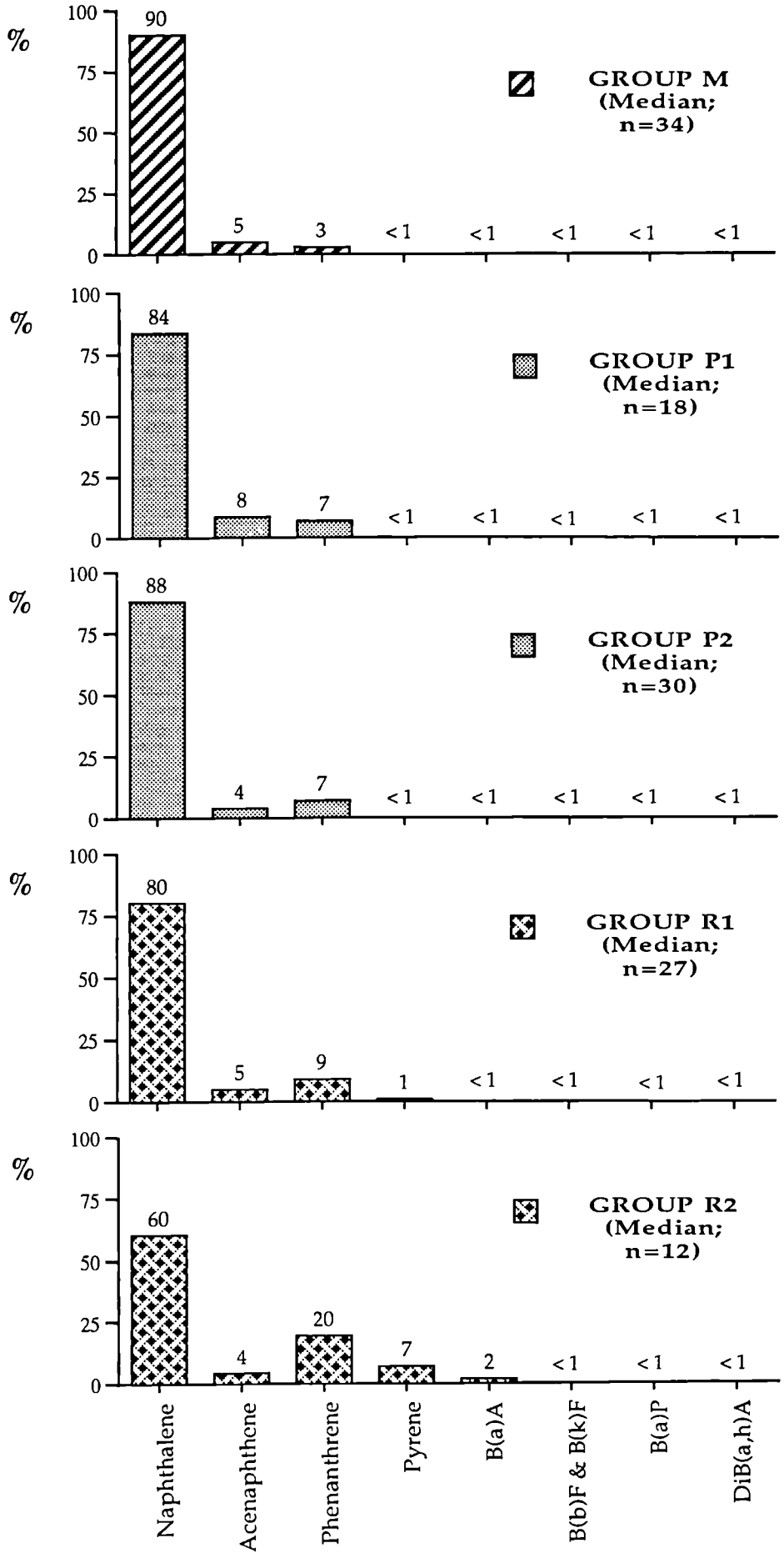


Figure : 12.1b Profile of median percentage distribution of nine PAH-species in personal air samples of pavers and roofers groups

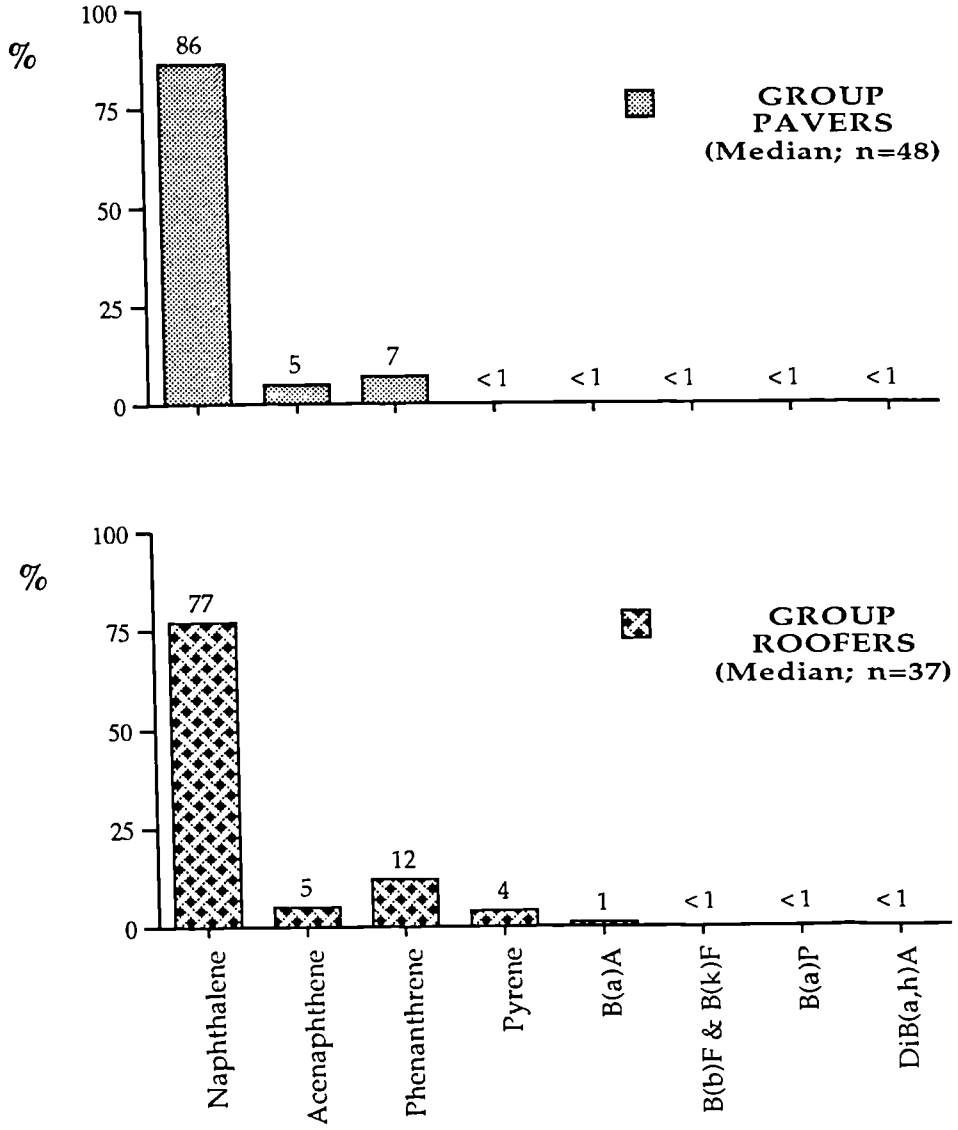


Figure : 12.2a Profile of TWA concentrations of nine PAH-species in personal air samples of groups M, P1, P2, R1 and R2

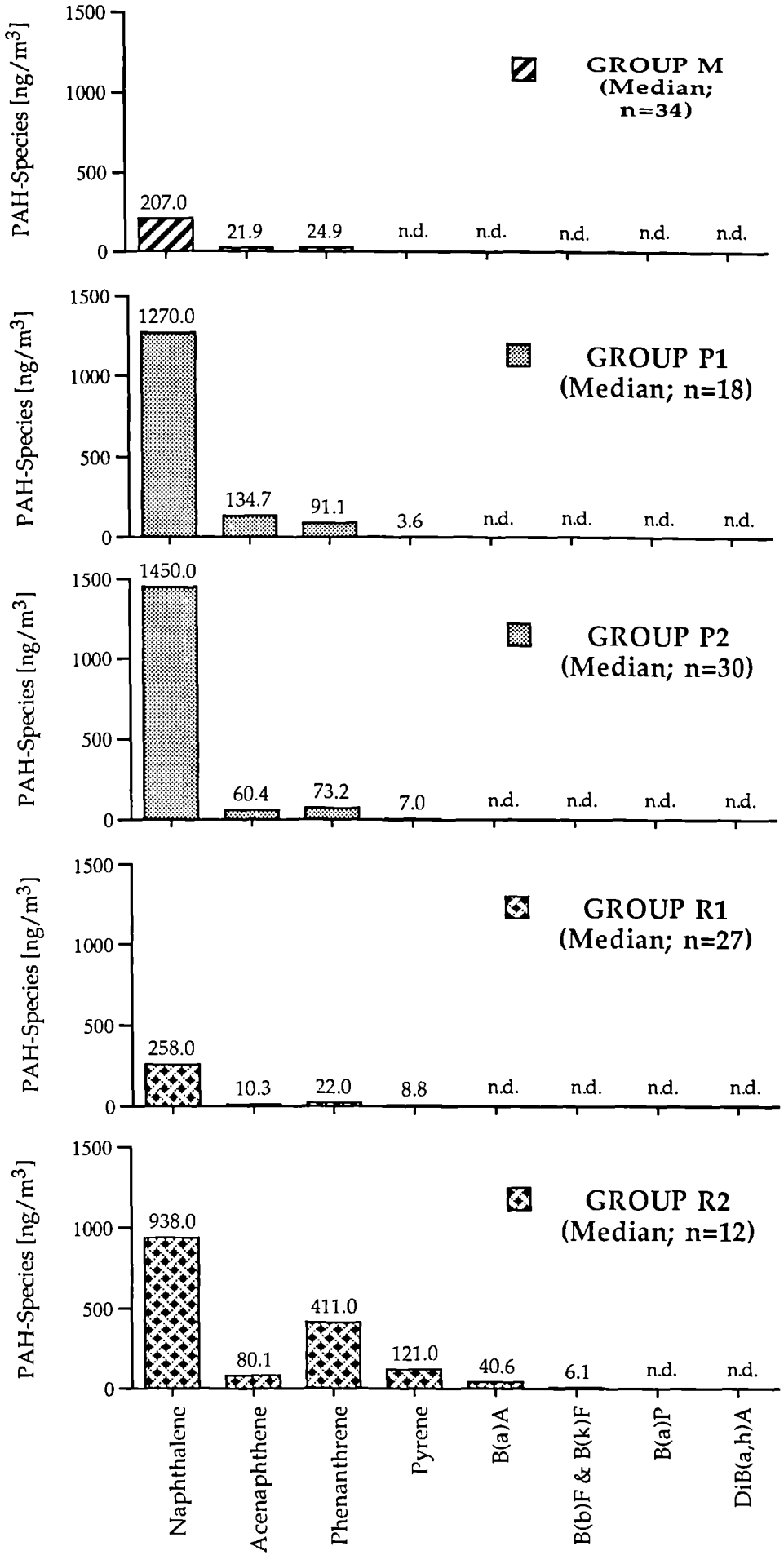
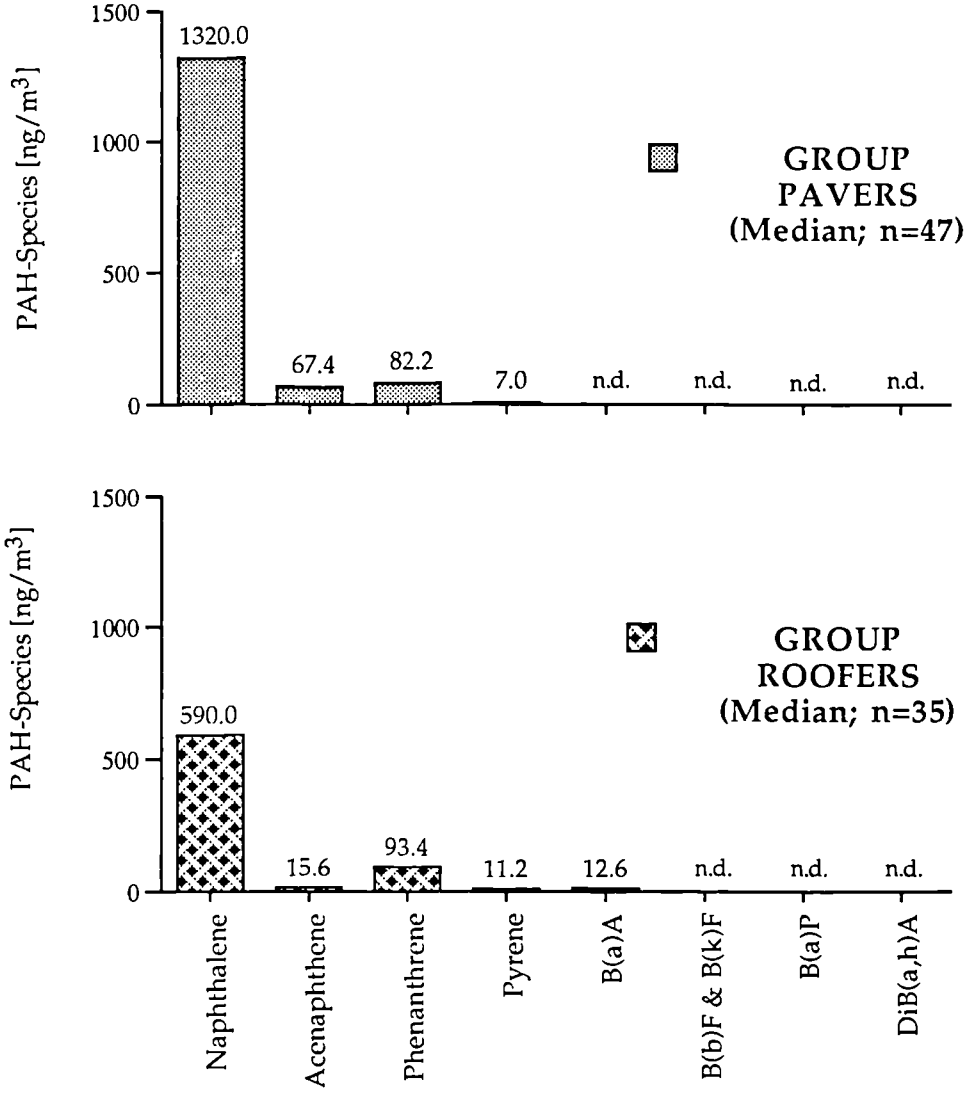


Figure : 12.2b Profile of TWA concentrations of nine PAH-species in personal air samples of pavers and roofers groups



CHAPTER 13

DISCUSSION OF AMBIENT EXPOSURE TO PAHs

13.1 INTRODUCTION

In this chapter the results obtained from the ambient monitoring data are discussed. It will examine profiles of PAH-species in personal air samples collected on different surveys from the different groups and discusses the personal exposure of individuals in relation to their job description. It will also analyse the correlations existing between different airborne PAH-exposure indices in personal air samples of group M, P1, P2, R1 and P2 as well as the combined groups PV and RF. The effect of weather conditions recorded on different surveys will be taken into consideration as an important uncontrollable factor affecting exposure.

13.2 Profile of PAH-Species

13.2.1 Profile of Two Ringed PAHs

Naphthalene was detected in a large percentage of personal air samples in the non-occupationally PAH-exposed group M. These percentages were similar to those of groups of pavers and roofers. The observed median TWA concentration of naphthalene in personal air samples in manual workers was 7 and 22 % of those seen in groups P1, P2 and R2 while it was approximately 80 % of group R1. This suggests that the relative contribution of background pollution and sources of PAH-emission other than hot bitumen to the amounts of naphthalene collected during paving and roofing operation is considerable.

Moreover, if bitumen fume is a major source of naphthalene in air, then one would we expect that group R2 should have had the highest median TWA concentration and not the groups of pavers, since roofers in group R2 had the highest median TWA concentration of total PAHs. This means that there is a source, other than background pollution and bitumen fume, contributing to the pavers' high naphthalene-exposure. This source could be gasoline or diesel exhausts of vehicles used by the pavers or general transport on the road. The paving machine driver, who is usually exposed to PAHs from bitumen fume and paving machine exhaust (Monarca *et al.* 1987), had an extremely high naphthalene TWA concentration on days 1 and 2 (12100 and 28600 ng/m^3, respectively)

(appendix II). Our conclusion here is in parallel with that of Brandt *et al.* (1993) who also concluded that naphthalene stems not from bitumen, but from other sources.

Naphthalene was exclusively found in gaseous form in all personal air samples of all groups regardless of the source and the occupation. This was observed by other investigators in air samples collected during paving operations (Lesage *et al.* 1987, Brandt and Cordingley 1992). This implies that the use of sorbent tubes to sample naphthalene in air is essential to characterise its profile. The no detectable levels of naphthalene reported by Greenspan *et al.* (1995) during a paving operation were due to air sampling on Teflon filters only. In samples collected on Chromosorb adsorbent tubes naphthalene was detectable in high concentrations especially for the paver operator.

Personal sampling and determination of naphthalene exposure is of use when characterising the risk from exposure to PAHs in bitumen fume. In our study the median TWA concentration of naphthalene was about 1320 ng/m^3 for the pavers group. This is similar to that which Brandt and Cordingley (1992) observed during paving operations (1609 ng/m^3); while those observed by Monarca *et al.* (1987) in area samples during paving operations, were approximately 6 fold lower than in this study. Therefore, exposure to much higher concentrations may suggest that there are sources other than bitumen fume in the industrial environment adding to the workers' external dose of PAHs.

In conclusion, the amount of naphthalene in the general environment adds to the workers' occupational exposure to this volatile PAH. It appears that pavers are exposed to higher quantities than roofers. This could be due to exposure to vehicle exhaust. Since the toxicity of a PAHs-mixture varies with its profile which in turn varies considerably with the source of emission (Scheepers and Bos, 1992), it is important to consider PAH-exposure from different sources of emission as well as bitumen fume in a risk assessment of occupational exposure.

13.2.2 Profile of Three Ringed PAHs

The percentage of samples with detectable levels of either of the 3

ringed PAHs (acenaphthene and phenanthrene) measured in personal air samples of group M was considerably lower than all groups except group R1.

The median TWA concentration of acenaphthene in personal air samples of group M was about 3 to 4 times lower than groups P1, P2 and R2 with that of P1 being the highest. If bitumen fume was the main source of acenaphthene exposure we would have expected it to be highest in samples of group R2 for two reasons. First, purely through a subjective assessment this group seemed to be more exposed to a visible intensity of bitumen fume and secondly their Σ8PAHs was the highest. Hence, the higher median TWA concentration of acenaphthene in group P1 is difficult to explain. The use of different types of bitumen would not justify this observation for two reasons. First, it was shown by Eldridge *et al.* (1982) that acenaphthene was not normally observed in GC/MS traces of PAH emissions from paving asphalt collected on an adsorbent material under controlled laboratory conditions. Secondly, Brandt *et al.* (1985) have shown that the PAH profile is not significantly different in bitumen fume generated, under controlled laboratory conditions, from penetration grade bitumen and oxidised grade bitumen at temperatures similar to application temperatures. Therefore the differences in acenaphthene profile between these two groups indicate that the source of acenaphthene was not mainly the bitumen fume and the higher acenaphthene levels observed in group P1 could be due to PAH emission from vehicle exhaust; however we are uncertain. It is worth noticing that the percentage of samples with detectable levels of acenaphthene are higher in occupationally PAH-exposed groups than in group M.

In contrast to acenaphthene, phenanthrene is normally detected in GC/MS traces of PAH emissions from paving asphalt (Eldridge *et al.* 1982). Brandt *et al.* (1985) concluded that phenanthrene constituted well over 50 % of the total measured PAHs in bitumen fumes; however in their study they did not measure naphthalene and acenaphthene levels. The results of personal air samples of group R2 seem to confirm what the above mentioned investigators reported if naphthalene levels are ignored; while those of groups of pavers do not. Pooling group R2 and R1 changes this observation and median TWA concentration of phenanthrene would be reported as equivalent to that of acenaphthene in air samples collected

during mastic asphalt roofing. This example shows the impact of combining ambient monitoring data from two surveys on risk evaluation. No studies have measured both acenaphthene and phenanthrene in a roofing industry to compare to our data. On the other hand, the results of studies that have investigated PAH profile during paving operations are conflicting. Some have shown that phenanthrene levels are higher than acenaphthene (Lesage *et al.* 1987 and Brandt and Cordingley, 1992) while others have shown the reverse (Monarca *et al.* 1987). Therefore it is difficult to resolve whether the distribution of phenanthrene levels with respect to total PAHs could be a potential indicator that the source of this PAH is largely attributed to bitumen fume; especially that a phenanthrene levels in outdoor air (54 ng/m^3) reported by Wilson *et al.* (1991) could be as high as those reported for groups M, R1, and P2.

The TWA concentration profile of acenaphthene and phenanthrene in different groups collated from all surveys are not really conclusive in regard to the source responsible for their presence in personal air samples. It is important to notice that the percentage of samples with detectable levels of either of the 3 ringed PAHs are higher in occupationally PAH-exposed groups than in group M. Therefore the percentage of samples with detectable levels might be potentially a better indicator than the profile, to justify that bitumen fume is possibly a main source of workers' exposure to these PAHs.

13.2.3 Profile of Four Ringed PAHs

The percentage of samples with detectable levels of pyrene was much lower in group M than in all other groups. Pyrene was higher in roofers group R1, who appeared to be exposed only to background levels of PAHs, was than both pavers. This shows that the roofers exposure to pyrene is probably attributed to occupational exposure to bitumen fume rather than other sources.

Pyrene is usually detectable in air samples collected during paving operations. Brandt and Cordingley (1992) reported a range (1.06 - 6.98 ng/m^3) for its TWA concentration in personal air samples of workers resurfacing a motorway. Monarca *et al.* (1987) and Brandt *et al.* (1993) did detect it in their ambient monitoring surveys of workers doing road

surfacing using penetration grade bitumen. Pyrene levels observed in our study, for groups of pavers (3.6 and 7.0 ng/m^3) were similar to those measured by Brandt and Cordingley, but lower than levels that was observed in outdoor air (9.4 ng/m^3) by Wilson *et al.* (1991). This may suggest that pyrene exposure of groups R1, P1 and P2 is unlikely to be due to bitumen fume exposure. On the other hand, the percentage of samples with a detectable level of pyrene in occupationally PAH-exposed groups (i.e. group P1, P2, R1 and R2) tends to be higher than that of group M suggesting that it was.

Median TWA concentration recorded for B(a)A at non-detectable levels for all groups except group R2 which was 40.2 ng/m^3. This observation seems to imply that group P1, P2 and R1 had a similar "background exposure" to this PAH; while that of R2 was possibly more linked with exposure to bitumen fume. The percentage with samples with detectable level of B(a)A in occupationally PAH-exposed groups (i.e. group P1, P2, R1 and R2) tends to be higher than that of group M suggesting that it could be emanating from exposure to bitumen fume.

13.2.4 Profile of Five Ringed PAHs

The concentrations of each of the 5 ringed PAHs (i.e. B(b)F & B(k)F, B(a)P and DiB(a,h)A) were lower than what was reported in other studies (Monarca *et al.* 1987, Brandt and Cordingley, 1992) and are even lower than what was measured by Wilson *et al.* (1991) in outdoor air. The main indication that exposure to 5 ringed PAHs could be due to occupational exposure to bitumen fumes is the higher percentage of samples with detectable levels of each of them in occupationally PAH-exposed groups in comparison to group M.

13.2.5 Summary of PAHs Profile

The profile of TWA concentrations of all 9 PAHs seemed to indicate that the exposure of groups R1, P1 and P2 are similar to that of group M; while that of group R2 was obviously different. This may indicate that the PAH-exposure of groups P1, P2 and R1 could be considered "environmental"; however that of group R2 is more likely to be occupational. A different conclusion may be reached if the percentage of

samples with detectable levels of individual PAHs is compared between groups. Hence interpretation of the PAH-profile for risk evaluation of exposure to bitumen fume should be looked at in more detail especially when levels are as low as those in this study. So, summarising PAH profiles in groups by looking at means or medians of TWA concentration may be slightly misleading especially if assessment of risk is to be done at such low and almost non-detectable levels of exposure. Moreover, the pooling of PAH-profile data of groups with similar occupations may significantly alter the overall conclusion reached from a risk assessment of PAH-exposure of a particular subgroup.

13.3 TWA Concentration of Airborne PAH-Exposure Indices

It is generally accepted that in industrial hygiene the results of air sampling are usually log normally distributed. Hence the data of the different indices of airborne PAH-exposure was summarised using medians and ranges of daily TWA concentration for all different groups. The coefficient of variation of the daily median TWA concentration was used as an indicator of inter-day variability in workers' PAH-exposure.

<u>∑9PAHs TWA concentration</u>: The main observation from this index was the higher variability in external exposure to PAH reflected by higher CVs in groups M and R1. This variability was probably related to their exposure to very low background "noise" levels of PAHs. This may be confirmed by the lower variability observed in groups P1, P2 and R2 whose exposure is thought to be more occupationally related which is normally less variable and less influenced by uncontrollable weather conditions.

The effect of combining data from different subgroups was clearly manifested in the observed CV of the roofers group which did not reflect those of the subgroups. The conclusion drawn above from the inter-day variability of external exposures regarding the source of PAH in the working environments of the subgroups will no more be valid. Actually, the reverse could be deduced. The CV calculated for the roofers group (RF) seems to resemble that of group M, which may indicate that the group RF is exposed to levels near enough to background concentration.

This index includes naphthalene concentration which did not originate from bitumen fume. This deduction about the source of naphthalene is reflected in the widest ranges of TWA concentrations seen on all three days for group P2. The wide ranges are less likely to be an artefact of sampling or analysis since the maximum TWA concentrations of Σ9PAHs was attributed to naphthalene. Moreover, the personal air samples with the highest concentrations belonged to the raker working behind the paving machine (P2 # 69) and the paving machine driver, who theoretically are exposed to higher concentrations of vehicle exhaust than other pavers.

Σ8PAHs TWA concentration: This index, as described before in the chapter 12, refers to the sum of TWA concentration of all PAHs except naphthalene. It was logical to consider this index along with Σ9PAHs as an airborne PAH-exposure index since our aim is to assess the risk of workers' exposure to PAHs emitted from hot bitumen with much less emphasis on the other sources such as diesel fumes.

What was observed and concluded from studying this index of exposure was similar to that of Σ9PAHs. The only difference was apparent in the ranges of TWA concentration of group P2. They were no more the highest among all groups. In fact the ranges reported for group R2 were about 6.5 and 3 fold higher than group P2 on days 1 and 2, respectively. This would somehow be expected since naphthalene, which is the main PAH-species behind the wide ranges in group P2, is believed to originate from sources other than bitumen fume. Therefore this index could be of more use for estimating the risk from ambient exposure to PAHs arising from bitumen at high temperatures.

Σ4,5 PAHs TWA concentration: This index, as described before in the chapter 12, refers to the sum of TWA concentration of 4 and 5 ringed PAHs analysed for in this study and which are classified as carcinogens by IARC. Obviously, this index is the most appropriate of the ones we studied for characterising the risk from exposure to carcinogens in bitumen fumes.

Four and five ringed PAHs emitted from paving asphalt under controlled laboratory conditions were found in minor quantities (Eldridge *et al* . 1982). In the study of Brandt and co-investigators, these PAHs were

found in bitumen fumes generated from penetration and oxidised bitumen in relatively low quantities in comparison to PAHs with MWt ≤ 202. The only exception was B(a)A which was present in comparable levels.

A number of field studies examining PAH-exposure during paving and roofing operations confirmed the results of these experimental studies. For instance 4 and 5 ringed PAHs were non-detectable in area air samples collected during the handling of hot bitumen by pavers and roofers (Lesage *et al.* 1987). Another study showed that the average of the sum of TWA concentrations Σ4,5 and 6 PAHs were measured at levels lower than 5 ng/m^3 during road paving (Brandt and Cordingley 1992). Average levels recorded for the sum Σ4,5 and 6 PAH during mastic asphalt roofing were at least 20 times higher than those measured for pavers (Brandt *et al.* 1985).

Results in this study are similar to what has been observed in regard to exposure to Σ4,5 PAHs in roofing and paving industries. Median TWA concentrations of this exposure index were at non-detectable levels for both groups of pavers. These non-detectable levels imply that these high molecular weight PAHs are present in very low concentrations in bitumen fume. The detection limit of the assay used for quantifying PAHs in our labs was 10 ng/m^3. The recoveries acheived for the these PAHs was lower than those of low molecular weight PAHs. This may explain why we did not observe levels as low as Brandt and Cordingley. As for the roofers group R2 but not R1 had median TWA concentrations (highest on day 1; 25.5 ng/m^3) comparable to those (56 ng/m^3) observed by Brandt *et al.* (1985).

The results of our ambient monitoring program are quite similar to what has been reported in the literature. Therefore the high percentage of samples with non-detectable levels of these 4 and 5 ringed PAHs in all groups except group R2 is less likely to be an artefact of the sampling or analytical procedure used for measuring them in personal air samples of workers. Therefore it possible to conclude that group M and R1 hardly had any exposure to these compounds. The pavers were obviously less exposed than the roofers in group R2.

One should be careful in interpreting results of risk assessment of exposure to a carcinogenic hazard. This is due to the fact that carcinogens are believed to have no "no effect threshold level". With respect to the risk assessment of exposure to high molecular weight PAHs in bitumen fume condensates there is some doubt whether the PAH-profile properly represents the carcinogenic potential of this exposure (Brandt *et al.* 1993). Therefore we can conclude that the level of exposure to carcinogenic PAHs is highest in group R2; however, the level of risk arising from such an exposure may not be highly elevated in comparison to the other groups.

13.4 PAHs External Exposure in Different Job Categories

Three-day-geometric mean and 95 % confidence intervals were used to summarise the external exposure of individuals to different airborne PAH-exposure indices quantified in their personal air samples; while means ± SEMs of individual geometric means were used to summarise the group's average external exposure to these indices.

13.4.1 External exposure to Σ9PAHs

It was observed that the mean of individual 3-day GM of TWA concentrations of Σ9PAHs was found in the following increasing order between the groups:

$$R1 \leq M < P1 < P2^{*} \leq R2^{*}$$

(The groups denoted with a star had a mean exposure significantly different from groups M and R1). Although group P1 had mean exposure levels approximately 3 times higher than group M and R1 there difference was not significant. This is probably due to the small numbers found in that group.

Manual workers: It is quite obvious that some of the workers in the group M (M # 41 and 45) were exposed to PAH levels as high as occupationally PAH-exposed groups. This indicates that they were possibly misclassified as controls. Since direct on site observation was not possible we are not sure that their exposure is purely environmental since they

might have been working in the vicinity of mastic asphalt roofing process. Regardless of the source of their exposure this observation should be taken into consideration especially when looking at biomarkers of risk assessment.

In more recent literature, misclassification is being acknowledged by investigators assessing the risk of occupational exposure to PAHs. It is mainly related to the presence of considerable levels of PAH in the general environment. Hemminki (1992) observed relatively high PAH-DNA adducts in controls living in polluted areas resembling those from coke oven workers which suggested that environmental contamination by PAHs can contribute to the no difference observed between the two groups. Ferriera Junior *et al.* (1994) looking at benzo(a)pyrenediol epoxide adducts in PAH-exposed workers addressed the same issue as Hemminki. Omland *et al.* (1994) has addressed the possibility that high air pollutant PAHs exposure in a control group might obscure a real difference in PAHs exposure between occupationally exposed and occupationally unexposed workers. Brandt inferred that in some instances the average occupational exposure to PAHs could be only a factor of two higher than the exposure of an hypothetical individual standing on the side of the motorway, as measured in a static upwind "clean air" sample. These observation imply that it is important to know the personal exposures to PAHs of individuals categorised as controls (Brandt *et al.* 1993).

Group P1: From the results of this PAH-exposure index (Σ9PAHs) it appears that pavers working on the same site and alternating tasks may have different intensity of exposure. For example, the exposure of paver (P1 # 7) quantified by this exposure index was an order of magnitude higher than paver (P1 # 11). Assuming the two pavers are more or less spending the same amount of time on different jobs, then it appears that the workers have different working behaviours affecting their exposure to the fumes. Another factor that could possibly partially explain these differences is the position of the sampling head . For example, we suspect that a personal air sample might have higher quantities of pollutants if placed on the left shoulder of a right handed raker in comparison to a left handed one.

Group P2: In this group the external exposure summarised by the

3-day GM of TWA concentration of Σ9PAHs was in the following decreasing order between the pavers: paver operator >> raker (working behind Blow Knox) > screwman > rakers ≡ tractor driver ≡ foreman ≡ roller drivers. The workers in the vicinity of the paving machine were the most exposed. There are no studies available to directly compare our data to; however, a survey conducted by the Association of Danish Asphalt Industries measuring exposure to bitumen fume as TPM reported a similar pattern between different job description; whereby, the paver operator > screedman > raker (at end of screed) > foreman ≡ roller driver > raker (at a distance from paving machine) (Claydon *et al.* 1984).

It appears that the pavers working near the paving machine were the most exposed. Their high exposure could be due to two things. Firstly, total fume emission is greatly influenced by bitumen temperature and the rate of emission increases by a factor of two for every 11 °C increase in temperature (Brandt, 1992). Since bitumen temperature is suspected to be higher in the paving machine than when applied to the road surface then we expect that pavers working on or close to the machine will have a higher exposure. Secondly, the diesel exhaust of the machine may also contribute to the total external dose of PAHs.

Group R1: Specific job descriptions were not identified in this group of roofers who shared tasks according to need during the working shift. In line with our observation in group P1, it appeared that the Σ9PAH-exposure of roofers working on the same site and sharing the same tasks (e.g. R1 # 20 and 21) can differ by an order of magnitude. This observation indicates, as in group P1, the inaccuracy of relating loosely defined job description to external exposure to PAHs in bitumen fume.

Group R2: In this group the external exposure summarised by the 3-day-GM of TWA concentration of Σ9PAHs was in the following decreasing order between the roofers: kettleman/mixer operator > tipper > troweller (R2 # 89) > troweller (R2 # 88). The kettleman/mixer operator had a higher degree of exposure which was possibly due to the fact that bitumen temperature in his immediate working environment is higher than other roofers leading to higher fume emissions and thus more exposure. It is important to notice that the mixer operators pattern of exposure was not continuos over the whole working shift. He was exposed

to high concentrations of fumes during three short time intervals (10 - 15 minutes) when he fed the mixer with mastic asphalt blocks during the day (see fig. 11.2b). The fact that bitumen temperature is highest in the kettle and the mixer and his exposure is the highest and mainly arising from short acute exposure stresses relationship between exposure to fumes and bitumen temperature.

Group PV and RF: The 3-day-GM of TWA concentrations of Σ9PAHs of the pavers groups was higher than that of roofers group. This shows that the conclusions reached from analysing these summaries in isolation from the results of the subgroups would be different to those observations deduced from results of subgroups analysed separately.

13.4.2 External exposure to Σ8PAHs

The order of hierarchy of exposure between groups according to Σ8PAHs as an index of airborne PAH-exposure was as follows:

$$M < R1 < P2^{*} < P1 \leq R2^{*}$$

The exposure profile across the groups was slightly different from that observed when Σ9PAHs was used as the exposure index. The difference is largely due to the inclusion of the environmentally abundant PAH (naphthalene) in the exposure index Σ9PAHs. It is obvious that its exclusion showed that group R1 does not have similar exposure to group M; however it is higher. Also, the confounding effect of PAH-emission sources other than hot bitumen on the assessment of exposure to PAH from bitumen fume is minimised.

Manual workers: Manual worker (M # 41 and 45) were also shown using Σ8PAHs as a PAH-exposure index that they are probably misclassified as controls.

Group P1: Although the use of Σ8PAHs as an exposure index minimised the difference between the PAH-exposure of pavers working on the same sight and performing the same jobs. It did not totally eliminate them.

Group P2: Summarising pavers' external exposure using the 3-day GM of TWA concentration of Σ8PAHs still indicated that pavers working on the paving machine were more exposed than others. This was also concluded by Greenspan and co-workers (Greenspan *et al.* 1995). The ambient exposure profile across the ten pavers in group P2 was as follows: paver operator >> foreman > screwman > rakers ≅ tractor driver ≅ roller drivers. The reasons behind the relatively high exposure of the foreman is unknown.

The paver operator's average TPM TWA concentration in the study by the Association of Danish Asphalt Industries was 1.7 mg/m^3. If we calculate the Σ14PAH-exposure of the paver operator from his TPM value as suggested in heading 4.4.1 of chapter 4, we obtain a value of 1460 ng/m^3. This value is 2.5 times that of the paver driver in this study. They are more or less comparable especially that we have measured eight PAHs and not 14, which obviously augmented the observed difference. Similar values are obtained if the calculation are applied to exposure of other pavers. Therefore our suggestion that a TLV of 4.3 $\mu g/m^3$ for the Σ14PAHs would reflect the TLV of 5 mg/m^3 for bitumen fume is possible.

Group R1: The use of Σ8PAHs as an exposure index did not diminish the difference between the PAH-exposure of roofers working on the same sight and performing the same jobs. This may signal that naphthalene is present homogeneously in their working environment suggesting that it is originating mainly from one source (i.e. background pollution) and not several as in the case of group P1.

Group R2: The profile of external exposure between the four roofers in this group remained the same on using Σ8PAHs as an airborne PAH-exposure index instead of Σ9PAHs. This made us arrive to the same conclusion as the one figured from the results of group R1; whereby naphthalene levels are homogeneously distributed in their working environment and their exposure to it is most probably environmental.

Group PV and RF: On studying the results of external exposure to Σ8PAHs in the combined groups we arrived at the conclusions similar to the ones deduced from the results of subgroups. In general the roofers' exposure to PAHs is higher than that of pavers. This is in agreement with

the ranking that Brandt *et al.* (1985) found on comparing external exposure to bitumen fume in the paving and roofing industries using TWA concentration of TPM and BSM. Malaiyandi *et al.* (1982) also arrived at the same conclusion in their study. Our observation is also reflected in the results obtained from independent studies that have monitored external exposure to PAHs in both industries (see table 5.1). Overall these surveys show that roofers are more exposed to PAHs than pavers.

13.4.3 External exposure to Σ4,5 PAHs

In general the usefulness of measuring exposure to 4,5 ringed PAHs was of limited use at the low levels of exposure to PAHs seen in this study. This is largely because four and five ringed PAHs are present in low quantities in bitumen fume (Brandt *et al.* 1985) and analytical methods are insufficiently sensitive for detecting them in small air volumes samples such as those collected by personal air monitoring techniques. This does not mean that this index should overlooked since it best characterises the risk from the carcinogenic component of bitumen fume.

In group R2 the Σ4,5 PAHs profile of external exposure of the four roofers was again exactly the same as the profile for Σ8PAHs described above. The highest exposure according was that of the kettleman. As previously clarified this is related to the worker's proximity to the source of release (i.e. in this case the kettle or the mixer). Of course these contain bitumen at the highest temperature which is also has been related to degree of exposure. Our results are supported by those of Brandt *et al.* (1985) who also observed that the kettleman is exposed to higher TWA concentrations of Σ4,5 and 6 PAHs than other roofers. This however was not strictly the case in the study by Malaiyandi *et al.* (1982) where they did not always observed higher exposure levels for the kettleman in relation to the other operators. In general it seems that Σ4,5PAHs, used as an airborne PAH-exposure index, does reflect the overall exposure of the workers and could be useful in quantitatively assessing risk of exposure to bitumen fume especially when exposures are not very low.

13.5 Correlations Between PAH-species

Correlation were studied between logarithmic transformation of the TWA concentration of the sum of both phases of specific PAH-species and that of the sum of a selection from other quantified PAHs in personal air samples of subgroups, combined groups and all groups pooled together.

The purpose of this was to find the single PAH that would best predict the overall exposure to PAHs in bitumen fumes. This approach will eventually reduce the number of factors to be determined for while carrying a risk assessment of exposure to bitumen fume. The choice of the studied PAH-species depended mainly on its abundancy (i.e. if it could be detectable in a considerable number of samples). Other characteristics such as the availability of a selective biomarker for quantifying its internal dose were also taken into consideration.

Naphthalene, phenanthrene and pyrene were chosen. Naphthalene and phenanthrene were present at high levels in personal air samples from roofers and pavers. They both have selective biomarkers that have been studied (Bieniek, 1994 and Grimmer, *et al* 1993). Pyrene, although less abundant, was studied because in parallel the internal dose was determined by measuring its selective metabolite 1-HP. It would have been ideal to have studied B(a)P, since it would best reflect the carcinogenic risk involved in such exposure; however this was not possible since it was detected in a small number of samples. In addition, the results collated could be related to its occupational exposures limits of 5 and 10 $\mu g/m^3$ in Sweden and Finland, respectively (Jongeneelen, 1987[a]). Of course this was not possible because it was not detectable in most of the personal air samples.

Naphthalene levels correlated significantly with Σ8PAHs in samples of all groups except roofers group R2. This possibly indicates that when the PAH-exposure is more occupationally related (i.e. originating from exposure to bitumen fume) rather than environmentally, naphthalene ceases to be a good index for predicting the overall exposure to PAHs. This is even more clearly manifested in its non-significant correlations with sum of four and five ringed PAHs (Σ4,5PAHs), which are believed to stem from bitumen fume. This fact was hidden when

naphthalene was correlated with Σ8PAHs in personal air samples of the roofers group as well as all groups, which highlights the importance of manipulating the data at subgroup levels first before pooling and summarising it from several surveys.

In conclusion, exposure to naphthalene is not reliable in predicting the total exposure to PAHs in pavers and roofers since it is unlikely that hot bitumen is its source.

Phenanthrene appears to be better than naphthalene in predicting the overall exposure to PAHs in bitumen fume. Phenanthrene levels correlated highly significantly with Σ8PAHs, Σ8PAHs - [naphthalene] and more importantly Σ4,5PAHs in personal air samples of all groups. The only exception was the non-significant correlation with Σ4,5PAHs in personal air samples of group R1. This could be due to the very low level of PAH-exposure this group was subjected to.

The results suggest that the log-TWA concentration of phenanthrene is potentially a suitable index for predicting worker's exposure to ΣPAHs. This however should be investigated further in more detail in other bitumen user industries.

Pyrene was not significantly correlated with Σ8PAHs in personal air samples of all groups except group R2. The exclusion of naphthalene from Σ8PAHs made the correlation even more significant in group R2. The exclusion of the 3 ringed PAHs led to a significant correlation between pyrene and Σ4,5PAHs in group R1 as well as group R2. Two important deductions could be made from these observations. Firstly, the source of pyrene is more likely to be from bitumen fume rather than the general environment. Secondly, its significant correlation with Σ4,5PAHs in personal air samples of all subgroups and combined ones suggests that it is better than either of phenanthrene or naphthalene as an index for assessing the carcinogenic risk of exposure to bitumen fume. Its only drawback is its relative low abundance at low levels of exposure to bitumen fume when compared to phenanthrene.

13.6 Summary of Discussion

1) The results show that at low levels of PAH-exposure from bitumen fume the TWA concentration profile of PAHs is not the optimum tool for characterising workers' exposure with respect to the identity of the source of PAH-emission. In our case, the percentage of samples with detectable levels of PAHs was a better indicator whether the pavers and roofers exposure was mainly occupational or not.

2) The presence of high concentrations of naphthalene in personal air samples of pavers signalled the presence of a PAH-emission source in the occupational environment other than hot bitumen, especially that our results suggest that it does not mainly stem from bitumen fumes.

3) It was not possible to identify the source of the 3 ringed PAHs in the occupational environments studied; however there is some evidence that the presence of acenaphthene, but not phenanthrene, in personal air samples is principally due sources other than bitumen fume. The reverse was concluded for the 4 and 5 ringed PAHs which indicated that exposure is more likely to be due to occupational exposure to bitumen fume.

4) Sampling of PAHs from both the vapour and the particulate matter phase is important since it provides essential information about the PAH-profile and thus the toxicity of the PAH-mixture which is an important factor to consider while carrying a risk assessment. In this study the exposure profile was typical to Class 1 industries (Lesage *et al*, 1987). It was dominated by PAHs of low molecular weight in the gaseous phase indicating relatively low toxicity.

5) It seems of importance to quantify the environmental PAH level, on the day of personal air sampling, since it seemed to contribute to the workers' occupational PAH-exposure. The high CV of the median TWA concentration of airborne PAH-exposure indices in group M illustrated that this contribution could vary considerably between days. The measurement of environmental concentrations of PAHs will aid to avoid misclassification while allocating workers to exposed or unexposed groups.

6) Relating loosely defined job descriptions to exposure does not seem appropriate; while if properly determined (as was the case of group P2 and R2) it could aid in the overall risk assessment of PAH-exposure in the paving and roofing industries. In a way this shows that the subjective assessment of the working condition could be of great help for the hygienist in interpreting the results of field exposure measurements.

7) Among the pavers those working in the vicinity of the paving machine were the most exposed; among the roofer it was the kettleman/mixer operator. In the former group the high exposure was probably due to diesel exhaust; while in the latter it was the proximity of the worker to the source of emission.

8) There is a potential for higher PAH-exposure in the roofing industry more than in the paving industry. This was also the observation of other investigators (Malaiyandi, *et al.* 1982, Brandt *et al.* 1985). This is possibly attributed to the higher bitumen application temperature in the roofing industry.

9) Phenanthrene appears to be a most suitable single PAH for predicting worker's airborne exposure to the sum of selected PAHs in bitumen fume. This however should be further investigated in more detail in other bitumen user industries.

10) Weather condition recorded were fairly similar between different surveys and it was unlikely that they have considerable effects on the exposure of the workers.

11) Assessment of exposure to PAHs in bitumen fume during paving and roofing operations is quite complicated owing to three factors. First their presence at very low levels. Secondly, the presence of other PAH-emission sources in their occupational environment as well as the background environmental PAH-pollution that could confound our observations. Thirdly, complexity of the PAHs mixture present in bitumen fume. Therefore, this demands that careful data collection, handling, manipulation and interpretation is required. Studying the data should commence at its raw stages and conclusions should not only be drawn from summary results.

CHAPTER 14

RESULTS OF

URINARY THIOETHERS

14.1 INTRODUCTION

This chapter describes the results obtained from the urinary thioether assay. Pre-shift and post-shift urine samples collected on the first three days of each survey from subjects in groups C2, M, P1, P2, R1 and R2 were analysed for thioether using the method described in section II, chapter 7 of this thesis. The results are expressed as UTh. mmol/mol. creatinine.

Pre-shift and post-shift urinary thioether levels are compared between group C2 and M, P1, P2, R1, R2 as well as the pooled groups of pavers and roofers on all three days of the surveys.

The difference between the urinary thioether output in morning and afternoon urine samples (i.e. change in urinary thioether output) are calculated for each individual. The mean of these differences are compared within each group on all three days. They are also compared between groups as well as the pooled groups of pavers and roofers on all three days of the surveys.

Differences between mean values (i.e. mean of pre-shift samples, mean of post-shift samples and mean of change in output) of all subgroups and pooled groups is tested for statistical significance using analysis of variance (ANOVA single-factor). The null hypothesis (no difference between means) is rejected for variance ratios greater than the critical *F*-values. Significance is reported at 95% confidence level ($\alpha = 0.05$) with ($p < 0.05$).

14.2 Mean Pre- & Post-shift UTh. Output

Day One

Figure 14.1 presents the pre-shift and post-shift mean urinary excretion of thioether in group C2, M, P1, P2, R1, R2, pavers group (PV) and roofers group (RF) group on days one.

Manual worker M # 38 and # 41 pre-shift urine samples of day 1

were lost during analysis, therefore the total number of pre-shift samples in group M on day 1 was 14. Roofers R1 # 20 and # 21 did not provide either a pre-shift or a post-shift urine sample on day 1. The morning sample of roofer R2 # 36 was lost during analysis. Therefore the total number of pre-shift samples was six while the post-shift was seven in group R2.

The mean of pre-shift samples in group C2 (mean ± SEM; 4.68 ± 0.73) was lower than that of group M (5.23 ± 0.72) as well as those of all other occupationally PAH-exposed groups excluding group R2 (3.08 ± 0.48). The mean of pre-shift in pavers group (PV) (6.41 ± 1.09) was similar that of both groups P1 (6.36 ± 0.90) and P2 (6.44 ± 0.71). The mean of pre-shift in roofers group (RF) (6.41 ± 1.09) did not represent that of group R1 (8.09 ± 0.90) or that of group R2 (3.08 ± 0.48). The means of pre-shift UTh. output in all groups were not significantly different (ANOVA single-factor; $p > 0.05$).

The mean post-shift UTh. output in group C2 (4.40 ± 0.64) was lower than those of all occupationally PAH-exposed groups as well as group M (5.71 ± 0.68). The mean of UTh. excretion in afternoon samples of pavers group (PV) (4.57 ± 0.38) was close to that of both subgroups P1 (4.88 ± 0.70) and P2 (4.39 ± 0.46). Similarly, the mean of UTh. excretion in post-shift samples of roofers group (RF) (5.59 ± 0.94) close to the means of both subgroups R1 (6.14 ± 0.70) and R2 (4.87 ± 0.46). The difference between the means of pre-shift UTh. output in all groups did not reach statistical significance (ANOVA single-factor; $p > 0.05$).

Day Two

Figure 14.2 presents the pre-shift and post-shift mean urinary excretion of thioether in group C2, M, P1, P2, R1, R2, pavers group (PV) and roofers group (RF) group on day two.

The pre-shift urine sample of subject (M # 35) and paver (P1 # 11) of day 2 were lost during analysis. This meant that the total number of morning samples in group M and group P1 was 14 and 5, respectively.

The mean of pre-shift samples in group C2 (2.61 ± 0.36) was lower

than that of group M (3.73 ± 0.45) as well as those of all other occupationally PAH-exposed groups excluding group R2 (2.44 ± 0.98). The mean of pre-shift UTh. output in pavers group (PV) (3.78 ± 0.59) was between that of group P2 (2.75 ± 0.45) and P1 (5.49 ± 1.10). Similarly, the mean of UTh. excretion in pre-shift samples of roofers group (RF) (3.43 ± 0.68) was between that of group R2 (2.44 ± 0.98) and group R1 (4.19 ± 1.10). The means of pre-shift UTh. output in all groups were not significantly different (ANOVA single-factor; $p > 0.05$).

The mean post-shift UTh. output in group C2 (3.74 ± 1.20) was lower than those of all occupationally PAH-exposed groups as well as group M (4.69 ± 0.48). The mean of UTh. excretion in afternoon samples of pavers group (PV) (4.10 ± 0.62) was close to that of both subgroups P1 (4.77 ± 1.30) and P2 (3.77 ± 0.69). Similarly, the mean of UTh. excretion in post-shift samples of roofers group (RF) (5.59 ± 0.94) was close to the means of both subgroups R1 (6.17 ± 1.30) and R2 (4.61 ± 1.06). The difference between the means of pre-shift UTh. output in all groups did not reach statistical significance (ANOVA single-factor; $p > 0.05$).

Day Three

Figure 14.3 presents the pre-shift and post-shift mean urinary excretion of thioether in group C2, M, P1, P2, R1, R2, pavers group (PV) and roofers group (RF) group on days three.

Only manual worker number M # 35 did not provide a post-shift urine sample on day 3. This meant that the total number of afternoon urine samples for group M on day 3 was 14.

The mean of pre-shift samples in group C2 (2.65 ± 0.36) was lower than that of group M (4.22 ± 0.60) as well as those of all other occupationally PAH-exposed groups. The mean of pre-shift in pavers group (PV) (4.39 ± 0.69) was between that of group P2 (2.85 ± 0.42) and P1 (6.94 ± 1.11). Similarly, the mean of pre-shift in roofers group (RF) (3.29 ± 0.30) was between that of group R2 (2.75 ± 0.41) and group R1 (3.70 ± 1.11). The means of pre-shift UTh. output in all groups were not significantly different (ANOVA single-factor; $p > 0.05$).

This was the only occasion were the mean post-shift UTh. output in group C2 (4.40 ± 0.64) was higher than those of all occupationally PAH-exposed groups - except group P1 - as well as group M (4.61 ± 0.84). The mean of UTh. excretion in afternoon samples of pavers group (PV) (4.51 ± 0.62) was ~ half that of P1 (8.17 ± 2.76) and ~ double that of P2 (2.50 ± 0.34). It is worth noting here that two subjects in group P1 (P1 # 11 and # 13) had the UTh. output of 14.98 and 18.20, respectively. These were the highest two observation among all the samples of all volunteers on all days. The mean of UTh. excretion in post-shift samples of roofers group (RF) (5.05 ± 0.80) was very close to the means of both subgroups R1 (5.45 ± 2.76) and R2 (4.51 ± 1.20). The difference between the means of pre-shift UTh. output in all groups did not reach statistical significance (ANOVA single-factor; $p > 0.05$).

14.3 Change Between Pre-shift and Post-shift UTh. Output

Figure 14.4A shows the change (i.e. difference) in urinary thioether output between pre-shift and post-shift urine samples for each of the individuals of the office workers group (group C2) as well as the mean of these differences on days 1, 2 and 3. In parallel, figure 14.4B shows the differences and the mean of differences observed in the group of manual workers (group M) on all three days. Similarly, figures 14.5A and 14.5B show those of groups P1 and P2, respectively. Figure 14.6A and 14.6B present the difference in UTh. output between morning and afternoon urine samples for each of the individuals of the groups of roofers (group R1 and R2) as well as the mean of these differences on days 1, 2 and 3. Finally, figures 14.7A and 14.7B display these differences and their means observed in the pavers group and the roofers group, respectively.

Day One

Figure 14.4A shows that out of 6 subjects in group C2 3 had a positive difference and 3 had a negative difference. In group M thirteen differences were calculated. The results of two individuals M # 38 and # 41 were excluded from the analysis because they did not provide pre-shift urine samples. Out of the thirteen subjects, 4 had a negative difference (fig. 14.4B). In group P1, all differences were negative except one (fig. 14.5A) and

in group P2 the majority of the differences (7 out of 10) were also negative. From figure 14.6A, it can be observed that the 5 out of 6 differences were negative in group R1. In parallel, figure 14.6B shows that all changes between morning and afternoon UTh. output in group R2 were positive. Three out of all 16 differences in the pavers group were positive (fig. 14.7A); while 50 % of the 10 differences calculated for the roofers group were positive (fig. 14.7B).

The office workers group C2 had the smallest mean of the differences in absolute value (-0.28 ± 0.96; fig. 14.4A). The only two group with a positive mean for the differences were group M (0.45 ± 0.95; fig. 14.4B) and group R2 (1.80 ± 0.63; fig. 14.6B). Group P1, P2, and R1 had means for the differences of (-1.48 ± 0.54; fig. 14.5A), (-2.06 ± 1.59; fig. 14.5B) and (-1.95 ± 1.52; fig. 14.6A), respectively. Figures 14.7A and 14.7B show that the mean for the differences in the pavers group was (-1.84 ± 1.00) and that of the roofers group was (-0.45 ± 1.09), respectively. The null hypothesis was found to be true; whereby all the means of the differences in all groups were not significantly different from each other (ANOVA single-factor; $p > 0.05$).

Day Two

Figure 14.4A shows that out of 6 subjects in group C2 3 had a positive difference and 3 had a negative difference. In group M out of the 15 subjects, 5 had a negative difference (fig. 14.4B). In group P1, paver number (P1 # 11) did not supply a post-shift sample. The number of differences were 5 for that group on day 2. Out of these 5 changes, three were negative (fig. 14.5A). In group P2, the majority of the differences (7 out of 10) were positive. From figure 14.6A it can be observed that the 2 out of 9 individuals in group R1 had negative differences; while, figure 14.6B shows that 3 subjects in group R2 had positive changes between morning and afternoon UTh. output. Twenty five percent of the pavers group were observed to have negative changes (fig. 14.7A). A similar percentage (23 %) of subjects in the roofers group had also negative changes (fig. 14.7B).

The mean of the differences in the office workers (group C2) was (1.13 ± 1.17; fig. 14.4A). This was higher than the mean of the differences in

group M (0.47 ± 0.62; fig. 14.4B), group R1 (1.08 ± 0.83; fig. 14.6A), group P2 (1.03 ± 0.87; fig. 14.5B) and the pavers group (0.61 ± 0.73; fig. 14.7A). Both group R2 and roofers group had means of the differences higher than that of group C2 (fig. 14.6B and fig. 14.7B). The former's mean of the differences was (2.17 ± 1.74) and the latter's was (1.41 ± 0.75). The only group with a negative mean of the differences was group P1 (-0.22 ± 1.38; fig. 14.5A). It was found that there is no statistically significant difference between all the means of the differences between all groups on day 2 (ANOVA single-factor; $p > 0.05$).

Day Three

Figure 14.4A shows that all subjects in group C2 had a positive difference of UTh. excretion between pre-shift and post-shift urine samples. The changes for each of the individuals in group P1 and P2 are shown in figure 14.5A and 14.5B, respectively. There was two pavers in group P1 and four group P2 who had a negative change. The individuals' changes in group R1 and R2 are presented in figure 14.6A and 14.6B, respectively. In the former group there was four negative differences observed. In the latter group there was one out of the four roofers with a negative difference.

The mean of the differences in the office workers (group C2) shown in figure 14.4A (3.62 ± 0.25) was highest one between all group on all three days. The manual workers had a mean of the differences on day 3 (0.39 ± 0.82; fig. 14.4B) similar to days one and two. Figure 14.5A and 14.5B report a mean of the difference of (1.23 ± 2.18) for group P1 and a mean of (0.01 ± 0.39) for group P2, respectively. The means of the differences in group R1 and R2 were virtually equal (fig. 14.6A and 14.6B). Finally, the pavers group had a mean of the differences of (0.24 ± 0.85) while the mean of the difference in the roofers group was (1.75 ± 0.70). Once more the differences in the mean of changes between all group did not reach statistical significance inorder for rejecting the null hypothesis (ANOVA single-factor; $p > 0.05$).

Figure : 14.1

Pre-shift and post-shift mean urinary excretion of thioethers in office workers (C2), manual workers (M), pavers (P1 & P2), roofers (R1 &R2), pavers group (PV) and roofers group (RF) on day one

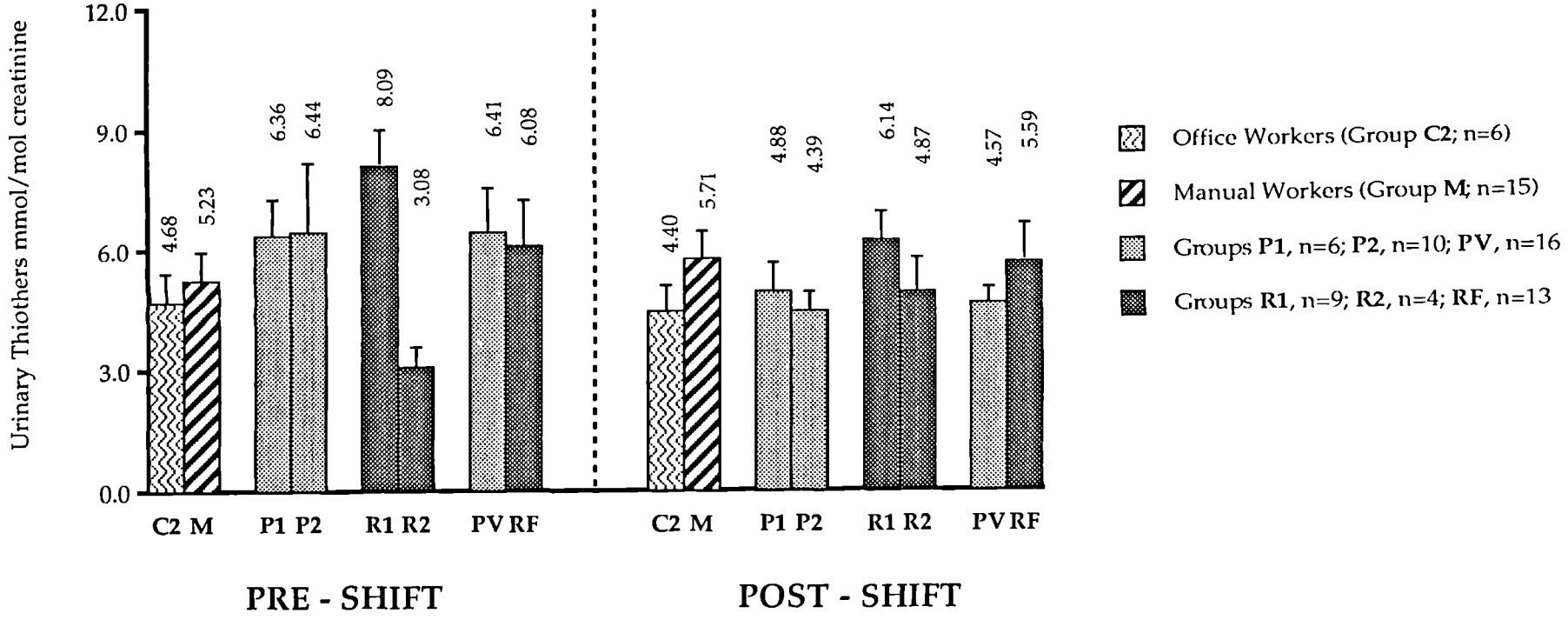


Figure : 14.2

Pre-shift and post-shift mean urinary excretion of thioethers in office workers (C2), manual workers (M), pavers (P1 & P2), roofers (R1 &R2), pavers group (PV) and roofers group (RF) on day two

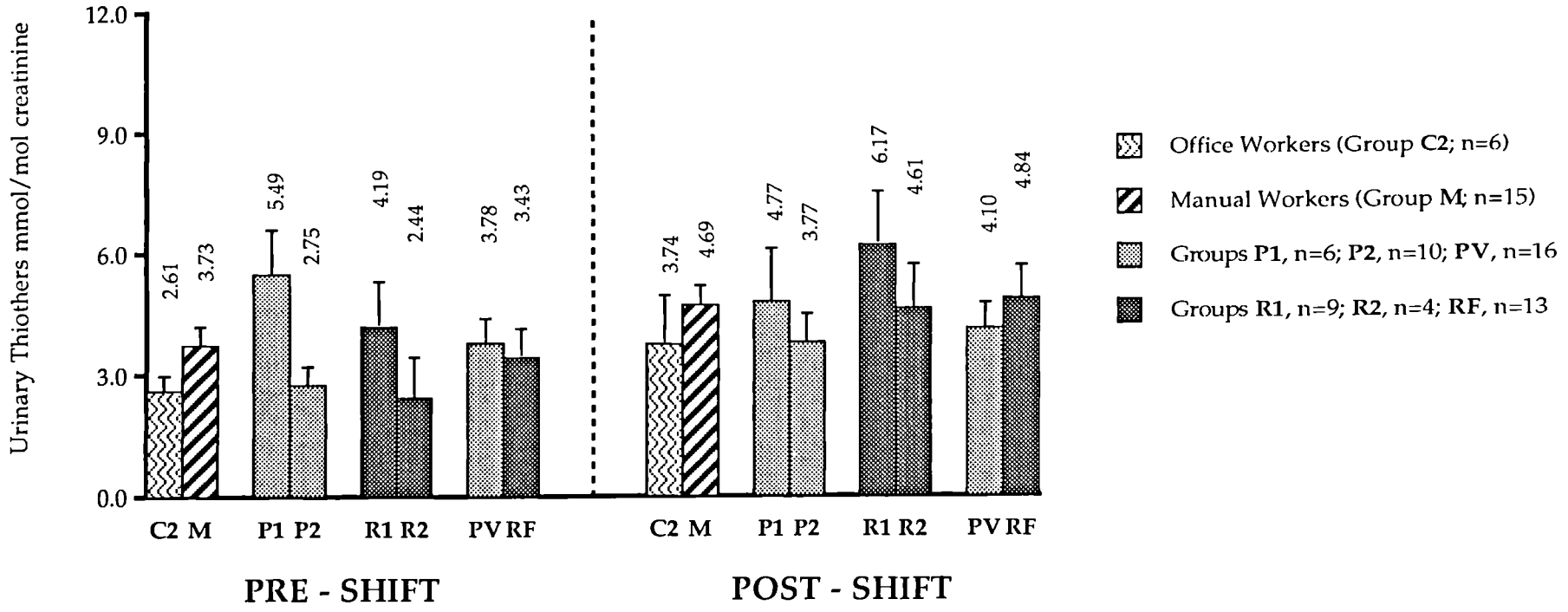


Figure : 14.3 Pre-shift and post-shift mean urinary excretion of thioethers in office workers (C2), manual workers (M), pavers (P1 & P2), roofers (R1 &R2), pavers group (PV) and roofers group (RF) on day three

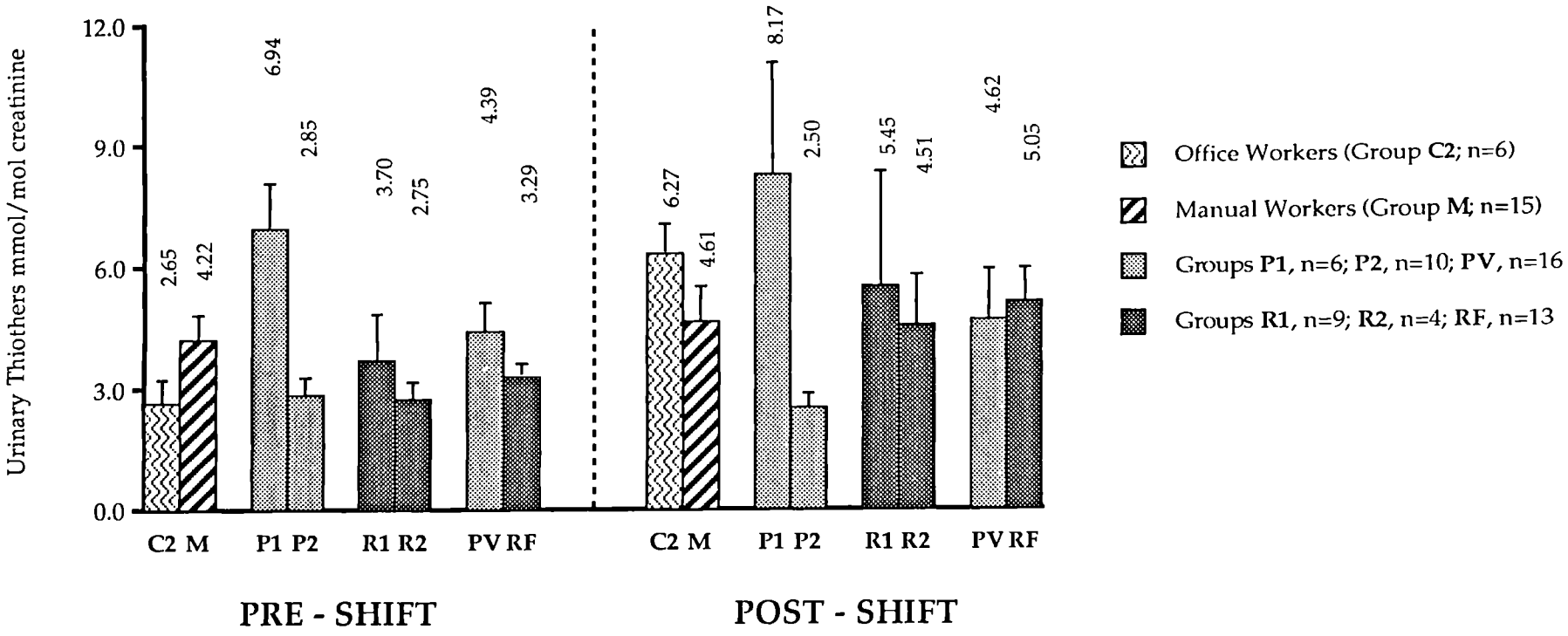


Figure : 14.4 Difference in urinary thioethers output between pre-shift and post-shift urine samples in office workers (group C2; n=6) and manual workers (group M; n=15) on days 1, 2 and 3
- Inter-individual differences and mean ± SEM of the differences -

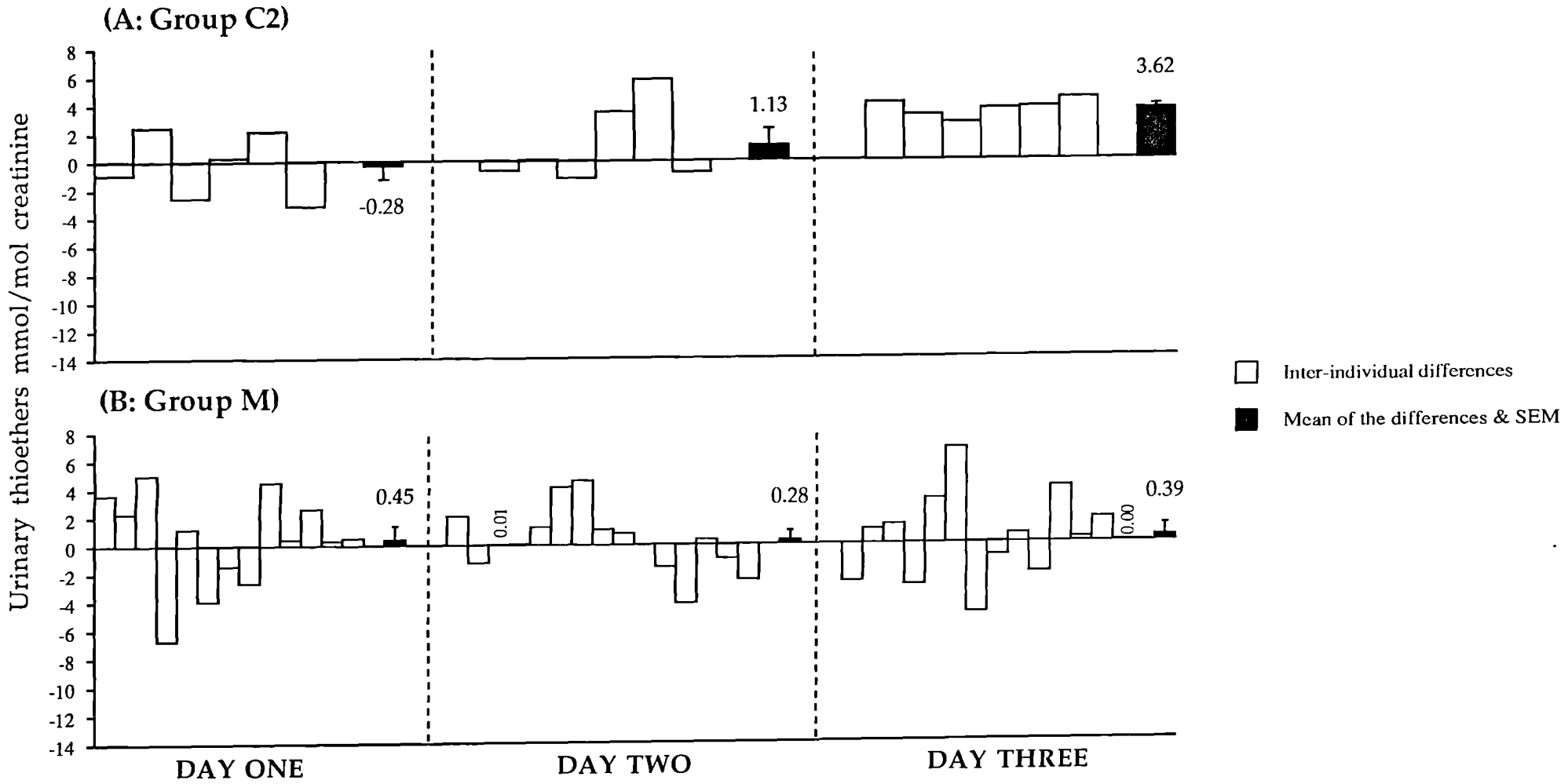


Figure : 14.5 Difference in urinary thioethers output between pre-shift and post-shift urine samples in pavers **group P1** (n=6) and pavers **group P2** (n=10) on days 1, 2 and 3
- Inter-individual differences and mean ± SEM of the differences -

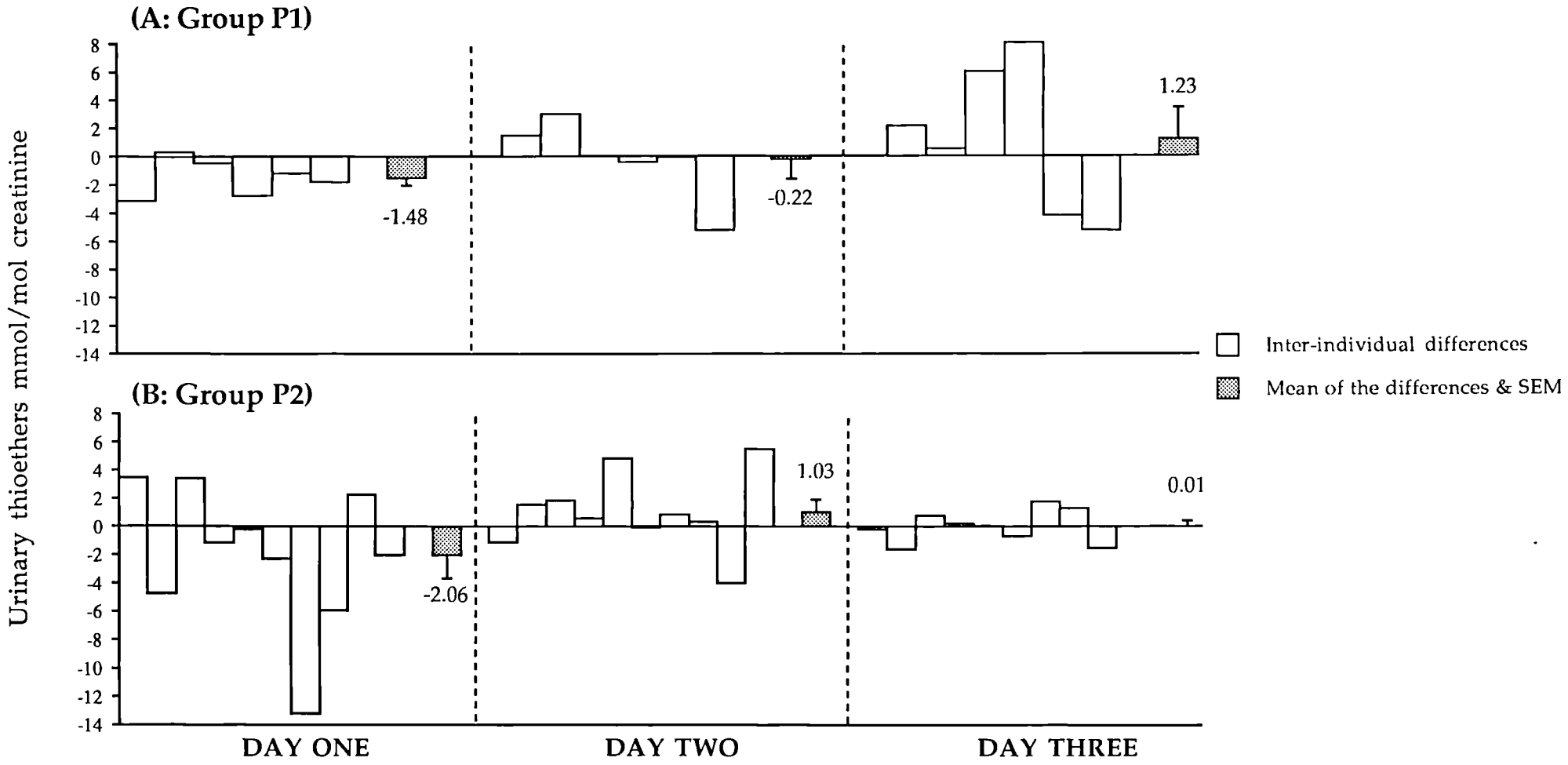


Figure : 14.6 Difference in urinary thioethers output between pre-shift and post-shift urine samples in roofers **group R1** (n=9) and roofers **group R2** (n=4) on days 1, 2 and 3
- Inter-individual differences and mean ± SEM of the differences -

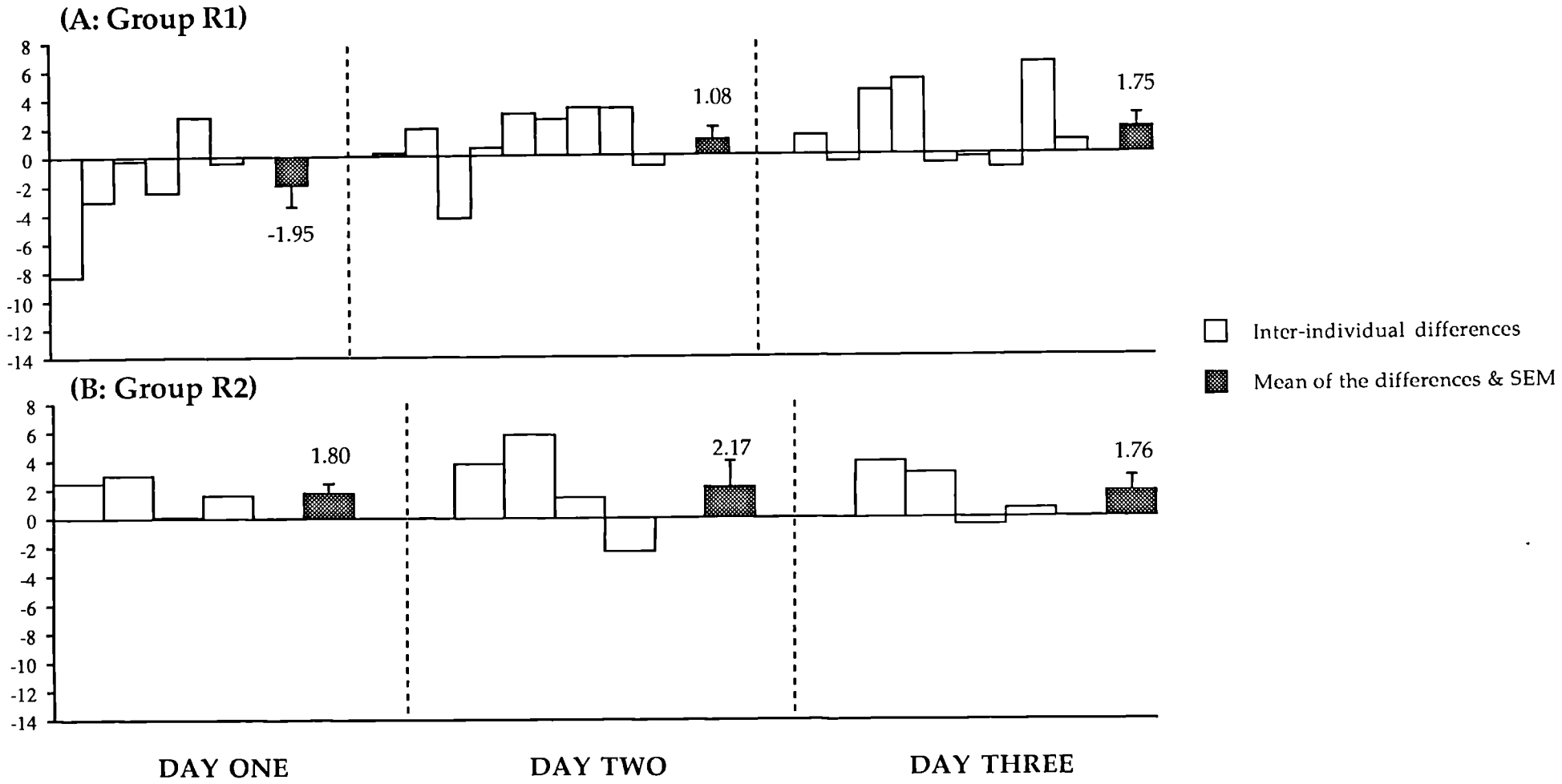
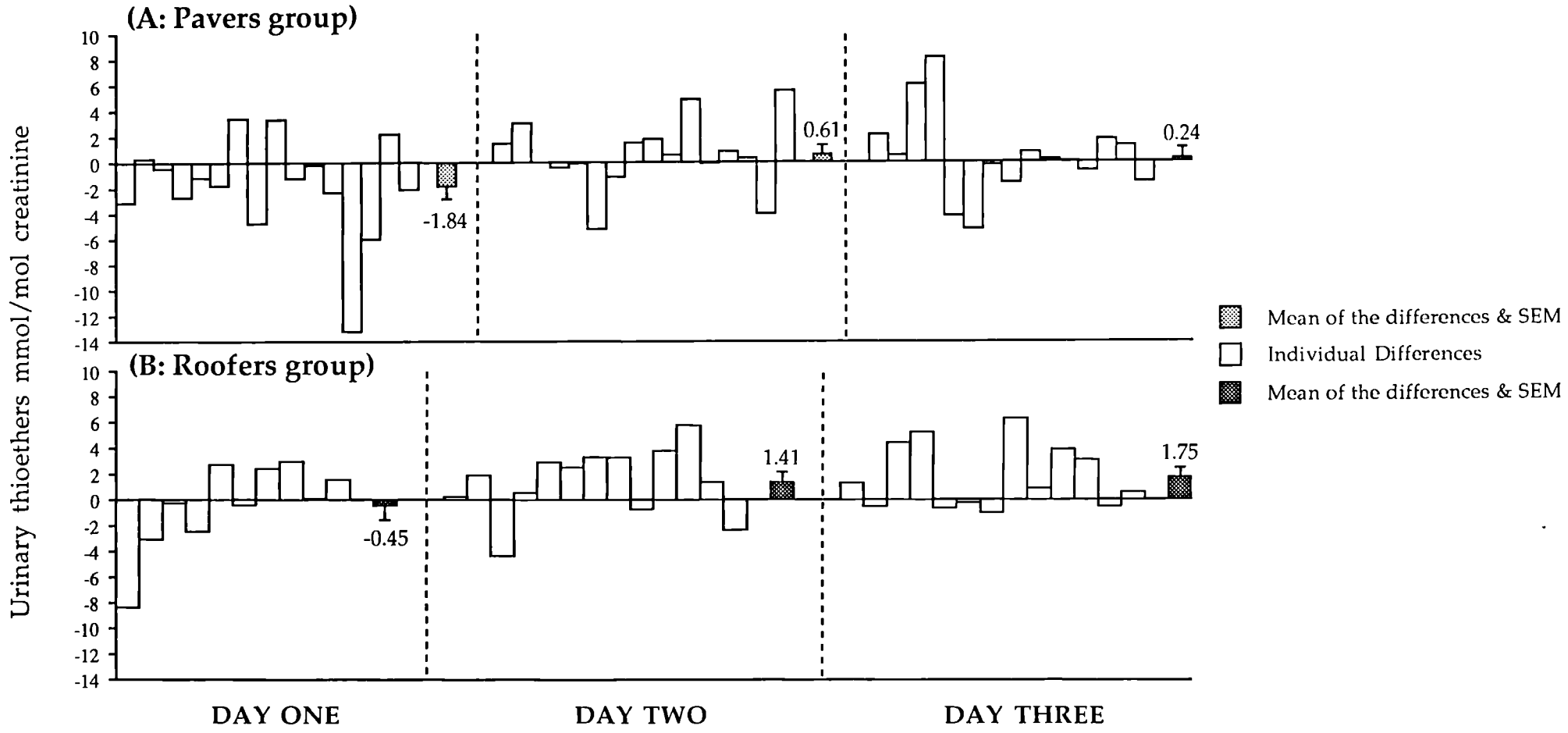


Figure : 14.7 Difference in urinary thioethers output between pre-shift and post-shift urine samples in **pavers group PV** (n=16) and **roofers group RF** (n=13) on days 1, 2 and 3
- Inter-individual differences and mean ± SEM of the differences -



CHAPTER 15

DISCUSSION OF URINARY THIOETHERS

15.1 Mean of Urinary Excretion of Thioether

Comparing the mean urinary excretion of UTh. demonstrated that exposure to PAH in bitumen fume did not results in statistically significant elevation of urinary thioether output between the studied groups. Results of previous studies which biologically monitored exposure to bitumen fume during road-paving using UTh. were contradictory. Scassellati Sforzolini *et al.* (1986), Burgaz *et al.* (1988) and Pasquini *et al.* (1989) found no significant difference in UTh. between controls groups and bitumen fume exposed groups even after controlling for the effect of smoking. In contrast, Burgaz *et al.* (1992) were able to show significant differences only after controlling for smoking. The significant positive results reported by Burgaz should be interpreted with care since there is evidence that urinary thioethers excretion in a large number of very highly PAH-exposed coke oven and graphite-electrode-production workers showed no significant elevation over control groups after statistically controlling for a number of confounding variables (Ferriera, Jr. 1994[a]).

The lack of discrimination between the control groups (C2 and M) and the occupationally PAH-exposed groups (P1, P2, R1 and R2) using the thioether technique may have a number of explanations:

* low biological uptake of electrophilic PAHs due to low exposure to PAHs in bitumen fume
* exposure too low for the sensitivity of the assay of UTh.
* confounding factors such as smoking and diet
* time of urine collection and route of excretion
* misclassification of individuals into respective groups
* relatively high coefficient variation of the assay
* too few individuals in the groups to attain statistically significant effect in the group analysis

Our ambient monitoring data has shown that exposure to PAHs in bitumen fumes was very low. It is also possible that the metabolism of the electrophilic compounds through glutathione conjugation was low and the analysis was not sensitive enough to detect such low rate. Moreover, Boyland and Sims in 1958 reported that only 'traces' of

mercapturic acid were found in human urine after application of a single dose of naphthalene 500 mg (van Doorn *et al.* 1981[b]). The levels of PAHs measured in personal air samples of groups P1, P2, R1 and R2 were probably insufficient to produce a significant rise in UTh. output.

It is unlikely that the confounding factor that were not statistically controlled for in this study accounted for the absence of a difference in UTh. between exposed and controls. The demographic characteristics summarised in table 11.2 show that the groups were fairly matched with respect to main confounding factors such as smoking habits and alcohol and coffee consumption. Secondly, the mean UTh output in the controls' pre-shift and post-shift samples on all days appeared to be normal (except pre-shift samples on day 3). This was close to the average excretion one would expect to observe in a non-exposed population (3.8 mmol/mol creatinine) (van Doorn *et al.* 1981[a]). In addition, thioether excretion in pre-shift and post-shift urine samples of occupationally exposed groups was similar to that which other investigator have observed in populations exposed to different electrophilic agents (Karakaya *et al.*, 1989, Pasquini *et al.* 1989, Edwards and Priestly, 1993).

Not much is known about the critical time of collection of urine samples for analysis of thioethers. Therefore at present, we have no idea whether the urine sampling times chosen for this study were optimum for the analysis of excretion of this non-specific biomarker. Alternatively, the glutathione conjugates may have been excreted via the bile (Reuterwall *et al.* 1991).

Misclassification of individuals could possibly explain the higher but insignificant levels of mean UTh. excretion in manual workers urine when compared to group C2. Although, subjective mis-categorisation of workers into control and exposed groups is possible and may mask differences in UTh. output (Edwards, 1990), it is less probable that this was the main reason for non-significant differences reported between controls group C2 and pavers and roofers.

It is logical to suspect that the relatively high CV for the analytical technique may have introduced a fair amount of variability that might have decreased the chances of observing a significant differences.

The small number of individuals in each subgroup did not help in applying more robust statistical tests for picking significant differences between subgroups. The pooling of the groups of pavers into group PV and the groups of roofers into group RF did seem to have any impact on our conclusions.

15.2 Change in Urinary Thioether Output

There was no noticeable pattern in the change in UTh. output between pre-shift and post-shift samples at individual level on the first three days of the surveys. That led us to conclude that the enhanced mean UTh. excretion in group M, P1, P2, R1 and R2 is not due to a diurnal rhythm. From these findings we can conclude that there is no positive relationship between bitumen fume exposure during paving and roofing operations and the excretion of thioethers in urine.

15.3 Summary of Discussion

Urinary thioethers excretion was not influenced by occupational exposure to low levels of PAHs in bitumen fume, such as those in this risk assessment. Confounding factors, such as smoking, that can have a substantial effect on this biomarker were not controlled for statistically.

The fact that there was no difference between control and exposed groups and that urinary thioethers values were found within normal limits does not strictly imply that no or negligible exposure to bitumen fumes have occurred. It could be that the metabolism of electrophilic compounds through glutathione conjugation was low.

The apparent limited use of urinary thioethers in biomonitoring populations exposed to low levels of PAHs in bitumen fume does not mean that it should be dropped from such programs. Biological monitoring using urinary thioethers may signal exposure and absorption of a number of electrophiles found in working environments, such as that of pavers and roofers, which selective biomarkers may not detect. Therefore the coupling of this non-specific biomarker with other selective

biomarkers may turn out to be useful in assessing the overall risk from exposure to potentially genotoxic substances.

Finally, it is important to mention that identification of excreted thioethers would open possibilities for discovering selective biomarkers of exposure which will allow better and more direct assessment of internal exposure to specific chemicals.

CHAPTER 16

RESULTS OF

URINARY 1-HYDROXYPYRENE

16.1 INTRODUCTION

This chapter describes the results obtained from the assay of 1-hydroxypyrene in urine. Pre-shift and post-shift urine samples collected on the first three days of each survey from subjects in groups C2, M, P1, P2, R1 and R2 were analysed for 1-hydroxypyrene using the method described in section II, chapter 7 of this thesis. The results of the urinary 1-hydroxypyrene are presented in four parts.

Part I: In this part, urinary 1-HP (U1-HP) concentration was corrected using urinary creatinine concentration and results were reported as a ratio (U1-HP μmol/mol creatinine). The corrected 1-HP data on each subject over time (3 days) was summarised by their regression slope and mean slopes were compared between groups using ANOVA single-factor. This was followed by an unpaired Student's t-test using the pooled standard deviation to test for differences in mean slopes between group C2 or M and occupationally PAH-exposed groups {i.e. P1, P2, R1, R2, pavers group (PV) and roofers group (RF)}. Mean slopes were also compared to the null hypothesis using a one sample Student's t-test. The null hypothesis tested here implies that the mean rates of increase of U1-HP excretion over time (i.e. slopes) is zero.

Part II: In this part, urinary 1-HP concentration was adjusted using urinary creatinine concentration and results were reported as adjusted concentration of urinary 1-HP which were expressed as ($U1\text{-}HP_{adj}$ nmol/L). The adjustment was based on the calculated regression relationship between $\log_{10}$ of urinary 1-HP concentration and $\log_{10}$ of urinary creatinine concentration. The results of the adjusted urinary 1-HP concentration for each individual were summarised in the same manner as the corrected urinary excretion data.

Part III: The absolute urinary 1-HP concentration were expressed as (U1-HP nmol/L). The absolute values for each subject were summarised and compared in the same manner as in part I.

Part IV: The relationship between external exposure to PAHs assessed using personal air monitoring and urinary excretion of 1-hydroxpyrene was determined.

16.2 PART I *(Corrected values)*

16.2.1 Daily Excretion of Urinary 1-hydroxypyrene *(Corrected values)*

Tables 16.1A and 16.1B present the daily morning and afternoon corrected U1-HP output for all individuals in the office workers (C2) and manual workers (M) groups, respectively. The results of the daily pre-shift and post-shift U1-HP excretion for subjects in groups P1 and P2 are presented in table 16.2A and 16.2B, respectively; while those of groups R1 and R2 are given in tables 16.3A and 16.3B, respectively. The means U1-HP excretion for each day for the pavers group (PV) and the roofers group (RF) are shown in figures 16.3A and 16.3B, respectively.

The mean excretion of U1-HP in pre-shift samples of group C2 on day 1 (mean ± SEM; 0.79 ± 0.24) (table 16.1A) was lower than all groups except group M (0.54 ± 0.13; table 16.1B). The mean excretion of U1-HP on day one was similar in group P1, P2 and R2. The morning mean U1-HP for group R2 reported in table 16.3B was approximately 2.5 fold higher than group C2. The mean excretion of U1-HP in the pavers group (1.00 ± 0.19) shown in figure 16.3A was higher than that of group C2 and comparable to both of its subgroup P1 and P2. The roofers group had a pre-shift mean U1-HP of (1.48 ± 0.40) which was approximately twice that of group C2. The mean of U1-HP output in pre-shift samples on day 1 was different between all groups; however, the observed differences did not reach statistical significance (ANOVA single factor, $p>0.05$).

16.2.2 Rate of Increase of Excretion of Urinary 1-HP

16.2.2.1 Slope of the Mean U1-HP Excretion Over Time

In group C2, the rate of increase in mean U1-HP excretion over time (i.e. slope "ß") was virtually zero (ß=0.004; fig. 16.1A). This rate of increase was slower than the groups of pavers P1 (ß=0.068; fig. 16.1B) and P2 (ß=0.025; fig. 16.1C). On the other hand; it was equal to that of the group R1 (ß=0.004; fig. 16.2A) and very close to that of group R2 (ß=0.006; fig.

16.2B). Group M, had a moderate rate of increase in U1-HP excretion (ß=0.020; fig. 16.1A), which was higher than that of groups C, R1 and R2, similar to group P1 and less than half that of group P1. Group P1 had a distinctively high rate of increase of U1-HP excretion (ß=0.068; fig. 16.1B). Figure 16.3A shows that regression slope of mean U1-HP excretion over time in the pavers group (Group PV; ß=0.041) was higher than that of group C2 and M. The regression slope of the mean U1-HP output over time in the roofers group (Group RF) was very close to zero and even lower than group C2.

16.2.2.2 Individuals' Slope of U1-HP Excretion Over Time

Office workers: The U1-HP data on each subject in group C2 over time are presented in table 16.1A. Three (C2 #s 80, 83 and 86) out of the 6 subjects (i.e. 50 %) had a negative regression slope indicating that their rate of U1-HP excretion was decreasing with time. The other three office worker had a positive but relatively slow rate of increase for U1-HP excretion.

The group's mean slopes was nearly zero (ã=0.001 ± 0.006) which was dissimilar to the regression slope of the mean U1-HP output over time (ß=0.004; fig. 16.1A). This difference is due to the exclusion of two post-shift samples of individuals C2 # 84 and # 86 on day 1 and the pre-shift sample of the office worker C2 # 83 on day 3, which were not analysed due to insufficient volume of urine available for analysis.

The mean slopes of group C2 was not significantly different from the null hypothesis (one sample Student's t-test; $p>0.05$) meaning that their rate of increase of U1-HP output is not different from zero.

Manual workers: In group M, individual regression slopes of U1-HP excretion over time were calculated from all pre-shift and post-shift samples on the three days for fourteen manual workers out of fifteen. The rate of increase of U1-HP excretion in the slater (M # 35) was not calculated because 1-HP was quantified in only one sample (table 16.1B).

Three manual workers (M #s 30, 32 and 33) had negative rates of

increase for excretion of U1-HP. Manual workers (M # 15, 34, 41 and 48) had regression slopes quite close to zero (table 16.1B). Manual workers (M # 10, 14, 17, 37, 38 and 45 and 48) had comparable positive rate of increase of U1-HP output. The lowest of them had a regression slope similar to the highest in group C2. The manual worker painting metal on the roofs (M # 38) had by far the the highest rate of increase of U1-HP excretion (a=0.126). This regression slope was the second highest among all individuals in this study.

The mean slopes in the manual workers shown in table 16.1B was (ã=0.020 ± 0.009). It was equal to the regression slope of the mean U1-HP output over time in the all manual workers shown in figure 16.1A.

The mean slopes in group M was not significantly different from group C2 (unpaired Student's t-test; $p>0.05$); however, it was significantly different from the null hypothesis (one sample Student's t-test; $p<0.05$).

Group P1: Table 16.2A shows that pavers (P1 # 7) had the highest rate of increase of U1-HP excretion (a=0.181) in this study. His morning and afternoon samples on day 3 were about 5 times higher than those of day 1 and 2. His GM of ∑9PAHs TWA concentration was the highest in this group of pavers. The second highest regression slope shown in table 16.2A belonged to paver (P1 # 11) which was double that of paver (P1 # 18). The rate of increase of U1-HP excretion in pavers (P1 # 8, 13 and 19) were similar to the highest and relatively moderate positive regression slopes in group C2 and group M, respectively.

The mean slopes in the this group of pavers shown in table 16.2A was (ã=0.068 ± 0.028) which is equal to the regression slope of the mean U1-HP output over time in the all subjects (ß=0.068; fig. 16.1B).

The difference in the mean slopes between group P1 and group C2 was significant (unpaired Student's t-test; $p<0.05$). On the other hand, the mean slopes of group P1 was not significantly different from that of group M. The mean slopes of group P1 was significantly different from the null hypothesis (one sample Student's t-test; $p<0.05$).

Group P2: Raker (P2 # 60) who had the highest regression slope for UDGA excretion had also the highest regression slope for U1-HP excretion over time (a=0.090) (table 16.2B). Raker (P2 # 69) who was working behind the paving machine had the second highest rate of increase which nearly was half that of the highest. All the other pavers had a relatively low to moderate regression slopes ($0.006<a<0.023$) (table 16.2B) which were similar to those of group M.

The mean slopes in the this group of pavers shown in table 16.2B was ($\tilde{a}=0.025 \pm 0.008$) which is equal to the regression slope of the mean U1-HP output over time in the all subjects ($ß=0.025$; fig. 16.1C).

The difference in the mean slopes between group P2 and group C2 was not statistically significant (unpaired Student's t-test; $p>0.05$). It was a not much greater than that of group M. When compared against the null hypothesis it was found to be statistically significantly different (one sample Student's t-test; $p<0.05$).

Group R1: Individual regression slopes of U1-HP excretion over time were calculated from all pre-shift and post-shift samples on the three days for seven roofers out of nine. The rate of increase of U1-HP excretion in roofers (R1 # 20 and 21) was calculated from 4 urine sample since they provide neither a pre-shift nor post-shift urine sample on day 1 (table 16.3A).

Five roofers (R1 # 21, 36, 40, 42 and 43) had negative rates of increase of U1-HP excretion (table 16.3A). Roofer (R1 # 21) had the steepest negative regression slope. In contrast, his UDGA excretion had the highest positive regression slope. All other roofers in this group (table 16.3A) had low to moderate positive rates of increase of U1-HP output except R2 # 44 who had relatively steep positive regression slope (a=0.064).

The mean slopes in the this group of roofers shown in table 16.3A was virtually zero ($\tilde{a}=0.002 \pm 0.010$). It was different from the regression slope of the mean U1-HP output over time in the all subjects in this group of roofers ($ß=0.004$; fig. 16.2A). This was due to the missing data in individual regression slopes of roofer R1 # 20 and 21.

The difference in the mean slopes between group R1 and group C2 or group M was not statistically significant (unpaired Student's t-test; $p>0.05$). When the mean slopes was compared against the null hypothesis the difference did not reach statistical significance (one sample Student's t-test; $p>0.05$).

Group R2: Table 16.3B shows that the tipper (R2 # 90) had a relatively moderate positive rate of increase in U1-HP output over time in this group of roofers ($a=0.023$). His regression was the highest in group R2. The other three roofers had virtually no increase in U1-HP excretion which was similar to those of group C2. Their weak regression positive/ negative slopes are shown in table 16.3B.

The mean slopes in the this group of roofers shown in table 16 3B was ($\tilde{a}=0.006 \pm 0.007$). The mean slopes was equal to the regression slope of the mean U1-HP output ($ß=0.006$) illustrated in figure 16.2B.

The difference in the mean slopes between group R2 and group C2 or group M was not statistically significant (unpaired Student's t-test; $p>0.05$). The mean slopes was not significantly different from the null hypothesis (one sample Student's t-test; $p>0.05$).

Pavers group: This group is group P1 and P2 combined together. Therefore the total number of subjects in this group is sixteen. The mean slopes in this combined group was positive ($\tilde{a}=0.041 \pm 0.012$). This mean slopes had the same sign as the means slopes of its subgroups. It was smaller than that of group P1 but bigger than group P2. The mean slopes was identical to the regression slope of the mean U1-HP excretion on time shown in figure 16.3A ($ß=0.041$).

The difference in the mean slopes between the pavers group (PV) and either of its subgroups was not statistically significant (unpaired Student's t-test; $p>0.05$). In addition the mean slopes was not significantly different from either that of group C2 or group M (unpaired Student's t-test; $p>0.05$). Meanwhile, it was found that the mean slopes was significantly different from the null hypothesis (one sample Student's t-test; $p<0.05$).

Roofers group: This group is group R1 and R2 combined together. Therefore the total number of subjects in this group is thirteen. The mean slopes in this combined group was positive (ã=0.003 ± 0.007). This mean slopes was similar to the mean slopes of its subgroup R1 & R2 shown in table 16.3A and 16.3B. The mean slopes of group RF was not very different to the regression slope of the mean U1-HP excretion over time shown for that group in figure 16.3B (ß=0.002). Of course this minor difference was due to the missing data in individual regression slopes of roofer R1 # 20 and 21.

The difference in the mean slopes between the roofers group (RF) and either of its subgroups was not statistically significant (unpaired Student's t-test; $p>0.05$). In addition the mean slope was not significantly different from either that of group C2 or group M (unpaired Student's t-test; $p>0.05$). Also, the mean slopes was not statistically different from the null hypothesis (one sample Student's t-test; $p>0.05$).

All groups: Testing the difference between the mean slopes values of all group simultaneously using ANOVA single factor it was observed that these differences reached statistical significance ($p=0.0059$).

16.3 PART II *(Adjusted Values)*

16.3.1 Adjustment of Urinary 1-Hydroxypyrene Concentration

The adjustment used was based on the calculated regression relationship between urinary 1-hydroxypyrene and urinary creatinine in all the samples collected from all individuals in this investigation. It is similar to that applied in the article by Thompson *et al.* (1990).

The relationship between urinary 1-hydroxypyrene and urinary creatinine shown in figure 16.4 was calculated from 287 urine samples. The linear regression of U1-HP on UCr was given by the equation:

$$[\text{U1-HP}] = 0.678\,[\text{UCr}] + 7.197$$

The correlation coefficient was not strong r=0.175 but was shown to be statistically significant ($p<0.0001$). This implied that adjusting U1-HP values for UCr concentration could be effective in attenuating the inter-individual variation in U1-HP values arising from urinary volume in spot urine samples.

The logarithmic transformation of U1-HP and UCr tended to strengthen the association between the two variables. The linear regression of log [U1-HP] on log [UCr] shown in figure 16.5 was given by the equation:

$$\log [\text{U1-HP}] = 0.574 \log [\text{UCr}] + 0.367 \quad \text{-------} \ (1)$$

The correlation coefficient was stronger r=0.280 than its observed value in the previous relationship. It was also shown to be statistically significant ($p<0.0001$). The measured U1-HP concentrations were adjusted for urinary creatinine using equation (1), standardising the U1-HP values to the mean UCr concentration observed for all the urine samples (10.3 mmol/L) which is equal to 1.01 on $\log_{10}$ scale. The adjustment was based on this relationship:

$$\log [\text{U1-HP}_{\text{adj.}}] = \log [\text{U1-HP}] - 0.574 \times (\log [\text{UCr}] - 1.01) \quad \text{-----} \ (2)$$

whereby;

$\log [\text{U1-HP}_{\text{adj.}}]$; is the log of [adjusted urinary 1-hydroxypyrene value]
log [U1-HP]; is the log of [observed urinary 1-hydroxypyrene value]
0.574; is the slope of the regression in equation (1)
log [UCr]; is the log of [observed urinary creatinine value]
1.01; is the mean urinary creatinine value on $\log_{10}$ scale

An example of how the adjustment of an observed U1-HP value was done is shown in figure 16.6. The observed U1-HP value of 36.8 nmol/L and UCr value of 23.8 mmol/L are the co-ordinates of point named X in figures 16.4 and 16.6. The adjusted U1-HP value calculated from equation (2) is the y-axis co-ordinate of point Y in figure 16.6. This is the adjusted U1-HP value that would have been observed if the UCr concentration had

been equal to 10.3 mmol/L. The line named A in figure 16.6 is the value of (log [UCr] - 1.01) and 0.574 times line A is given by line B. Therefore the adjusted U1-HP concentration is the observed U1-HP less that value indicated by line B. Using equation (2) the adjusted U1-HP value would be 22.7 nmol/L shown in figure 16.6.

In brief, the principle of the method of adjustment is that the adjusted U1-HP concentration is that value would have been observed for U1-HP concentration had all the subjects had the same urinary creatinine concentration.

16.3.2 Daily Excretion of Urinary 1-HP

Tables 16.4A and 16.4B present the daily morning and afternoon adjusted U1-HP output for all individuals in the office workers (C2) and manual workers (M) groups, respectively. The results of the daily pre-shift and post-shift U1-HP excretion for subjects in groups P1 and P2 are presented in table 16.5A and 16.5B, respectively; while those of groups R1 and R2 are given in tables 16.6A and 16.6B, respectively. The means of daily U1-HP excretion in the pavers group and the roofers group are shown in figures 16.9A and 16.9B, respectively.

The mean of U1-HP output in pre-shift samples on day 1 was different between all groups; however, the observed differences did not reach statistical significance (ANOVA single factor, $p>0.05$). In general, the profile of adjusted U1-HP concentrations in pre-shift samples of all group on day 1 mirrored that of the corrected U1-HP values with subtle differences.

A close look at the data (figures and tables) shows us that the comparison of the regression slope of the mean adjusted U1-HP concentrations between all group mirrored that of the corrected U1-HP values with slight variation.

16.3.3 Individuals' Slope of U1-HP Excretion Over Time

Overall, there was a number of subtle differences in the profile of individuals' rate of increase of urinary excretion of 1-hydroxypyrene when calculated from adjusted U1-HP values in comparison to when calculated from corrected U1-HP values. These differences were mainly found in the results of group C2 and M.

Testing the difference between the mean slopes values (calculated from adjusted U1-HP concentrations) of all group simultaneously using ANOVA single factor it was observed that these differences reached statistical significance with a p-value (p=0.0022). This p-value was 2.7 time less than the computed from corrected U1-HP.

16.4 PART III *(Absolute values)*

16.4.1 Excretion of Urinary 1-HP

Tables 16.7A and 16.7B present the daily morning and afternoon absolute U1-HP output for all individuals in the office workers (C2) and manual workers (M) groups, respectively. They also present the rate of increase in excretion of absolute U1-HP for each individual as well as the mean slopes for each group. In parallel, tables 16.8A and 16.9B describes the results of groups P1 and P2 while tables 16.9A and table 16.9B describe those of groups R1 and R2.

The descriptive analysis of the above eight tables shows that the profile of the daily excretion of urinary 1-hydroxypyrene was virtually identical to that described for the results of the adjusted U1-Hp results. Similarly, the results of the regression slopes of mean U1-HP over time had a profile closely mirroring those of adjusted U1-Hp results. This is evident when comparing figures 16.10, 16.11 and 16.12 with the those of the adjusted results (fig. 16.7, 16.8 and 16.9). Again, the profile of the mean of individual regression slopes computed from the absolute U1-HP results in table 16.7, 16.8 and 16.9 was very similar to those of computed from the

adjusted U1-HP results presented in tables 16.5, 16.6 and 16.7.

Testing the difference between the mean slopes values (calculated from absolute U1-HP concentrations) of all group simultaneously using ANOVA single factor it was observed that these differences reached statistical significance with a p-value (p=0.0040). This p-value was lower than that of the corrected U1-HP results, but approximately 2 times higher than the computed from adjusted U1-HP data.

16.5 PART IV

This part studies the relationship between TWA concentration of different airborne exposure indices (Σ9PAHs, Σ8PAHs phenanthrene or pyrene) in personal air samples and the biological exposure indicator the change ($\underline{\Delta}$) in urinary 1-HP output over a three day period (1-HP concentration expressed as a ratio to urinary creatinine concentration). Multiple linear regression analysis was used to calculate the empirical mathematical relationship existing between {day-3-post-shift U1-HP} as the dependant variable and {day-1-pre-shift U1-HP} and {3-day geometric mean of the TWA concentration of one of the airborne exposure indices} as the predictor variables. The model is given below:

for example if Σ9PAHs is the external exposure index then;

$$\text{Day 3 Post-shift U1-HP} = \alpha + \beta_1(\text{Day 1, pre-shift U1-HP}) + \beta_2(\text{TWA conc. of } \Sigma 9\text{PAHs})$$

whereby;

Day 3 post-shift U1-HP: is the concentration of U1-HP in post-shift urine sample of day 3 expressed as U1-HP μmol/mol creatinine

Day 1 pre-shift U1-HP: is the concentration of U1-HP in pre-shift urine sample of day 1 expressed as U1-HP μmol/mol creatinine

TWA conc. of Σ9PAHs: is 3-day geometric mean of TWA concentration of Σ9PAHs

α: is constant

β_1: is regression coefficient of pre-shift U1-HP

β_2: is regression coefficient of airborne exposure index

All the data of the individuals in group P1, P2 and R2 were included in the calculation of the empirical mathematical relationship. Subjects M # 30, 32 and 48 were excluded from the calculations because their external exposure was not monitored. Since roofers R2 # 20 and 21 did not provide urine sample on the morning of day 1; the value of their {day 1 pre-shift U1-HP} was assumed to be half the mean of pre-shift U1-HP of group R2 on day 1. Therefore the total number subjects included in the multiple regression analysis was n=40. The total number was 31 when pyrene was one of the predictor variables.

16.5.1 ∑9PAHs as a Predictor Variable

When the 3-day geometric mean of the TWA concentration of ∑9PAHs was used as one of the predictor variables it was observed that the correlation coefficient r^2 of the model was not significant when applied to the results of group M and group RF (R1 + R2) combined (multiple linear regression; $p>0.05$) (table 16.12). However, it was significant when applied to group M and group PV (P1 + P2) combined or all groups pooled together (multiple linear regression; $p=0.039$ and $p=0.027$, respectively) (table 16.11 and 16.10). These two tables also show the regression coefficient "$ß_2$" of the airborne PAH-exposure index was not significant for either of the significant correlations ($p > 0.05$). While the regression coefficient "$ß_1$" of day-1-pre-shift U1-HP was not significant for all groups pooled (table 16.10) it was statistically significant when studied in groups M and PV pooled together ($ß_1$ = value ± SD; = 1.60 ± 0.75; $p=0.042$) (table 16.11).

16.5.2 ∑8PAHs as a Predictor Variable

When the 3-day geometric mean of the TWA concentration of ∑8PAHs (i.e. excluding naphthalene) was used as one of the predictor variables it was observed that the correlation coefficient r^2 of the model was not significant when applied to the results of the group M and group RF (R1 + R2) combined (multiple linear regression; $p>0.05$) (table 16.12). For group M and PV combined r^2 was equal to 0.432. The correlation coefficient was the highest observed for all models and most significant ($p=0.001$) (table 16.11). In this model "$ß_2$" was (0.009 ± 0.001) which was the

most significant (p=0.007) (table 16.11) suggesting that the Σ8PAHs is the most appropriate external exposure index to use for predicting U1-HP excretion. The regression coefficient "$ß_1$" was equal to (1.07 ± 0.63) but was not statistically significance (p=0.099) (table 16.11). As for the pooled group (M+PV+RF) (table 16.10) although the goodness of the fit of the model was significant (p=0.036) it was relatively weak (r^2 = 0.165). In this model neither of the predictor variables' regression coefficient was significant (p>0.05) (table 16.10).

16.5.3 Phenanthrene as a Predictor Variable

When the 3-day geometric mean of the TWA concentration of phenanthrene was used as one of the predictor variables; it was observed that the correlation coefficient r^2 of the model was not significant when applied to the results of the group M and group RF (R1 + R2) combined (p>0.05) (table 16.12). For group M and PV combined r^2 was equal to 0.373. This correlation coefficient was the second highest and second most significant (p=0.004) (table 16.11) among all calculated models. In this model "$ß_2$" was (0.015 ± 0.006) (table 16.10) which was the second most significant (p=0.026) among all other models (table 16.12) suggesting that phenanthrene is an appropriate external exposure index to use for predicting U1-HP excretion. Table 16.11 also shows that the regression coefficient "$ß_1$" was equal to (1.01 ± 0.67) but was not statistically significance (p=0.101) (table 16.12b). As for the pooled group (M+PV+RF) (table 16.10) although the goodness of the fit of the model was of borderline significance (p=0.056) it was relatively weak (r^2 = 0.145). In this model neither of the predictor variables' regression coefficient was significant (p>0.05).

16.5.3 Pyrene as a Predictor Variable

Nine individuals were excluded from the calculations of the model because they had no detectable levels of pyrene in any of their personal air samples on all three days. A 2-day GM of pyrene TWA concentration was used as a measure of subjects' external dose when pyrene was detected in two personal air samples. For individuals with one

personal air sample with detectable pyrene levels; the pyrene TWA concentration in that sample was used as the individual's external dose index.

When the TWA concentration of pyrene was used as one of the predictor variables; it was observed that the correlation coefficient r^2 for the results of any of the combined group was not statistically significant (table 16.10, 16.11 and 16.12). This suggests that the external exposure index quantified as the TWA concentration of pyrene in personal air samples is not a good predictor of U1-HP excretion over a three day period.

Figure : 16.1 Mean urinary excretion of 1-hydroxypyrene in office workers (C2), manual workers (M) and groups of pavers (P1 and P2) over a three days period
- Mean ± SEM and rate of increase of excretion "ß" -
(Corrected values)

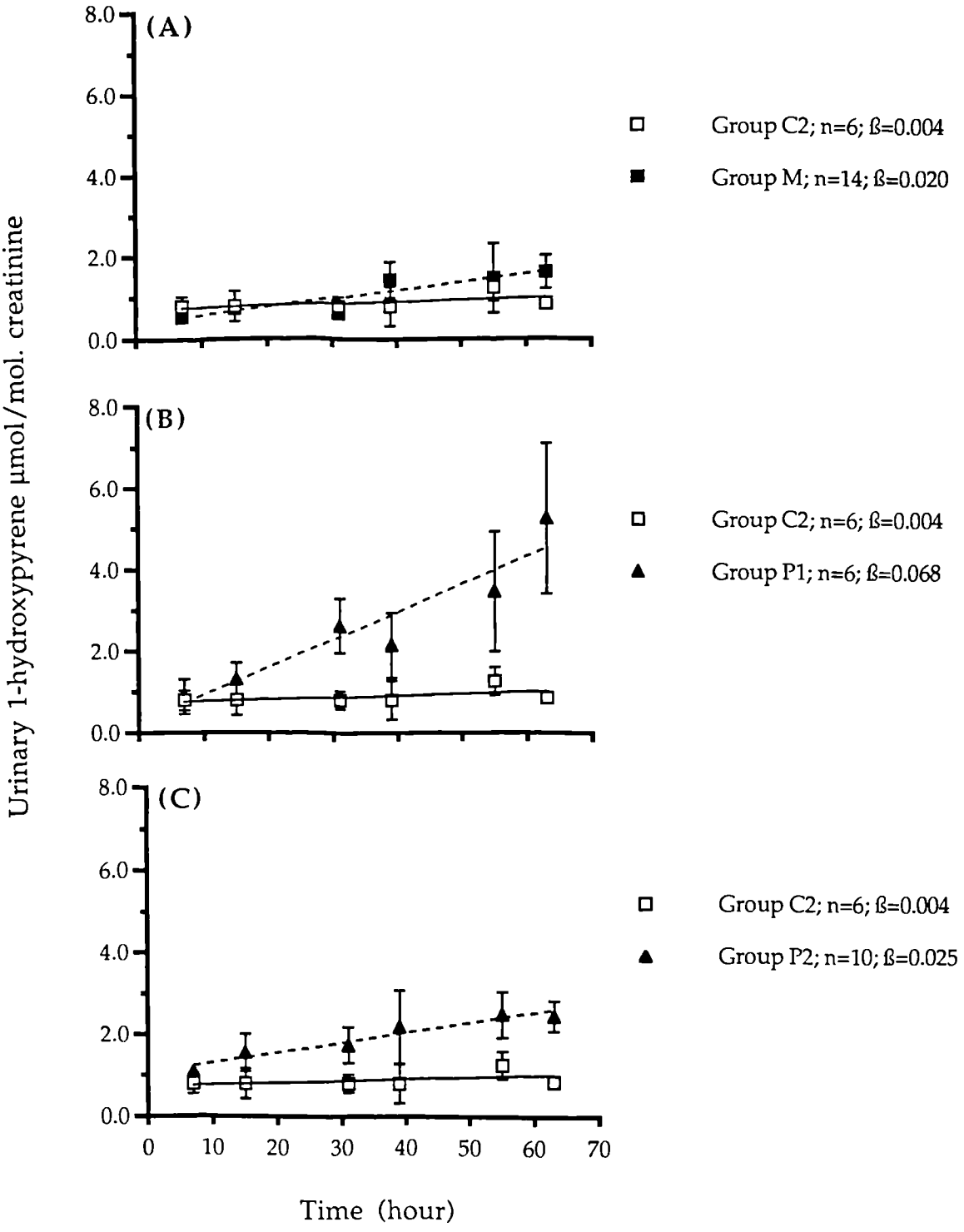


Figure : 16.2 Mean urinary excretion of 1-hydroxypyrene in office workers (**C2**) and groups of roofers (**R1 and R2**) over a three days period - Mean ± SEM and rate of increase of excretion "ß" - *(Corrected values)*

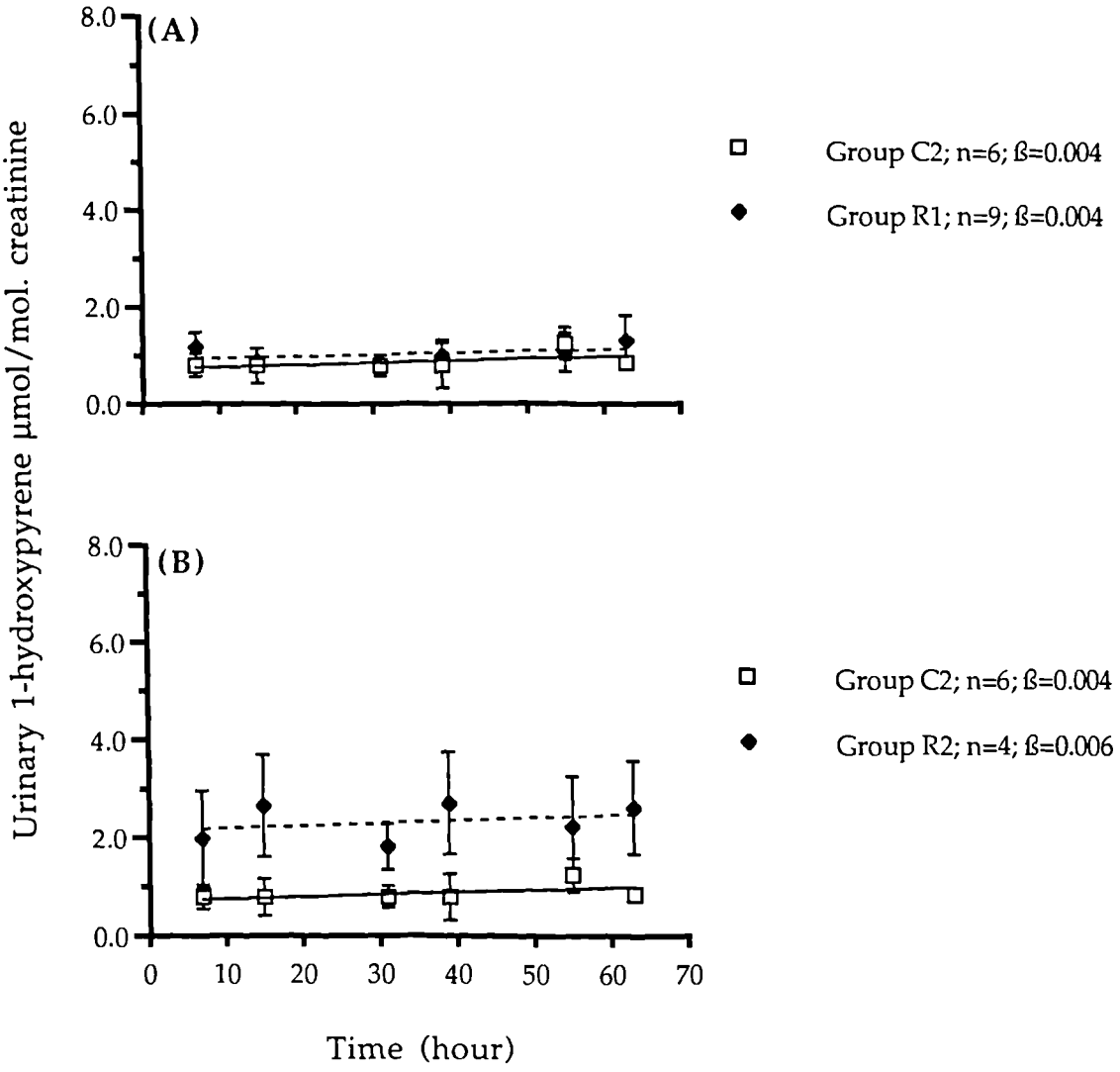


Figure : 16.3 Mean urinary excretion of 1-hydroxypyrene in office workers (**C2**), pavers group (**PV**) and roofers group (**RF**) over a three days period
- Mean ± SEM and rate of increase of excretion "ß" -
(Corrected values)

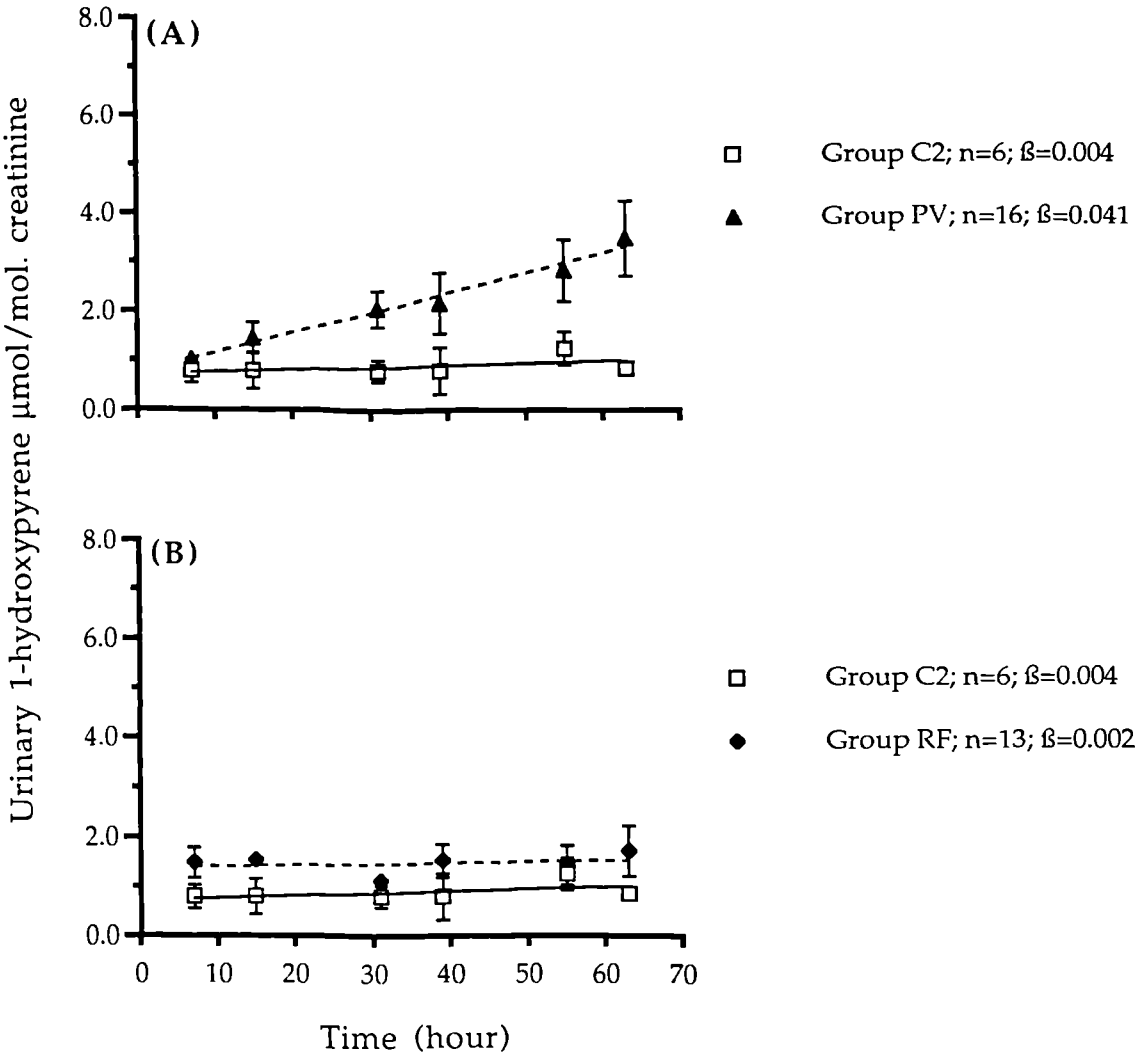


Figure : 16.4 The relationship between urinary 1-hydroxypyrene and urinary creatinine in all urine samples collected from groups C2, M, P1, P2, R1, and R2

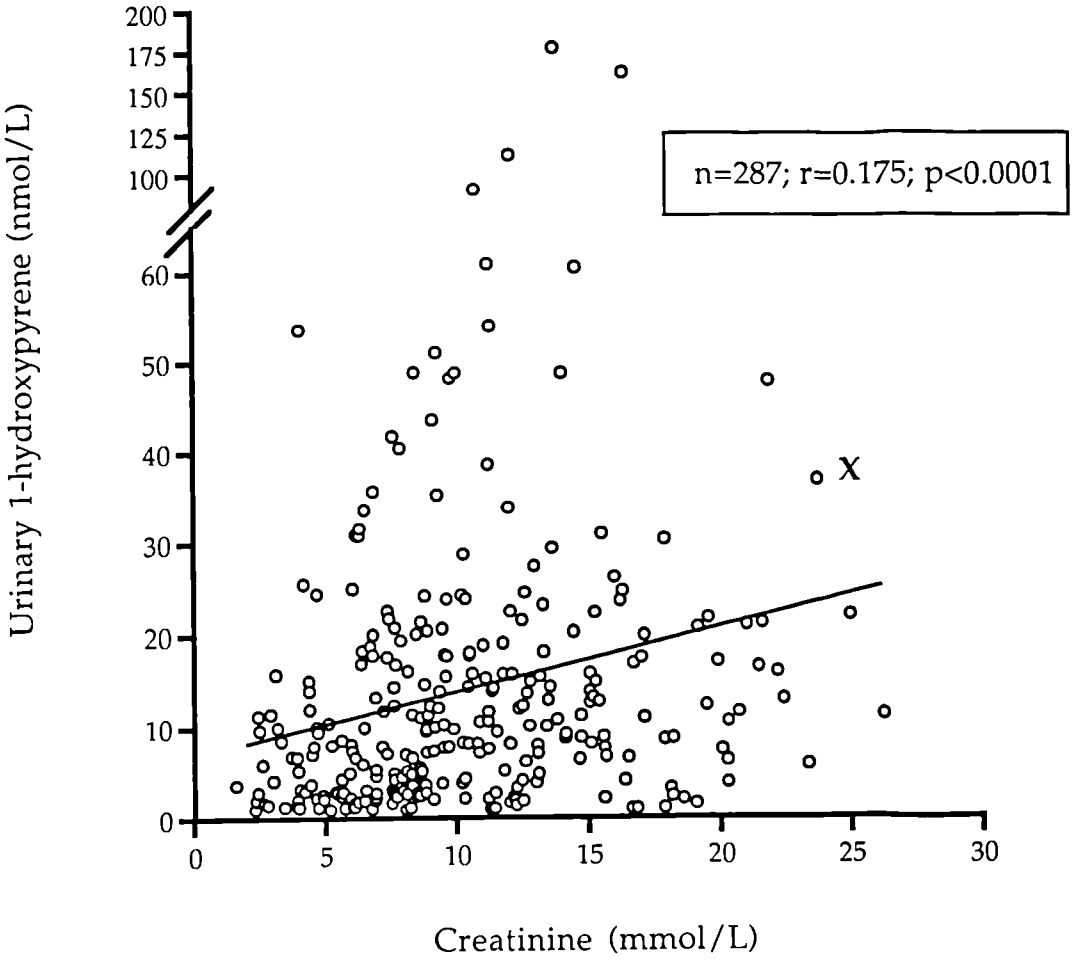


Figure : 16.5 The relationship between the logarithmic transformation of urinary 1-hydroxypyrene and urinary creatinine in all urine samples collected from groups C2, M, P1, P2, R1 and R2

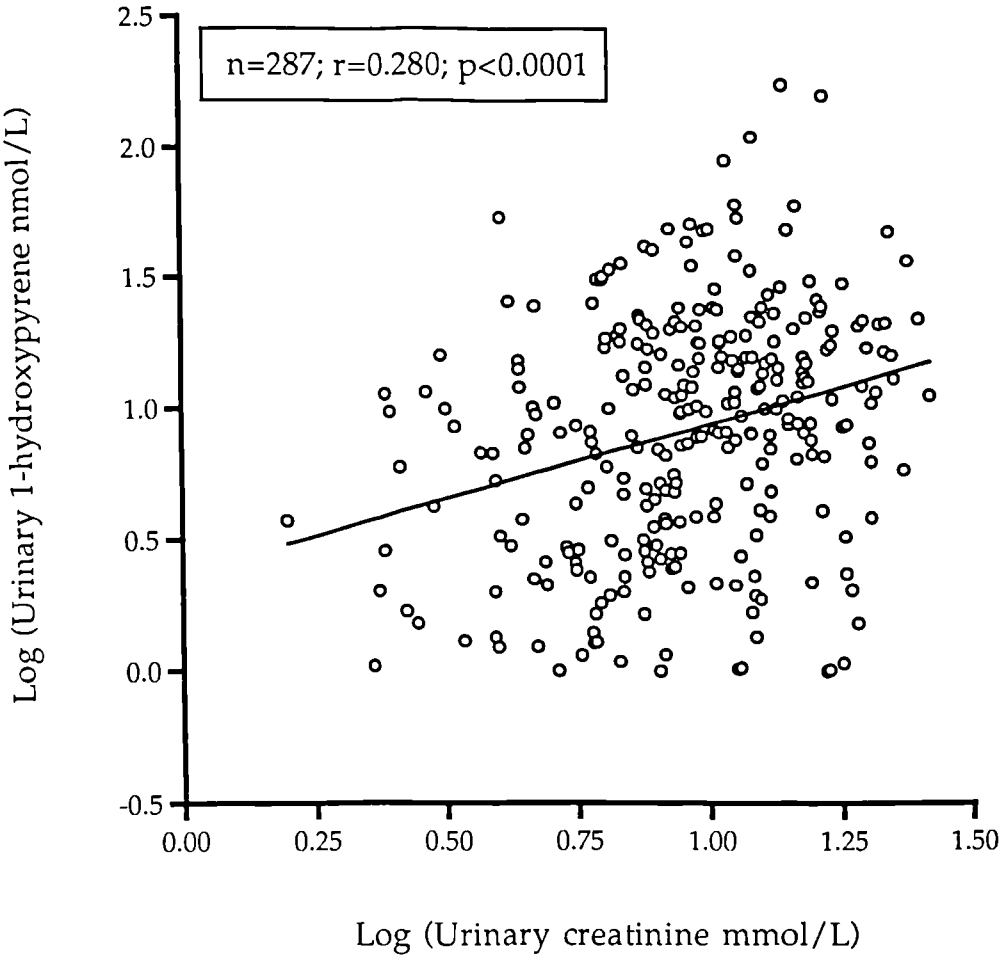


Figure : 16.6 An example of the method of adjustment of urinary 1-hydroxypyrene using urinary creatinine concentration

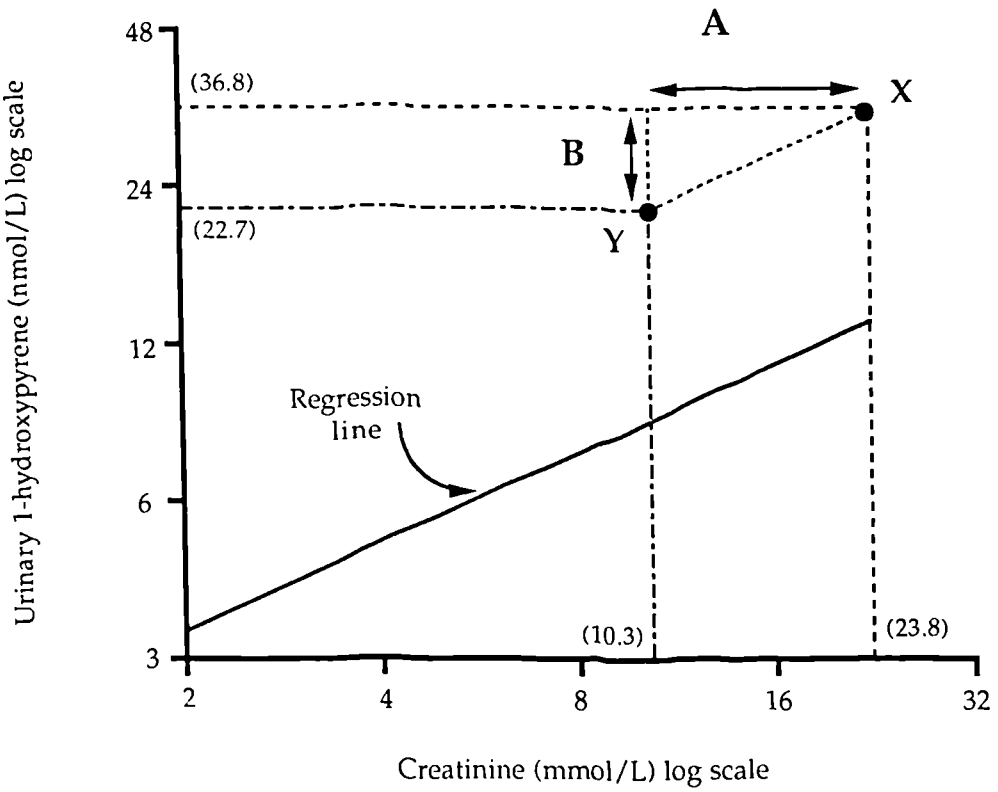


Illustration of the method for adjusting the concentration of urinary 1-hydroxypyrene using urinary creatinine concentration. The coordinates of point labelled **X** become those of point **Y** after the adjustment. The line joining the two points is parallel to the regression line of log urinary 1-hydroxypyrene on log urinary creatinine (see text for detailed explanation).

Figure : 16.7 Mean urinary excretion of 1-hydroxypyrene in office workers (C2), manual workers (M) and groups of pavers (P1 and P2) over a three days period
- Mean ± SEM and rate of increase of excretion "ß" -
(Adjusted values)

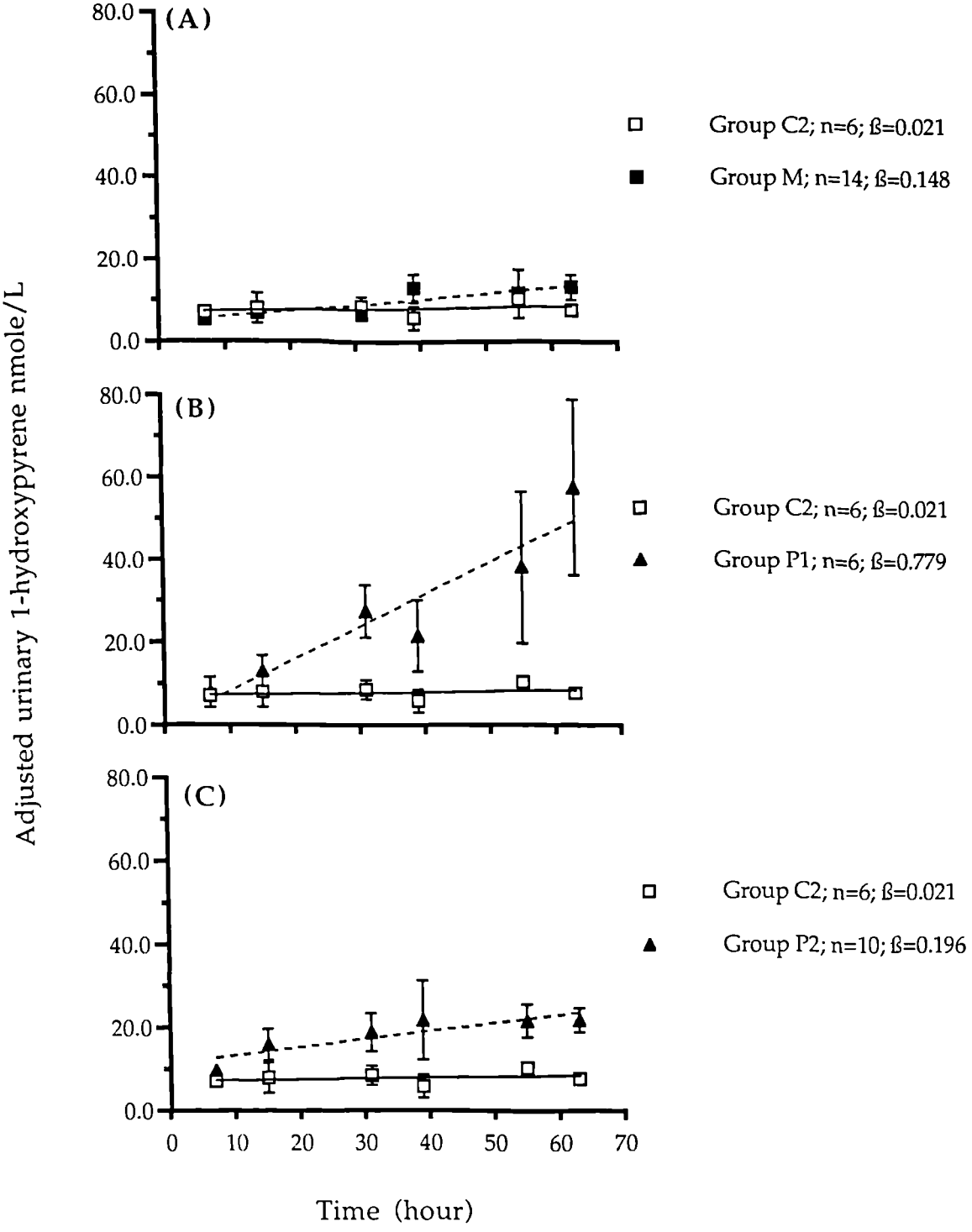


Figure : 16.8 Mean urinary excretion of 1-hydroxypyrene in office workers (**C2**) and groups of roofers (**R1 and R2**) over a three days period - Mean ± SEM and rate of increase of excretion "ß" - *(Adjusted values)*

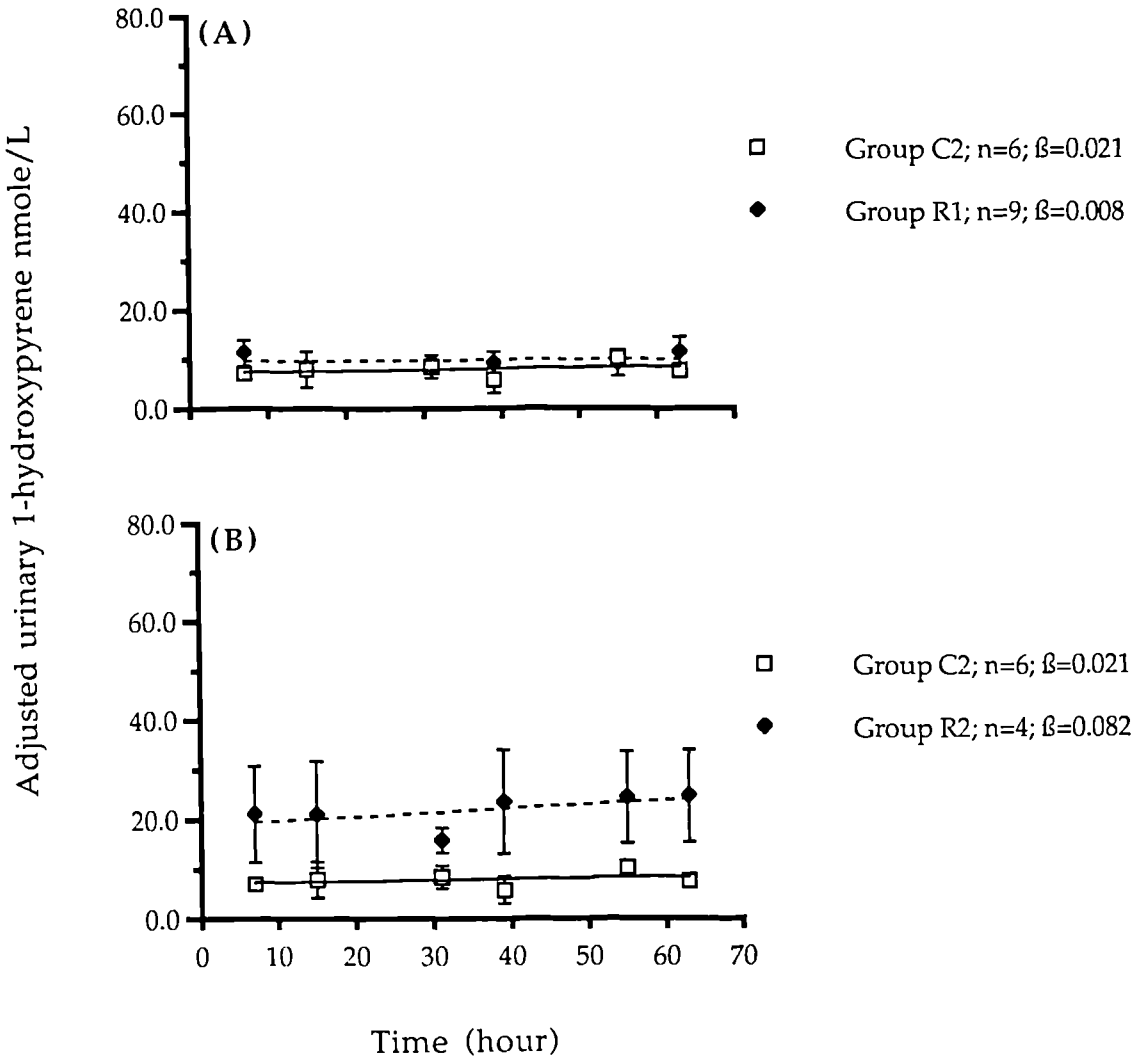


Figure : 16.9 Mean urinary excretion of 1-hydroxypyrene in office workers(**C2**), pavers group (**PV**) and roofers group(**RF**) over a three days period - Mean ± SEM and rate of increase of excretion "ß" - *(Adjusted values)*

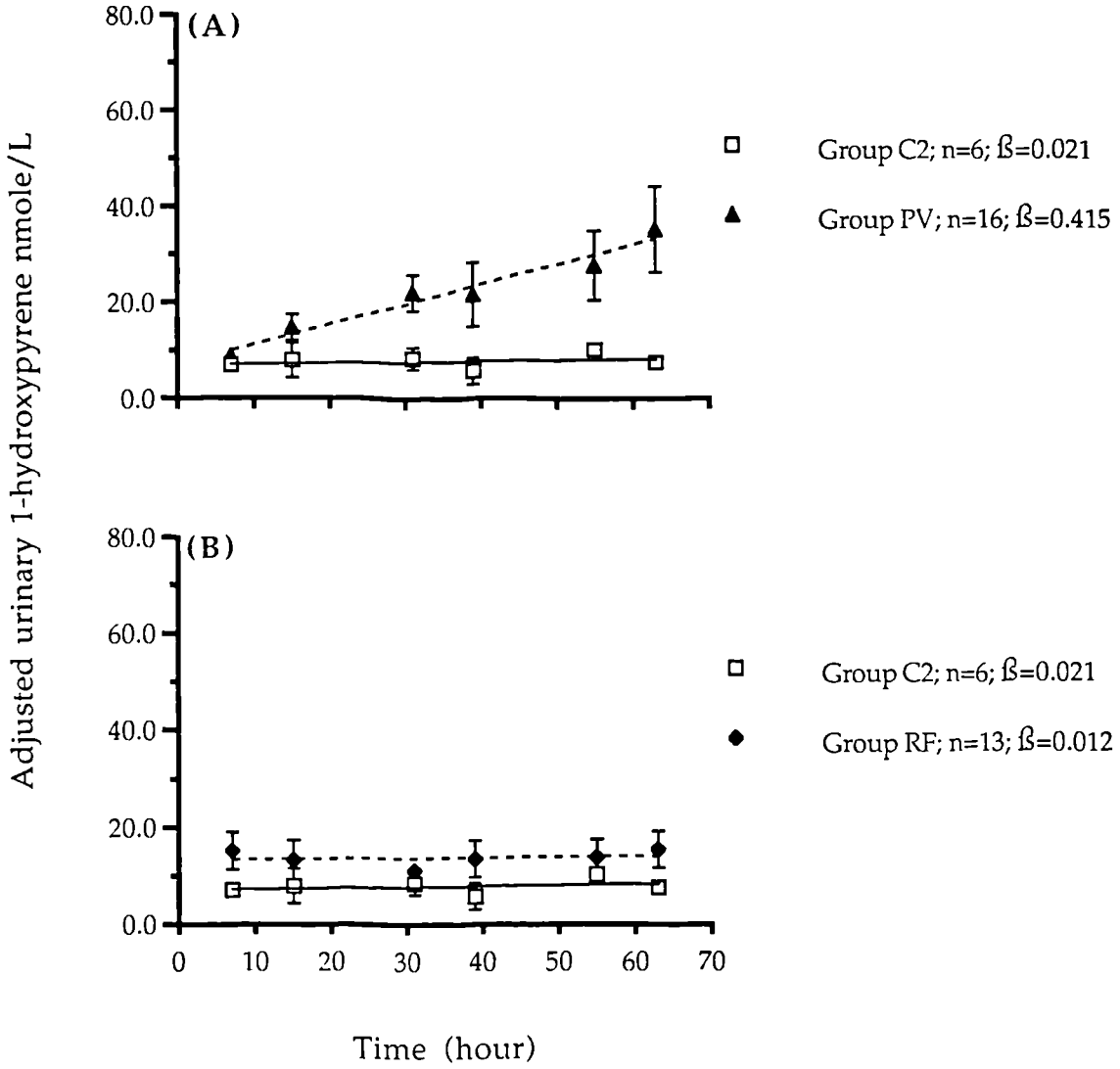


Figure : 16.10 Mean urinary excretion of 1-hydroxypyrene in office workers (**C2**), manual workers (**M**) and groups of pavers (**P1 and P2**) over a three days period
- Mean ± SEM and rate of increase of excretion "ß" -
(Absolute values)

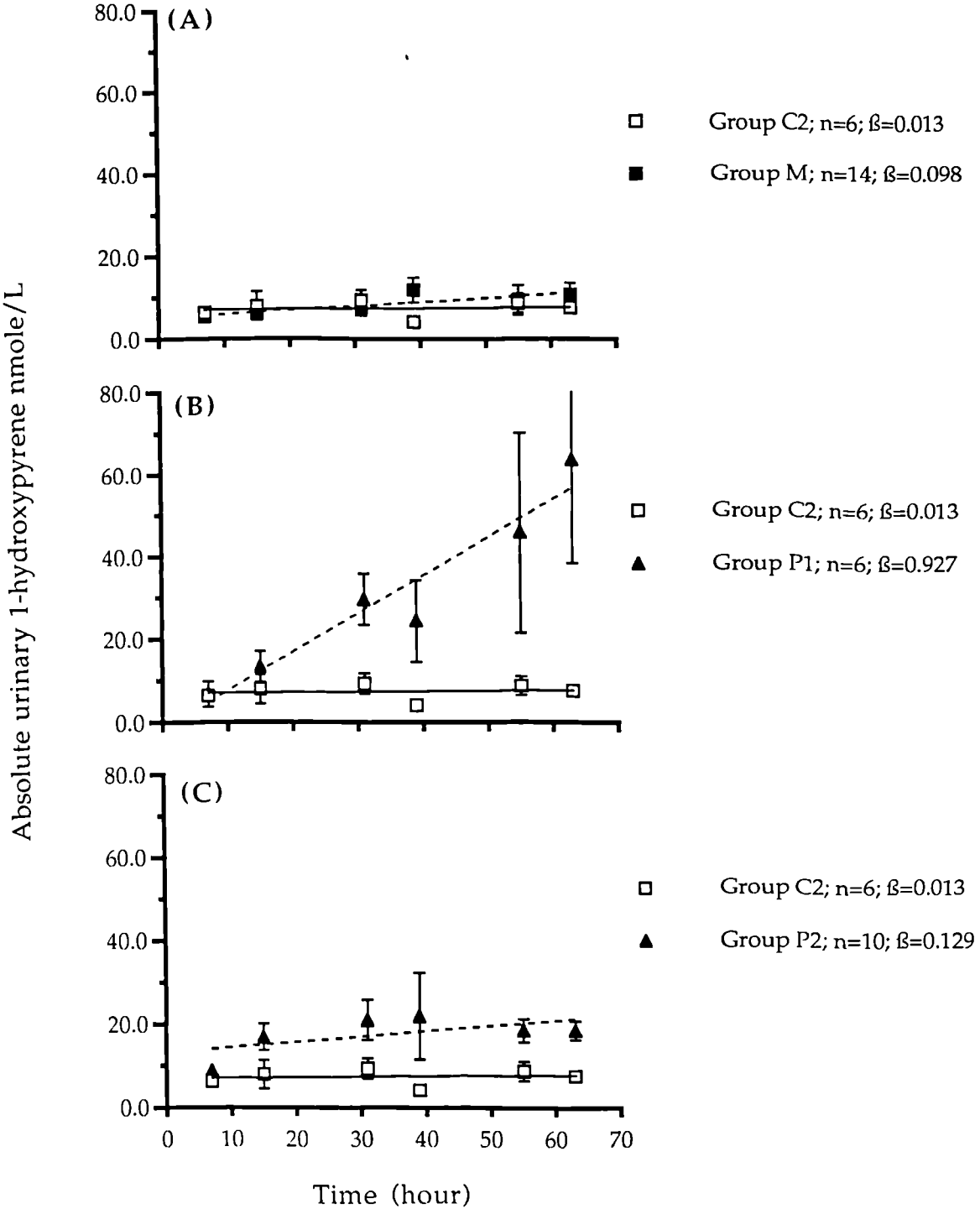


Figure : 16.11 Mean urinary excretion of 1-hydroxypyrene in office workers (**C2**) and groups of roofers (**R1 and R2**) over a three days period - Mean ± SEM and rate of increase of excretion "ß" - *(Absolute values)*

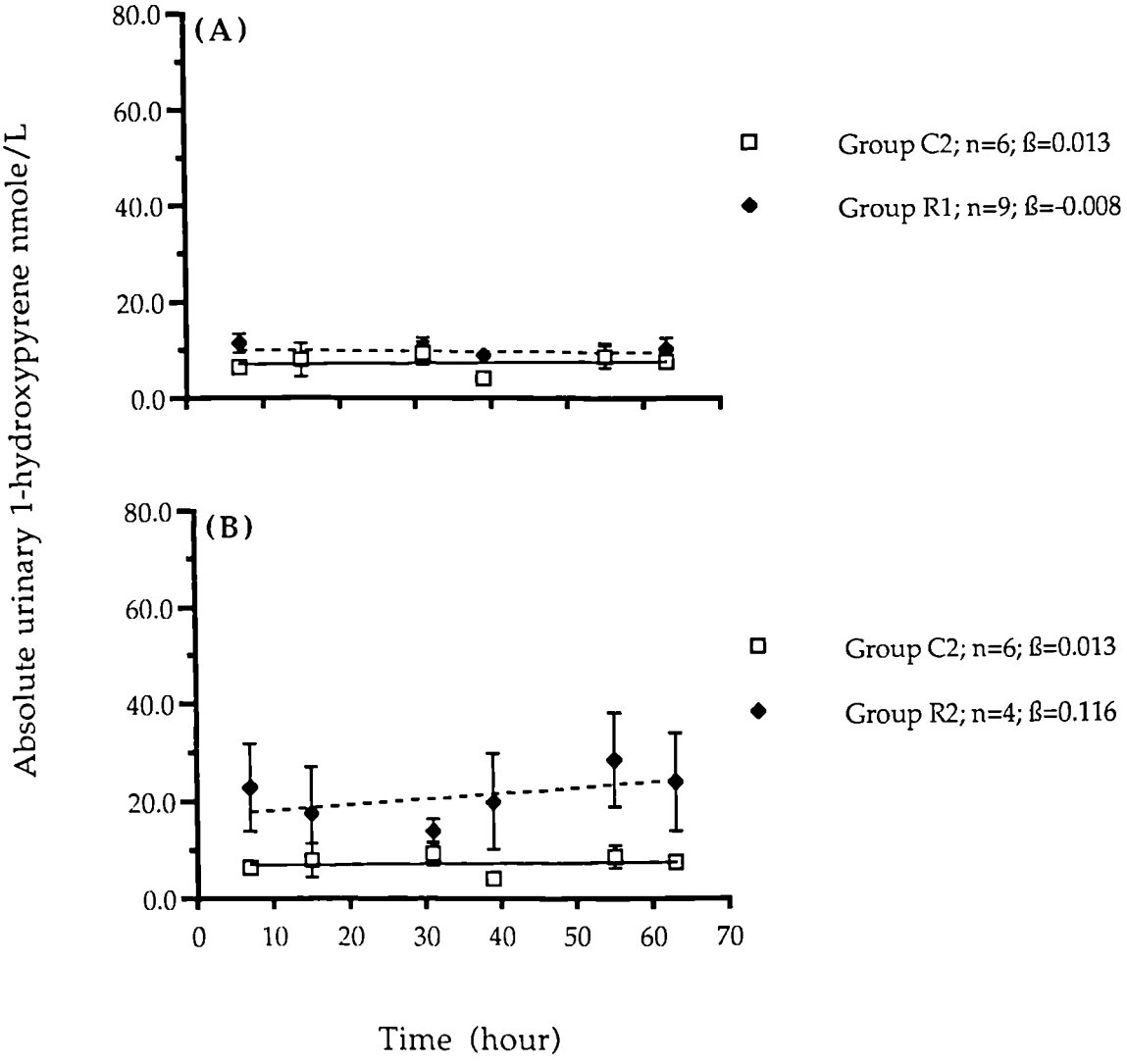


Figure : 16.12 Mean urinary excretion of 1-hydroxypyrene in office workers(**C2**), pavers group (**PV**) and roofers group(**RF**) over a three days period - Mean ± SEM and rate of increase of excretion "ß" - *(Absolute values)*

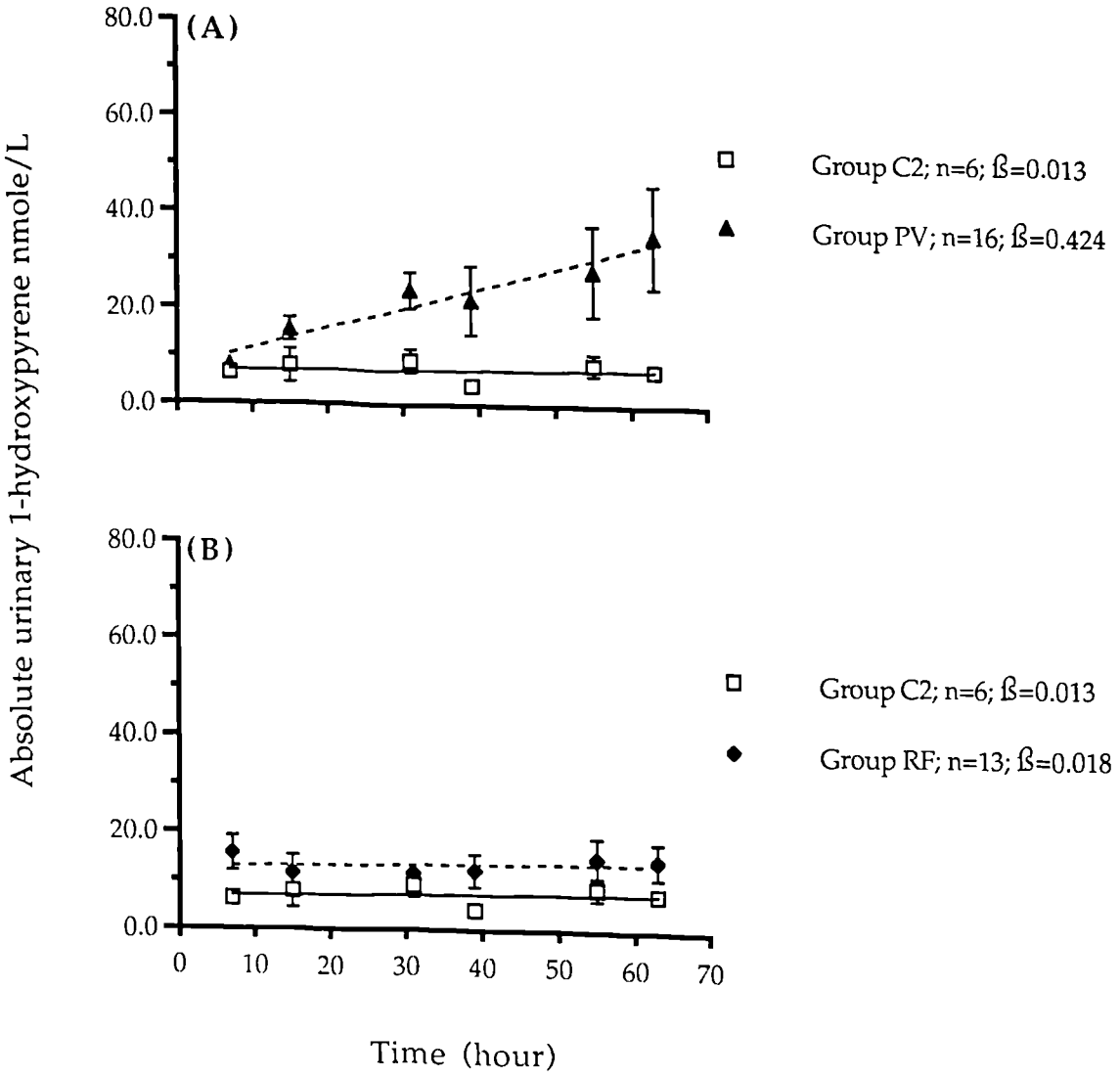


Table : 16.1 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase of its excretion (i.e. slope "a") in office workers (C2) and manual workers (M) over a three days period (Corrected values)

(A)

Office worker		Corrected 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
80	Office work	1.30	1.87	0.42	0.57	0.93	0.68	-0.015
81	Office work	0.48	0.65	0.38	0.14	0.87	n.v.	0.003
82	Office work	0.16	0.44	0.66	0.19	1.03	0.82	0.012
83	Office work	0.65	0.19	0.37	0.33	n.v.	0.31	-0.003
84	Sales Rep.	0.41	n.v.	1.18	3.14	2.58	1.09	0.023
86	Office work	1.72	n.v.	1.70	0.34	0.77	1.37	-0.012
	Mean	**0.79**	**0.79**	**0.79**	**0.79**	**1.24**	**0.85**	**0.001**
	SEM	*0.24*	*0.37*	*0.22*	*0.47*	*0.34*	*0.18*	*0.006*

(B)

Manual worker		Corrected 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
10	Sewer Maintenance	0.25	1.09	0.40	0.51	1.34	2.47	0.028
14	Handling cement	0.21	2.93	0.29	2.39	0.38	3.43	0.020
15	Handling cement	0.13	0.55	0.82	0.51	0.18	0.59	0.002
16	Sewer Maintenance	0.63	0.29	1.49	1.69	1.10	1.23	0.014
17	Sewer Maintenance	2.11	0.40	0.77	5.20	0.42	4.63	0.037
30	Site supervisor	0.56	0.44	0.23	0.09	0.19	0.34	-0.005
32	Sewer Maintenance	0.27	0.48	0.48	0.30	0.33	0.32	-0.001
33	Site Supervisor	0.33	0.11	0.08	0.02	0.06	0.06	-0.004
34	Site Supervisor	0.21	0.43	0.12	0.09	0.44	0.29	0.001
35	Slatter	n.v.	n.v.	n.s.	n.v.	1.09*	n.s.	N.C.
37	Laying Insulation	0.85	0.38	1.53	2.37	0.81	3.90	0.041
38	Painting Metal	0.52	1.48	1.05	4.12	13.24	2.46	0.126
41	Painting Metal	0.38	0.49	0.38	0.33	0.71	0.46	0.003
45	Stripping Lead	0.49	0.56	0.98	0.97	1.62	1.84	0.025
48	Laying Insulation	0.60	0.78	0.06	1.45	1.08	0.62	0.005
	Mean	**0.54**	**0.74**	**0.62**	**1.43**	**1.47**	**1.62**	**0.020**
	SEM	*0.13*	*0.19*	*0.13*	*0.43*	*0.91*	*0.41*	*0.009*

N.C.: Not calculated (less than four samples analysed)

n.v.: Not enough volume to carry out analysis

n.s.: No sample provided M: Morning A: Afternoon

Table : 16.2 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase in excretion (i.e. slope "a") in groups of pavers (P1 & P2) over a three days period (Corrected values)

(A)

Paver		Corrected 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
7	Paver *	2.71	1.86	2.15	0.11	9.83	12.80	0.181
8	Paver *	0.16	0.46	1.55	0.43	0.88	1.63	0.019
11	Paver *	0.23	2.79	4.87	4.77	5.49	8.36	0.117
13	Paver *	1.57	1.72	3.05	4.14	1.83	2.47	0.013
18	Paver *	0.43	0.65	3.79	2.16	2.18	4.76	0.061
19	Paver *	0.20	0.29	0.27	1.23	0.45	1.37	0.017
	Mean	**0.88**	**1.30**	**2.61**	**2.14**	**3.44**	**5.23**	**0.068**
	SEM	*0.43*	*0.40*	*0.67*	*0.79*	*1.47*	*1.85*	*0.028*

* Exact job description not specified

(B)

Paver		Corrected 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
60	Raker	0.67	0.54	1.72	2.01	5.01	5.16	0.090
61	Raker	2.05	0.15	0.97	0.84	1.74	2.75	0.021
62	Raker	0.64	0.87	0.48	0.99	1.09	0.98	0.007
63	Tractor Driver	1.01	0.56	1.30	0.97	0.78	1.38	0.006
64	Screwman	1.36	0.72	0.84	0.75	1.06	2.73	0.019
65	Roller Driver	0.63	1.17	1.11	0.14	1.36	1.92	0.015
66	Foreman	0.24	1.64	5.40	0.81	1.98	2.62	0.023
67	Paver	0.78	4.99	2.30	5.20	2.82	2.93	0.012
68	Roller Driver	1.40	1.99	1.51	0.86	2.86	1.63	0.009
69	Raker	1.89	2.79	1.71	9.19	6.08	2.38	0.046
	Mean	**1.07**	**1.54**	**1.73**	**2.18**	**2.48**	**2.45**	**0.025**
	SEM	*0.19*	*0.46*	*0.44*	*0.90*	*0.56*	*0.37*	*0.008*

M: Morning

A: Afternoon

Table : 16.3 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase in excretion (i.e. slope "a") in groups of roofers (R1 & R2) over a three days period (Corrected values)

(A)

Roofer		Corrected 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
20	Roofer*	n.s.	n.s.	0.58	0.43	0.25	0.98	0.008
21	Roofer*	n.s.	n.s.	1.94	0.30	0.31	0.37	-0.039
36	Roofer*	1.34	1.10	0.96	1.25	1.16	0.81	-0.005
39	Roofer*	0.14	0.19	0.21	0.26	0.22	0.31	0.002
40	Roofer*	1.28	0.81	0.61	0.79	0.53	0.76	-0.008
42	Roofer*	0.82	0.56	0.57	0.54	0.54	0.42	-0.005
43	Roofer*	2.66	1.38	0.68	1.27	0.85	0.95	-0.023
44	Roofer*	1.62	1.54	1.04	3.45	3.95	5.08	0.064
46	Roofer*	0.52	0.76	0.80	0.64	1.74	2.19	0.027
	Mean	**1.17**	**0.87**	**0.82**	**0.99**	**1.06**	**1.32**	**0.002**
	SEM	*0.31*	*0.18*	*0.16*	*0.33*	*0.40*	*0.51*	*0.010*

* Exact job description not specified

(B)

Roofer		Corrected 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
87	Mixer Operator	4.91	5.49	3.20	5.78	5.13	4.89	0.001
88	Troweller	0.68	2.35	1.40	1.08	0.60	1.18	-0.009
89	Troweller	0.83	0.45	1.54	2.28	1.00	0.92	0.007
90	Tipper	1.46	2.30	1.08	1.67	2.18	3.47	0.023
	Mean	**1.97**	**2.65**	**1.81**	**2.70**	**2.23**	**2.62**	**0.006**
	SEM	*0.99*	*1.05*	*0.47*	*1.05*	*1.02*	*0.95*	*0.007*

n.s.: No sample provided

M: Morning

A: Afternoon

Table : 16.4 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase of excretion (i.e. slope "a") in office workers (C2) and manual workers (M) over a three days period (Adjusted values)

(A)

Office worker		Adjusted 1-Hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
80	Office work	12.77	18.57	4.03	5.22	7.80	5.88	-0.168
81	Office work	6.23	5.86	3.88	1.31	8.03	4.63	-0.005
82	Office work	1.37	5.24	6.93	2.60	13.09	8.83	0.145
83	Office work	6.17	2.09	5.04	3.06	n.v.	2.81	-0.035
84	Sales Rep.	4.05	n.v.	13.44	19.50	16.30	10.64	0.145
86	Office work	11.67	n.v.	17.58	3.07	6.09	12.82	-0.057
	Mean	7.04	7.94	8.48	5.79	10.26	7.60	0.004
	SEM	*1.80*	*3.64*	*2.33*	*2.79*	*1.91*	*1.56*	*0.05*

(B)

Manual worker		Adjusted 1-Hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
10	Sewer Maintenance	3.13	8.96	5.02	4.05	9.14	23.53	0.238
14	Handling cement	1.72	26.15	2.40	21.26	3.09	36.53	0.245
15	Handling cement	1.70	4.28	7.89	3.47	2.35	5.52	0.021
16	Sewer Maintenance	3.64	2.76	15.48	21.95	9.66	10.03	0.143
17	Sewer Maintenance	23.88	3.47	10.80	38.16	4.31	25.70	0.074
30	Site supervisor	5.05	4.78	1.88	0.88	1.45	2.31	-0.061
32	Sewer Maintenance	2.21	3.51	4.06	2.53	2.86	3.07	0.002
33	Site Supervisor	3.68	1.45	1.07	0.41	0.75	0.76	-0.041
34	Site Supervisor	2.15	3.22	1.11	0.97	4.12	2.51	0.011
35	Slatter	n.v.	n.v.	n.s.	n.v.	0.96*	n.s.	N.C.
37	Laying Insulation	6.10	2.44	12.12	22.38	5.57	21.82	0.227
38	Painting Metal	4.06	14.58	12.67	33.73	91.29	22.52	0.890
41	Painting Metal	3.42	5.48	3.49	3.12	4.98	3.64	-0.002
45	Stripping Lead	6.00	5.32	11.89	10.75	17.60	18.35	0.244
48	Laying Insulation	7.16	8.05	0.78	18.05	14.44	7.27	0.083
	Mean	5.28	6.75	6.48	12.98	11.50	13.11	0.148
	SEM	*1.50*	*1.74*	*1.38*	*3.44*	*5.84*	*3.04*	*0.064*

n.c.: Not calculated (< 3 samples provided) M: Morning

n.v.: Not enough volume to carry out analysis A: Afternoon

n.s.: Sample not provided *: Not included in the mean and SEM

Table : 16.5 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase of excretion (i.e. slope "a") in groups of pavers (P1 & P2) over a three days period (Adjusted values)

(A)

Paver		Adjusted 1-Hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
7	Paver *	24.51	20.41	24.88	1.21	123.07	148.78	**2.266**
8	Paver *	1.76	4.29	22.71	3.41	13.17	20.18	**0.257**
11	Paver *	2.24	23.87	49.35	46.49	53.89	87.46	**1.240**
13	Paver *	11.31	19.16	27.15	49.27	12.14	24.64	**0.134**
18	Paver *	5.27	7.63	37.24	15.79	21.58	50.86	**0.612**
19	Paver *	1.60	2.74	2.98	13.15	4.12	13.26	**0.165**
	Mean	**7.78**	**13.02**	**27.39**	**21.55**	**38.00**	**57.53**	**0.779**
	SEM	*3.67*	*3.75*	*6.33*	*8.63*	*18.43*	*21.37*	*0.342*

* Exact job description not specified

(B)

Paver		Adjusted 1-Hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
60	Raker	7.35	6.52	17.79	14.77	41.33	43.58	**0.708**
61	Raker	15.59	1.67	8.61	6.80	12.59	26.39	**0.214**
62	Raker	5.93	10.53	6.28	9.98	10.47	10.92	**0.064**
63	Tractor Driver	12.74	7.74	14.29	10.18	9.07	14.62	**0.026**
64	Screwman	11.06	10.25	10.13	8.61	12.22	19.48	**0.112**
65	Roller Driver	8.48	13.17	14.97	1.53	15.57	16.61	**0.092**
66	Foreman	2.58	14.45	57.56	8.10	18.38	22.54	**0.183**
67	Paver	6.75	41.61	22.17	44.88	30.88	25.22	**0.164**
68	Roller Driver	8.49	24.32	18.85	11.68	23.96	14.69	**0.057**
69	Raker	17.04	28.62	18.06	101.00	42.53	24.32	**0.339**
	Mean	**9.60**	**15.89**	**18.87**	**21.75**	**21.70**	**21.84**	**0.196**
	SEM	**1.42**	*3.83*	*4.58*	*9.56*	*3.97*	*2.92*	*0.064*

M: Morning

A: Afternoon

Table : 16.6 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase of excretion (i.e. slope "a") in groups of roofers (R1 & R2) over a three days period (Adjusted values)

(A)

Roofer		Adjusted 1-hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
20	Roofer*	n.s.	n.s.	8.29	6.57	3.64	13.79	0.101
21	Roofer*	n.s.	n.s.	21.69	3.40	4.24	4.21	-0.427
36	Roofer*	14.55	10.59	11.04	13.39	14.78	8.29	-0.034
39	Roofer*	1.71	2.02	1.72	1.91	1.97	2.12	0.005
40	Roofer*	12.18	7.89	6.92	8.88	3.96	9.08	-0.066
42	Roofer*	8.13	5.32	6.97	3.18	6.13	3.77	-0.050
43	Roofer*	22.24	14.24	7.23	12.25	4.66	10.10	-0.213
44	Roofer*	16.13	12.96	11.06	24.57	23.66	31.30	0.289
46	Roofer*	7.12	7.95	7.49	8.15	19.90	19.83	0.249
	Mean	**11.25**	**8.40**	**9.16**	**9.14**	**9.22**	**11.39**	**-0.016**
	SEM	*2.55*	*1.62*	*1.81*	*2.34*	*2.69*	*3.09*	*0.073*

* Exact job description not specified

(B)

Roofer		Adjusted 1-hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
87	Mixer Operator	49.33	49.49	22.82	54.49	46.97	40.70	-0.069
88	Troweller	7.44	10.85	16.59	10.71	5.72	6.53	-0.063
89	Troweller	10.11	0.19	11.84	12.99	13.91	11.09	0.128
90	Tipper	17.71	23.67	12.10	16.01	30.85	40.62	0.334
	Mean	**21.15**	**21.05**	**15.84**	**23.55**	**24.36**	**24.74**	**0.082**
	SEM	*9.64*	*10.63*	*2.57*	*10.37*	*9.17*	*9.24*	*0.095*

M: Morning

A: Afternoon

Table : 16.7 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase of excretion (i.e. slope "a") in office workers (C2) and manual workers (M) over a three days period (Absolute values)

(A)

Office worker		Absolute 1-Hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
80	Office work	12.09	17.84	3.70	4.50	5.96	4.69	-0.184
81	Office work	8.58	4.94	3.88	1.15	6.99	n.v.	-0.046
82	Office work	1.08	6.44	7.17	3.85	17.52	9.45	0.184
83	Office work	5.58	2.31	7.42	2.67	n.v.	2.39	-0.038
84	Sales Rep.	3.87	n.v.	15.53	9.95	8.51	10.00	0.067
86	Office work	6.71	n.v.	17.83	2.60	4.31	11.37	-0.008
	Mean	6.32	7.88	9.25	4.12	8.66	7.58	-0.004
	SEM	*1.55*	*3.43*	*2.45*	*1.26*	*2.32*	*1.72*	*0.050*

(B)

Manual worker		Absolute 1-Hydroxypyrene nmol/L						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
10	Sewer Maintenance	4.09	6.67	6.60	2.88	5.29	21.38	0.181
14	Handling cement	1.28	21.74	1.81	17.60	2.26	38.56	0.291
15	Handling cement	2.36	2.95	7.26	2.00	3.26	4.88	0.023
16	Sewer Maintenance	1.68	2.50	15.79	30.27	7.87	7.39	0.145
17	Sewer Maintenance	27.36	2.77	16.53	24.39	4.33	11.27	-0.167
30	Site supervisor	4.27	5.19	1.39	1.02	0.98	1.34	-0.072
32	Sewer Maintenance	1.64	2.23	3.13	1.94	2.28	2.82	0.012
33	Site Supervisor	4.12	2.04	1.52	1.01	1.00	1.01	-0.047
34	Site Supervisor	2.16	2.12	0.97	1.03	3.65	2.00	0.011
35	Slatter	n.v.	n.v.	n.s.	n.v.	1.02	n.s.	N.C.
37	Laying Insulation	3.77	1.30	8.58	20.07	3.26	9.67	0.108
38	Painting Metal	2.82	13.85	15.81	24.97	53.62	19.38	0.568
41	Painting Metal	2.87	6.18	3.02	2.80	2.99	2.57	-0.031
45	Stripping Lead	7.64	4.82	14.96	11.98	19.07	17.72	0.238
48	Laying Insulation	8.82	8.14	1.07	23.52	20.69	8.75	0.151
	Mean	5.35	5.89	7.03	11.82	9.32	10.62	0.101
	SEM	*1.79*	*1.51*	*1.66*	*2.98*	*3.57*	*2.82*	*0.050*

N.C.: Not calculated (< 3 samples analysed) M: Morning

n.v.: Not enough volume to carry analysis A: Afternoon

n.s.: No sample provided *: Not included in mean and SEM

Table : 16.8 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase of excretion (i.e. slope "a") in groups of pavers (P1 & P2) over a three days period (Absolute values)

(A)

Paver		Absolute 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
7	Paver *	20.76	22.43	29.36	1.35	161.52	176.64	**2.931**
8	Paver *	1.95	3.80	36.85	2.41	21.99	26.10	**0.376**
11	Paver *	2.09	18.76	48.70	43.54	50.96	90.11	**1.287**
13	Paver *	7.04	21.49	22.51	60.37	6.77	23.81	**0.136**
18	Paver *	6.73	9.19	35.26	10.04	20.64	53.92	**0.604**
19	Paver *	1.14	2.46	3.31	13.94	3.54	12.31	**0.157**
	Mean	**6.67**	**13.30**	**29.57**	**24.38**	**45.77**	**63.70**	**0.915**
	SEM	*3.01*	*3.67*	*6.30*	*9.92*	*24.43*	*25.29*	*0.439*

* Exact job description not specified

(B)

Paver		Absolute 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
60	Raker	8.06	8.14	18.06	9.45	30.91	33.64	**0.474**
61	Raker	10.46	1.88	7.11	4.96	7.90	24.20	**0.207**
62	Raker	5.18	13.21	8.73	9.78	9.62	12.25	**0.054**
63	Tractor Driver	16.88	11.59	15.73	10.54	10.76	15.32	**-0.034**
64	Screwman	8.12	15.98	12.63	10.05	14.35	11.98	**0.027**
65	Roller Driver	12.27	14.96	21.70	1.68	18.10	13.25	**0.004**
66	Foreman	2.75	11.81	60.80	7.85	16.12	17.84	**0.124**
67	Paver	5.38	31.59	20.45	35.67	33.84	19.98	**0.214**
68	Roller Driver	4.20	30.90	24.63	17.11	18.30	12.39	**-0.022**
69	Raker	14.36	28.71	18.84	111.20	25.48	24.28	**0.243**
	Mean	**8.76**	**16.88**	**20.87**	**21.83**	**18.54**	**18.51**	**0.129**
	SEM	*1.47*	*3.21*	*4.78*	*10.36*	*2.81*	*2.25*	*0.050*

M: Morning

A: Afternoon

Table : 16.9 Daily morning and afternoon urinary output of 1-hydroxypyrene and the rate of increase of excretion (i.e. slope "a") in groups of roofers (R1 & R2) over a three days period (Absolute values)

(A)

Roofer		Absolute 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
20	Roofer*	n.s.	n.s.	13.01	11.28	5.84	21.18	0.136
21	Roofer*	n.s.	n.s.	24.44	3.90	6.28	4.84	-0.460
36	Roofer*	15.77	9.75	12.93	14.24	19.86	8.29	-0.001
39	Roofer*	2.18	2.13	1.27	1.23	1.65	1.23	-0.015
40	Roofer*	11.05	7.39	7.96	10.07	2.59	11.18	-0.035
42	Roofer*	7.79	4.82	8.87	1.51	7.06	3.16	-0.048
43	Roofer*	16.94	14.41	7.62	11.30	2.01	10.63	-0.171
44	Roofer*	15.54	9.96	11.65	15.08	11.49	15.80	0.022
46	Roofer*	10.55	8.20	6.64	10.95	23.12	16.82	0.205
	Mean	**11.40**	**8.09**	**10.49**	**8.84**	**8.88**	**10.35**	**-0.041**
	SEM	*1.99*	*1.49*	*2.13*	*1.76*	*2.61*	*2.23*	*0.064*

* Exact job description not specified

(B)

Roofer		Absolute 1-HP µmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
87	Mixer Operator	48.12	41.72	14.02	48.78	40.42	30.81	-0.155
88	Trowellor	8.15	3.71	20.22	10.26	5.20	2.86	-0.066
89	Trowellor	12.77	1.04	8.05	5.91	21.02	13.84	0.175
90	Tipper	22.28	23.85	13.68	14.66	47.74	48.69	0.509
	Mean	**22.83**	**17.58**	**13.99**	**19.90**	**28.60**	**24.05**	**0.116**
	SEM	*8.93*	*9.52*	*2.48*	*9.79*	*9.62*	*10.03*	*0.148*

M: Morning

A: Afternoon

Table : 16.10 Multiple linear regression analysis for Day- 3-post-shift urinary 1-hydroxypyrene concentration of all groups (M, P1, P2, R1, R2)

	Regression Coefficient		Intercept		Corr. Coeff. of Model	
Predictor variable	"ß" ± SEM	p-value	alpha	p-value	r-squared	p-value
Day-1-pre-shift U1-HP	0.57 ± 0.43	0.193	1.27 ± 0.56	0.03	0.178	0.027
3-day-GM ∑9PAHs	0.001±0.0002	0.116				
D-1-pre-shift U1-HP	0.48 ± 0.48	0.318	1.47 ± 0.54	0.009	0.165	0.036
3-day-GM ∑8PAHs	0.002 ± 0.002	0.168				
D-1-pre-shift U1-HP	0.61 ± 0.48	0.219	1.51 ± 0.54	0.008	0.145	0.056
3-day-GM Phenan.	0.003 ± 0.003	0.311				
D-1-pre-shift U1-HP	0.93 ± 0.51	0.080	1.92 ± 0.54	0.008	0.107	0.204
3-day-GM Pyrene	0.005 ± 0.005	0.343				

Corr. Coeff.: Correlation coefficient

"ß": is "ß1" or "ß2". "ß1" for D-1-pre-shift U1-HP and "ß2" for airborne PAH-exposure index

Table : 16.11

Multiple linear regression analysis for Day- 3-post-shift urinary 1-hydroxypyrene concentration of manual workers and pavers

	Regression Coefficient		Intercept		Corr. Coeff. of Model	
Predictor variable	"ß" ± SEM	p-value	alpha	p-value	r-squared	p-value
Day-1-pre-shift U1-HP	1.60 ± 0.75	0.042	1.25 ± 0.77	0.115	0.236	0.039
3-day-GM ∑9PAHs	0.0002 ± 0.0004	0.584				
D-1-pre-shift U1-HP	1.07 ± 0.63	0.099	0.47 ± 0.69	0.503	0.432	0.001
3-day-GM ∑8PAHs	0.009 ± 0.003	0.007				
D-1-pre-shift U1-HP	1.40 ± 0.67	0.115	0.68 ± 0.72	0.351	0.373	0.004
3-day-GM Phenan.	0.015 ± 0.006	0.026				
D-1-pre-shift U1-HP	1.71 ± 0.88	0.069	1.92 ± 1.10	0.096	0.209	0.136
3-day-GM Pyrene	0.009 ± 0.010	0.407				

Corr. Coeff.: Correlation coefficient

"ß": is "ß1" or "ß2". "ß1" for D-1-pre-shift U1-HP and "ß2" for airborne PAH-exposure index

Table : 16.12

Multiple linear regression analysis for Day- 3-post-shift urinary 1-hydroxypyrene concentration of manual workers and roofers

	Regression Coefficient		Intercept		Corr. Coeff. of Model	
Predictor variable	"ß" ± SEM	p-value	alpha	p-value	r-squared	p-value
Day-1-pre-shift U1-HP	0.61 ± 0.42	0.161	1.06 ± 0.43	0.022	0.229	0.065
3-day-GM ∑9PAHs	0.0001 ± 0.0005	0.750				
D-1-pre-shift U1-HP	0.66 ± 0.39	0.104	1.09 ± 0.42	0.018	0.227	0.067
3-day-GM ∑8PAHs	0.0002 ± 0.0013	0.857				
D-1-pre-shift U1-HP	0.64 ± 0.38	0.101	1.08 ± 0.47	0.018	0.228	0.066
3-day-GM Phenan.	0.0006 ± 0.002	0.787				
D-1-pre-shift U1-HP	0.61 ± 0.38	0.132	0.91 ± 0.59	0.147	0.246	0.184
3-day-GM Pyrene	0.001 ± 0.003	0.837				

Corr. Coeff.: Correlation coefficient

"ß": is "ß1" or "ß2". "ß1" for D-1-pre-shift U1-HP and "ß2" for airborne PAH-exposure index

CHAPTER 17

DISCUSSION OF

URINARY 1-HYDROXYPYRENE

17.1 INTRODUCTION

This chapter discusses the results presented in chapter 16 and is divided into three parts.

Part I: This part will consider the results of U1-HP corrected for creatinine that were used to determine differences between groups of pavers and roofers and non-occupationally PAH-exposed controls with respect to the internal dose of pyrene/PAHs.

Part II: The usefulness of correcting concentration of urinary biomarkers have been addressed in this thesis (chapter 9). Using the results of U1-HP presented in chapter 16 this phenomenon has been tested. Justification of why the use of U1-HP values corrected for creatinine may not be the best way to express the results of this biomarker is presented.

Part III: Investigators are continuously trying to carry out a dose-response assessment between external exposure to PAH and U1-HP as a selective biomarker of internal exposure to pyrene/PAHs. In this study we made use of the observation of Jongeneelen (1992) that the sample at the end of a 3-day working period can give a reliable indication of the dose over that period. By way of multiple linear regression analysis and using the results of corrected U1-HP results, we studied the relationship between day-1-pre-shift U1-HP and 3-day-GM of different airborne PAH-exposure indices as predictor variables and day-3-post-shift as the dependant variable.

17.2 PART I

17.2.1 Baseline Excretion of Urinary 1-Hydroxypyrene

It was necessary prior to summarising the U1-HP excretion over time to identify the baseline values for mean U1-HP excretion in the populations and relate them to levels reported in the literature. Knowledge of baseline values are essential for the interpretation of results

of the excretion of a biological marker over time since in certain circumstances high background output may indicate that excretion have approached steady state due to saturation of metabolic pathways.

There was no difference between urinary 1-hydroxypyrene excretion on the first day. Although occupationally PAH-exposed (both pavers and roofers) had a relatively higher baseline which suggested that their internal dose of pyrene was not fully cleared during the occupational PAH-exposure free period (i.e. weekend). They were still within the limits of background levels of urinary 1-hydroxypyrene (0 - 1.2 μmol/mol creatinine) reported in the literature (Jongeneelen, 1992) but slightly higher than baseline pre-exposure U1-HP of paving workers (~ 0.62 μmol/mol creatinine) (Jongeneelen, 1988[b]).

Only values measured in group R2 were higher than 1.2 μmol/mol creatinine. There are a number of explanations for these results. Firstly, the percentage of smokers in this group was higher than the other groups which could have contributed to their elevated level. This effect however could be argued because roofers group R1 had a baseline twice as high as the manual workers although both groups had similar external PAH-exposure profiles and percentage of smokers. Their external exposure during the week prior to the survey might have been relatively high and have contributed to the background values observed. It could simply be due to inter-individual variation in pyrene toxicokinetics. As we see it is difficult to attribute this observation to one factor and not the other and much more information is needed inorder to arrive to a sensible explanation.

The influence of this result on the study of rate of increase of U1-HP excretion is minimal since it is obvious that this level is by no means approaching steady state. Firstly, because mean U1-HP excretion in the day-3-post-shift samples of pavers were three time as high and secondly investigators have observed an increased rate of excretion with baseline values as high as 5 μmol/mol creatinine reaching a peak excretion after four days of exposure a higher as 10 μmol/mol creatinine in creosote workers (Jongeneelen, 1985).

17.2.2 Rate of Increase of Excretion of Urinary 1-Hydroxypyrene

Office workers (group C2): The no build up in U1-HP excretion in control group C2 was expected since their external dose to PAH was virtually none existing. The zero rate of increase was statistically confirmed. The profile of U1-HP excretion in control groups is as expected. Jongeneelen (1992) presumed that this profile is true and assumed that the end-of-work values of U1-HP in controls are equal to pre-work ones for his data analysis. This may suggest that comparing the rate of increase of excretion of occupationally PAH-exposed groups to the null hypothesis could be sufficient in establishing a build up in the dose of pyrene/PAHs.

Manual workers: The highest rate of increase of U1-HP excretion was for pavers group P1. The build up in this group of pavers was significantly greater than control group C2 but not group M. The statistically significant build up of U1-HP excretion in group M in relation to a zero rate of increase was the reason for the this non-significant difference. The misclassification mentioned earlier (chapter 13) in our case was not the reason behind this significant increase particularly since the manual workers (M # 41 and 45) who were suspected to be misclassified according to the ambient monitoring assessment did not have the highest rate of increase (table 16.1b). A more logical and possible explanation for the positive rate of increase in group M was their external exposure to PAHs. This shows that the exposure of group M to appreciable levels of PAHs from environmental pollution or unidentified PAH-emission sources in their working environment should be considered when interpreting the results of the biological monitoring program. Group M is fairly tightly matched with the occupationally PAH-exposed groups with respect to confounding factors (work load, socio-economic class, respiratory ventilation rate) that are usually disregarded in risk assessments such as the one in this study. Therefore, in theory, they should serve as a better referent group than the office workers. However, in this study this was not where the differences were quite small due to the low external exposure to PAHs in pavers and roofers.

Group P1 & P2: Group P1 had a higher rate of increase of U1-HP excretion in comparison to group P2. This may be explained by considering the multiple linear regression analysis. In the most significant model

obtained, the regression coefficient between the 3-day-GM of Σ8PAHs (i.e. Σ9PAHs - [naphthalene]) and change in U1-HP was highly significant. This meant that using this airborne PAH-exposure index we can significantly predict the change in U1-HP over the three working days period (see table 16.11). If we look more closely at the ambient monitoring results we observe that the mean of the 3-day-GM of the TWA concentration of Σ8PAHs of group P1 is higher than that of group P2. This observation is a reasonable explanation for the higher rate of increase observed in group P1. It is interesting to note that the same argument does not hold for the airborne PAH-exposure index (3-day-GM of TWA concentration Σ9PAHs) which suggests that the inclusion of naphthalene concentration weakens our estimation of the internal dose of PAH from ambient exposure levels in the paving industry.

These results may show that ambient monitoring programs assessing external exposure to PAHs during paving operation can provide an estimate of the internal dose of PAHs. The fact that there was a significant relationship between the airborne PAH-exposure index Σ8PAHs and the rate of excretion U1-HP suggest that the inhalation route was an important route of exposure. From these results we are not able to comment on the relative importance of the dermal route in relation to the pulmonary route of absorption since the former was not assessed.

Group R1 & R2: The rate of increase of U1-HP excretion among the groups of roofers was very small and not different from controls as well as the null hypothesis (zero rate of increase). This result was not expected since pyrene levels in their personal air samples was either equal (that of group R1) or an order of magnitude (that of group R2) higher than the pavers. The relationships observed between airborne PAH-exposure indices and U1-HP excretion in the roofers were not significant (table 16.12). The absence of the relationship may indicate that their exposure was by a different route from the pavers possibly the dermal route rather than the pulmonary route. The absorption rate through skin is expected to be lower and more delayed than by inhalation. Absolute levels of absorbed pyrene might be lower with less fluctuation contributing to lower 1-HP in the urine and less bioaccumulation. This is reflected in the profile of the excretion of U1-HP in group R2.

Studies have shown that there is a relationship between PAHs residues on skin and PAHs in personal air samples (Wolff *et al.* 1989). Together the observation of Wolff and co-workers and our supposition that exposure in the roofing industry was through skin may explain why group R1 had a zero rate of increase of U1-HP excretion and no elevation in their baseline values.

Studies have shown that exposure to PAHs in coal tar through the skin in dermatological patients does give rise to a significant increase of U1-HP over time (van Rooij *et al.* 1993). Similar findings were reported in creosote workers, who were also thought to be mainly exposed to PAHs by the dermal route (Jongeneelen *et al.* 1985). These observation however contrast with our results in roofers. In the experimental study on dermatological patient the pyrene doses applied to the skin were much higher than the doses that the roofers could have been exposed to even if efficiency in absorption was assumed to be at 100 % . In the case of creosote workers the concentration of PAHs in personal air samples were an order of magnitude higher than what we measured in personal air samples for group R2. Dermal absorption is influenced by the vehicle and may have been greater for coal tar or creosote directly in contact with the skin than bitumen fume.

The results are not supported by the subjective occupational hygiene assessment; where we observed that neither of the groups used any respiratory or personal protective equipment and the roofers were closer than the pavers to the source of PAH-emission due the nature of their job. On the basis of these observation it does not seem reasonable to assume that for the roofers the route of absorption be mainly dermal while that for the pavers it was through pulmonary uptake.

In conclusion it seems unreasonable to find a clinical justification for the profile of U1-HP excretion in group R2 and our suggestion that it could be due to inter-individual variation appears to be the most sensible explanation.

<u>Pavers and roofers group</u>: The pooling of the groups did alter the profiles of U1-HP results and therefore did not alter our conclusions. This may suggest that impact of combining data of biomarkers monitored in

groups of workers carrying out the same type of operation is possible.

17.3 PART II

17.3.1 Adjustment of Urinary 1-HP

There have been suggestions in the literature that using the ratio of urinary concentration of biomarkers to creatinine concentration in order to correct variation in urinary volume in spot urine samples may be inappropriate. A recent study monitoring the excretion of 1-naphthol, a selective biomarker of exposure to naphthalene, acknowledged this problem (Bieniek, 1993). The author studied the correlation between the results of naphthalene in air and those of 1-naphthol in urine expressed as absolute and corrected for either creatinine concentration or specific gravity.

Thompson *et al.* (1990) have suggested an alternative method for compensating for these variation. They showed that expressing urinary concentration of cotinine as adjusted values using their method improved the correlation between serum and urinary cotinine. We used their adjustment method to study U1-HP data.

In order to do so we have summarised the corrected, adjusted and absolute data of U1-HP identically. The comparison of the profiles of the U1-HP results showed subtle differences between the three methods of data presentation. These subtle differences were statistically confirmed in the analysis of variance conducted on the data. The significant differences in the rate of increase of excretion of U1-HP between groups was lowest when calculated using the corrected values (ANOVA, $p = 0.0059$). The differences were slightly more significant using the absolute value (ANOVA, $p = 0.0040$); while the adjusted values show the most significant results (ANOVA, $p = 0.0022$). Therefore it seems that reporting of the concentrations U1-HP as a ratio to creatinine concentration is not satisfactory. Although, the absolute value indicate that the excretion of U1-HP is not affected by the variation of urinary dilution between individuals. It appears that the method of adjustment suggested by

Thompson was the most appropriate way to handle data of U1-HP.

It is important to note there are major limitations in using of this adjustment method. Firstly, considerably large numbers of samples are needed in order to figure out the relationship existing between the concentration of the biomarker and creatinine and secondly, the relationship could not be assumed to hold true for all populations sampled and should be calculated for each study.

17.4 PART III

17.4.1 Correlation Between Urinary 1-HP Excretion and External Exposure to PAHs-species

When combining the results of the manual workers, pavers and roofers we found that none of the of the regression coefficients were statistically significant. The suspected variation in the route of absorption may be the reason behind the absence of any relationship; however, more importantly is the variation in the PAHs profile in personal air samples of pavers and roofers which was the main reason. That latter suggestion was possibly justified when the relationships were tested for in data collated for either of the occupations separately. In the roofers none of the models tested were significant which meant that the relationship between internal dose of PAHs dose not reflect the ambient exposures the roofers subjected to. This may suggest as previously mentioned that the roofers route of exposure is probably more dermal. The results of the pavers also supported our suggestions that their route of exposure was through the pulmonary route. This is shown in the significant model calculated between the predictor variables (Day-1-pre-shift U1-HP and 3-day-GM Σ8PAHs) and the dependant (day-3-post-shift U1-HP). In this model we observe that the airborne PAH-exposure index did significantly predict the internal dose which may signify the contribution of the respiratory route to the absorbed dose. The relationship was not significant when Σ9PAHs was used instead of Σ8PAHs. The inclusion of naphthalene concentration in the calculations of the airborne PAH-exposure index may have lead to this, especially that it was shown the external exposure to that PAHs is

highly variable not only between group but also within groups. The use of phenanthrene as an airborne PAH-exposure index lead to a significant model as well. The presence of phenanthrene in vapour form in the personal air samples of the pavers and the significance of its relationship with internal dose together strongly suggest that the route of absorption was most probably due to inhalation. The non-significant model obtained upon the use of pyrene as a predictor variable could be due to its presence in particulate form and thus affecting its bioavailability and the efficiency of its resorption in the lungs. Moreover, being mainly in particulate form might decrease its access to the lower respiratory tract and therefore it absorption into the circulatory system. For this conclusion to be justified we should study the particle size distribution by measuring the inhalable and respirable dust fractions during the industrial operations by using an air sampling instruments such as a cascade impactor.

CHAPTER 18

RESULTS OF

URINARY D-GLUCARIC ACID

18.1 INTRODUCTION

This chapter describes the results obtained for the urinary d-glucaric acid measurements. Pre-shift and post-shift urine samples collected on the first three days of each survey from subjects in groups C2, M, P1, P2, R1 and R2 were analysed for d-glucaric acid using the method described in section II, chapter 8 of this thesis. The results were expressed as UDGA mmol/mol. creatinine.

The mean ± SEM of the UDGA excretion in pre-shift and post-shift samples on all three days were calculated and presented separately in tables for each of the groups. The regression slope "ß" of the mean UDGA output over time is presented in figures for each of the groups.

The UDGA data on each subject over time was summarised by their regression slope " a " and mean slopes " ã "were compared between groups using ANOVA single-factor. This was followed by an unpaired Student's t-test using the pooled standard deviation to test for differences in mean slopes between group C2 or M and occupationally PAH-exposed groups (i.e. P1, P2, R1, R2, pavers group and roofers group). The mean slopes "ã" of each group is separately compared against the null hypothesis of zero slope (i.e. zero rate of increase of UDGA excretion; ã=0) using a one sample Student's t-test.

The value of the mean slopes "ã" is identical to regression slope of the mean UDGA excretion over time "ß" when there is no missing samples in the set of data (e.g. groups C2, P1, P2 and R2). In the case were there are few missing samples the value of "ß" is a close estimate of the value of the mean of individual regression slopes "ã".

18.1.1 Daily Excretion of Urinary D-Glucaric Acid

Tables 18.1A and 18.1B present the daily morning and afternoon UDGA output for all individuals in the office workers (C2) and manual workers (M) groups, respectively. The results of the daily pre-shift and post-shift UDGA excretion for subjects in groups P1 and P2 are presented in table 18.2A and 18.2B, respectively; while those of groups R1 and R2 are

given in tables 18.3A and 18.3B, respectively. Only means of daily UDGA excretion in the pavers group and the roofers group are presented in figures 18.3A and B, respectively.

The mean excretion of UDGA in pre-shift samples of group C2 on day 1 (mean ± SEM; 1.75 ± 0.21) was similar to that of group P1 (1.79 ± 0.42). These were lower than those of group M (2.27 ± 0.39) and group R1 (2.24 ± 0.44). Group P2 had the lowest mean of UDGA output (0.71 ± 0.13) in the pre-shift sample on day 1; while that of group R2 (4.33 ± 1.04) was the highest. This mean observed for group R2 was the highest mean of UDGA excretion between all the pre-shift and post-shift samples for all groups on all days. The mean excretion of UDGA in the pavers group (1.12 ± 0.22) was lower than that of group C2; while that of roofers group (3.00 ± 0.22) was ~ 1.7 time higher than group C2. The mean of UDGA output in pre-shift samples between all groups on day 1 was different, however the observed differences did not reach statistical significance (ANOVA single factor, $p>0.05$).

18.1.2 Rate of of Increase of Excretion of Urinary D-Glucaric Acid

18.1.2.1 Mean UDGA Excretion Over Time

In group C2, the rate of increase in mean UDGA excretion over time (i.e. slope "ß") was virtually zero (ß=0.001; fig. 18.1A). This rate of increase was slower than those of all groups. Group M, had a moderate rate of increase in UDGA excretion indicated by the regression slope of the means of morning and afternoon samples over time (ß=0.020; fig. 18.1A). Figures 18.2A and 18.1C show respectively, the rate of increase in UDGA output over time in groups R1 and P2. Both groups had a regression slope ß higher than that of group C2, but similar to that of the manual workers (fig. 18.1A). Group P1 had a distinctively high rate of increase of UDGA excretion (ß=0.060; fig. 18.1B). Group R2 had a negative regression slope (ß=-0.025) shown in figure 18.2B. The regression slope of group R2 would have been positive (ß=0.008) if the mean of their pre-shift samples on day 1 was excluded from calculations. Figure 18.3A shows that regression slope of mean UDGA excretion over time in the pavers group (Group PV; ß=0.035) was higher than that of group C2 and M. The regression slope of

the mean UDGA output over time in the roofers group (Group RF; ß=0.011) was approximately half that of group M and obviously higher than that of group C2.

18.1.2.2 Individuals' Slope of UDGA Excretion Over Time

Office workers: The UDGA data on each subject in group C2 over time are presented in table 18.1A. Three (C2 #s 81, 82, 84) out of the 6 subjects (i.e. 50 %) had a negative regression slope indicating that their rate of UDGA excretion was decreasing with time. The other three office worker had a positive but relatively slow rate of increase for UDGA excretion.

The group's mean of the regression slopes was nearly zero (ã = mean ± SEM; ã=0.001 ± 0.011) which is equal to the regression slope of the mean UDGA output over time in the all subjects (ß=0.001; fig. 18.1A).

The mean slopes of group C2 was not significantly different from the null hypothesis (one sample Student's t-test; $p>0.05$) meaning that their rate of increase of UDGA output is not different from zero.

Manual workers: In group M, individual regression slopes of UDGA excretion over time were calculated from all pre-shift and post-shift samples on the three days for twelve manual workers out of fifteen. The rate of increase of UDGA excretion in the slatter (M # 35) was calculated from 4 urine sample since he did not provide a pre-shift and a post-shift urine sample on days 2 and 3, respectively (table 18.1B). Manual workers M # 38 and 41 pre-shift samples on day 1 were lost during analysis and their regression slopes were computed from five data points (table 18.1B).

Thirty percent of the manual workers (M #s 16, 32, 35, 45 and 41) had negative rates of increase in excretion of UDGA. The latter of these five manual workers with negative regression slopes had the highest GM of ∑9PAHs TWA concentration, respectively (table 11.4a). Manual workers (M # 10, 34, 37, 38 and 48) had comparable positive regression slopes. The former four did not have comparable external exposure quantified in their respective GMs of ∑9PAHs TWA concentrations (table 11.4a). The two

manual workers handling cement (M #s 14 and 15) had the highest rate of increase of UDGA excretion (a=0.070) and (a=0.089), respectively. The second highest two positive regression slopes were recorded for manual worker (M #s 17 and 30). The former did sewer maintenance and the latter was a site supervisor (table 18.1B). The lowest positive rate of increase observed (a=0.012) was that of subject number M # 32 whose job was site supervision.

The mean slopes in the manual workers shown in table 18.1B was ($\tilde{a}$=0.018 ± 0.010). It was slightly different from the regression slope of the mean UDGA output over time in the all manual workers (ß=0.020). This was due to the few missing data in individual regression slopes.

The mean slopes in group M was not significantly different from group C2 (unpaired Student's t-test; $p>0.05$). It was also observed that the mean slopes of group M was not significantly different the null hypothesis (one sample Student's t-test; $p<0.05$).

Group P1: Table 18.2A shows that pavers (P1 # 7 and 8) had the highest rate of increase of UDGA excretion (a=0.109 and 0.105, respectively). It is worth noting that UDGA concentration in post-shift samples these two pavers on day 3 were relatively high (10.64 and 10.00 UDGA mmol/mol creatinine, respectively). The values of these samples influenced the regression slope quite considerably. These rate of increase in UDGA excretion observed were the second highest among all subjects in this study after that of (R1 # 21) who had a regression slope of (a=0.140). Paver (P1 # 7) had the highest external exposure to Σ9PAHs (table 11.4b). Paver (P1 # 13) was the only one with a negative regression slope in this group while his external exposure to PAHs quantified by the GM of Σ9PAHs TWA concentration was the highest.

The mean slopes in the this group of pavers shown in table 18.2A was ($\tilde{a}$=0.060 ± 0.018) which is equal to the regression slope of the mean UDGA output over time in the all subjects (ß=0.060; fig. 18.1B).

The difference in the mean slopes between group P1 and group C2 was significant (unpaired Student's t-test; $p<0.05$). On the other hand, the mean slopes of group P1 was not significantly different from that of group

M. The mean slopes of group P1 was significantly different from the null hypothesis (one sample Student's t-test; $p<0.05$).

Group P2: In this group, raker (P2 # 60) and the roller driver (P2 # 68) had the highest two regression slope (a=0.059 and 0.047), respectively (table 18.2B). Although these rates of increase in UDGA excretion were high in relation to this group they were equivalent to relatively moderate ones in group P1 (table 18.2A). The rate of increase in UDGA excretion calculated for the foreman (P2 # 66) was approximately half that of the roller driver (table 18.2B). All the other pavers in this group had comparable regression slopes (table 18.2B).

The mean slopes in the this group of pavers shown in table 18.2B was ($\tilde{a}=0.020 \pm 0.006$) which is equal to the regression slope of the mean UDGA output over time in the all subjects ($ß=0.020$; fig. 18.1C).

The difference in the mean slopes between group P2 and group C2 was not statistically significant (unpaired Student's t-test; $p>0.05$). It was equal to that group of group M. When compared against the null hypothesis it was found to be statistically significantly different (one sample Student's t-test; $p<0.05$).

Group R1: Individual regression slopes of UDGA excretion over time were calculated from all pre-shift and post-shift samples on the three days for seven roofers out of nine. The rate of increase of UDGA excretion in roofers (R1 # 20 and 21) was calculated from 4 urine sample since they provide neither a pre-shift nor post-shift urine sample on day 1 (table 18.3A).

Thirty percent of the roofers (R1 # 36, 40 and 42) had negative rates of increase of UDGA excretion (table 18.3A). As mentioned earlier roofer (R1 # 21) had the highest rate of increase of UDGA excretion among all subjects in this study. He had distinctly the highest GM of Σ9PAHs in this group of roofers; however, in comparison to external exposure to PAHs in group R2 his was relatively low. Roofers R1 # 42 and 43 had relatively high rate of increase (a=0.080) and (a=0.075), respectively (table 18.3A). Roofers R1 # 20, 36 and 46 had regression slopes approximately half that of R1 # 42 and 43 (table 18.3A).

The mean slopes in the this group of roofers shown in table 18.3A was (ã=0.040 ± 0.018). It was different from the regression slope of the mean UDGA output over time in the all subjects in this group of roofers (ß=0.029; fig. 18.2B). This was due to the missing data in individual regression slopes of roofer R1 # 20 and 21.

The difference in the mean slopes between group R1 and group C2 or group M was not statistically significant (unpaired Student's t-test; p>0.05). When the mean slopes was compared against the null hypothesis it was found to be different with borderline significance (one sample Student's t-test; p=0.056).

Group R2: Table 18.3B shows that only the tipper (R2 # 90) had a positive rate of increase in UDGA output overtime in this group of roofers (a=0.003). The other three roofers had negative regression slopes of different magnitude. UDGA excretion over time was decreasing at the highest in troweller (R2 # 88) followed by the kettleman/mixer operator (R2 # 87) and troweller (R2 # 89) (table 18.3B).

The mean slopes in the this group of roofers shown in table 18.3A was (ã=-0.025 ± 0.014). The mean slopes was negative in this group due to the high UDGA excretion in morning samples of roofer R2 # 87 and 88. The mean slopes was equal to the regression slope of the mean UDGA output (ß=-0.025) illustrated in figure 18.2B.

The difference in the mean slopes between group R2 and group C2 or group M was not statistically significant (unpaired Student's t-test; p>0.05). The mean slopes was not significantly different from the null hypothesis (one sample Student's t-test; p=0.058).

Pavers group: This group is group P1 and P2 combined together. Therefore the total number of subjects in this group is sixteen. The mean slopes in this combined group was positive (ã=0.035 ± 0.009). This mean slopes had the same sign as the means slopes of its subgroups but it was smaller than that of group P1 but bigger than group P2. The mean slopes was identical to the regression slope of the mean UDGA excretion on time shown in figure 18.3A (ß=0.035).

The difference in the mean slopes between the pavers group (PV) and either of its subgroups was not significant. In addition the mean slope was not significantly different from either that of group C2 or group M (unpaired Student's t-test; $p>0.05$). Meanwhile, it was found that the mean slopes was significantly different from the null hypothesis (one sample Student's t-test; $p<0.05$).

Roofers group: This group is group R1 and R2 combined together. Therefore the total number of subjects in this group is thirteen. The mean slopes in this combined group was positive ($\tilde{a}=0.020 \pm 0.016$) (table 18.4). This mean slopes did not have the same sign as the mean slopes of subgroup R2 which had a negative mean slopes (table 18.3B). The mean slopes was dissimilar to the regression slope of the mean UDGA excretion on time shown in figure 18.3B ($ß=0.011$). Of course this was due to the missing data in individual regression slopes of roofer R1 # 20 and 21.

The difference in the mean slopes between the roofers group (RF) and either of its subgroups was not significant. In addition the mean slope was not significantly different from either that of group C2 or group M (unpaired Student's t-test; $p>0.05$). Also, the mean slopes was not statistically different from the null hypothesis (one sample Student's t-test; $p>0.05$).

Figure : 18.1 Mean urinary excretion of d-glucaric acid in office workers (C2), manualworkers (M) and groups of pavers (P1 or P2) over a three days period
- Mean ± SEM and rate of increase of excretion "ß" -

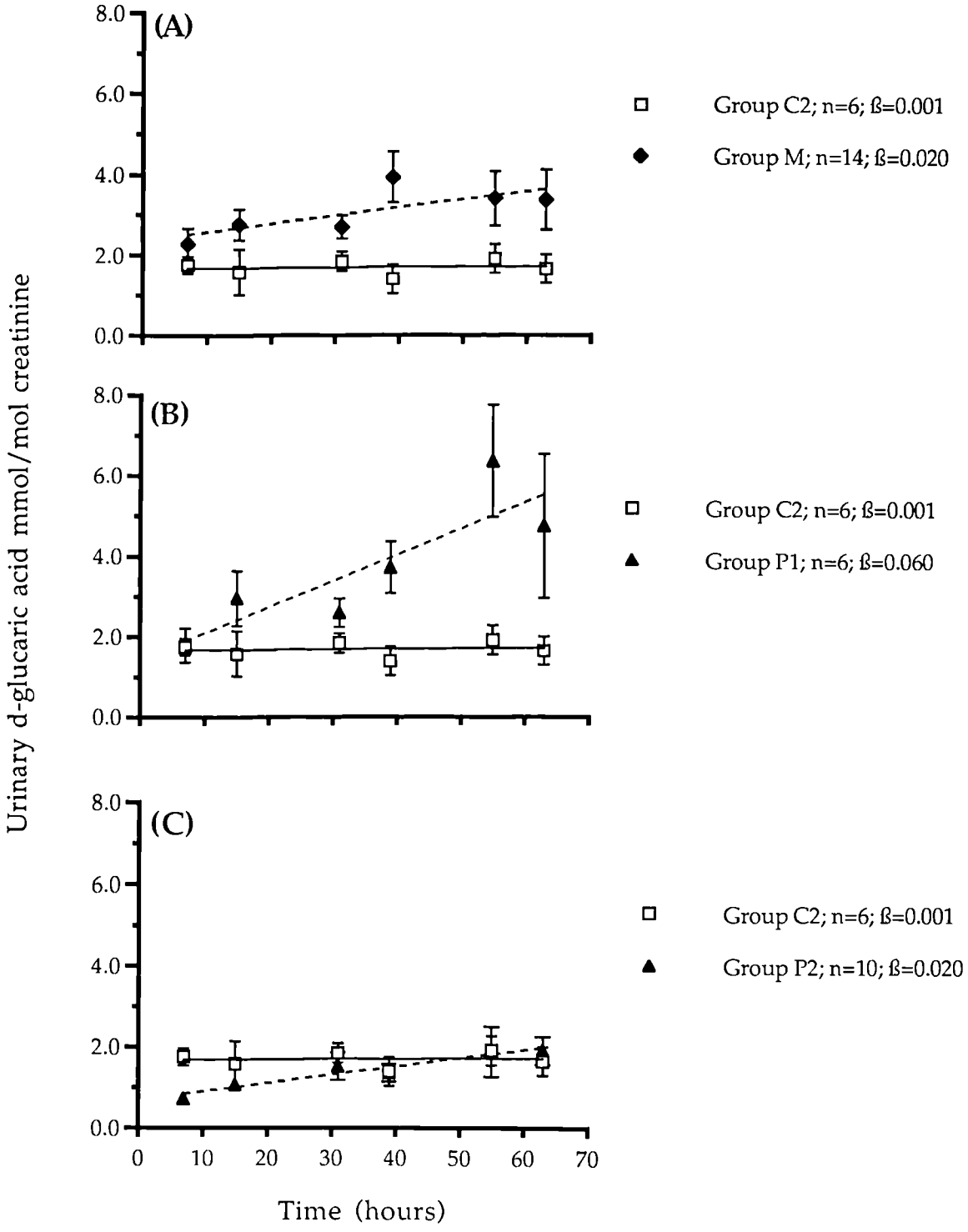


Figure : 18.2 Mean urinary excretion of d-glucaric acid in office workers (**C2**) and groups of roofers (**R1 or R2**) over a three days period - Mean ± SEM and rate of increase of excretion "ß" -

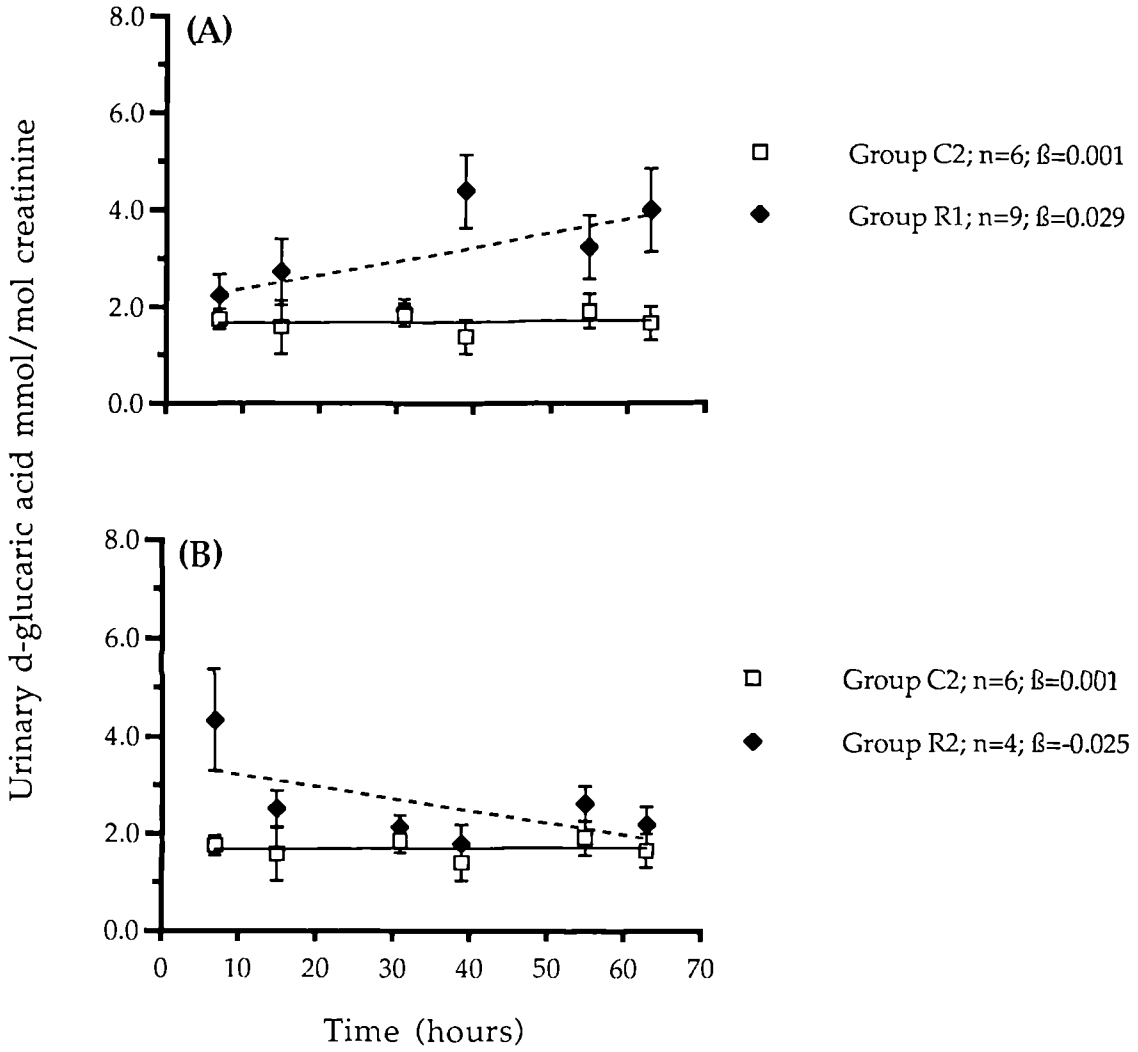


Figure : 18.3 Mean urinary excretion of d-glucaric acid in office workers (**C2**), pavers group (**PV**) and roofers group (**RF**) over a three days period - Mean ± SEM and rate of increase of excretion "ß" -

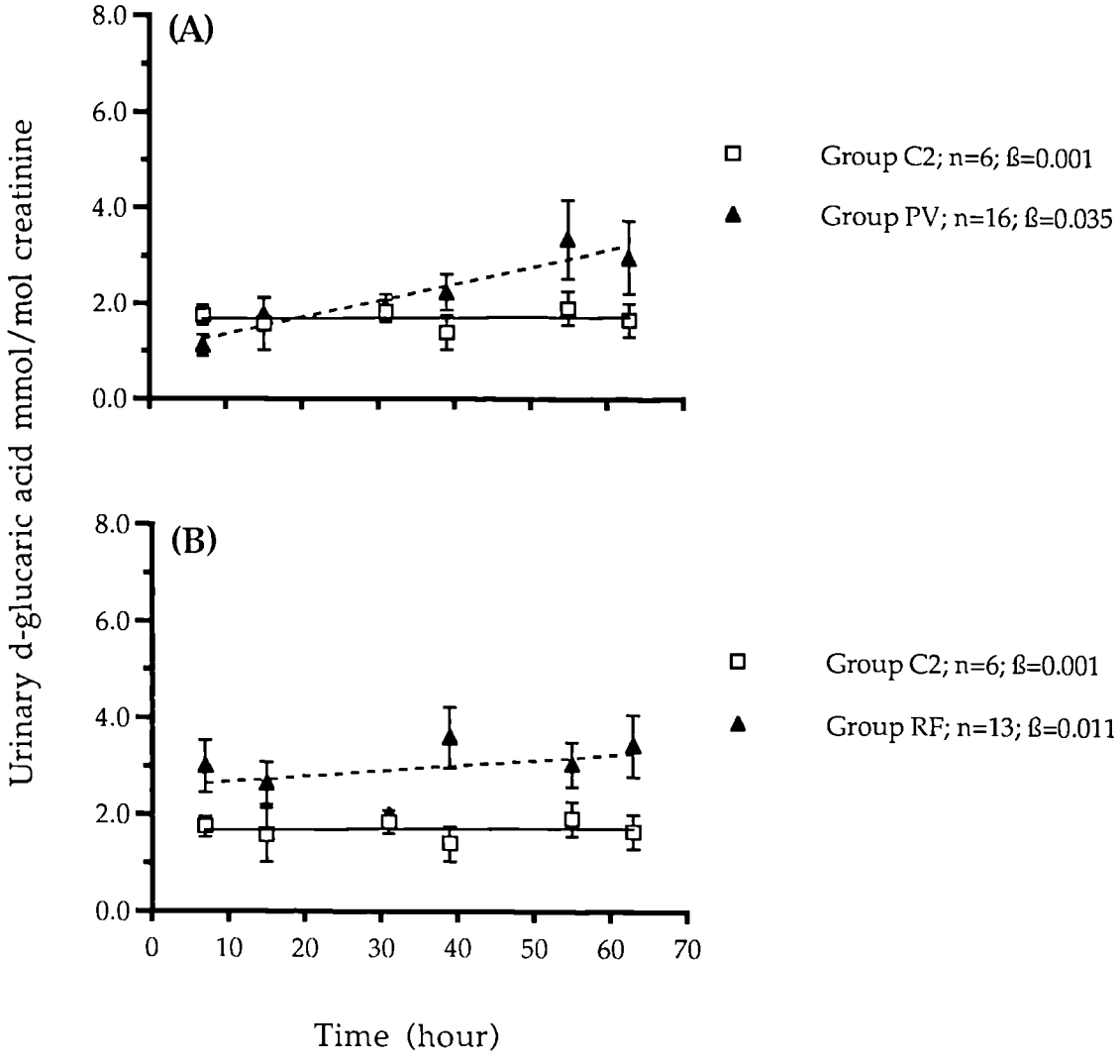


Table : 18.1 Daily morning and afternoon urinary output of d-glucaric acid and the rate of increase of excretion (i.e. slope "a") in office workers (C2) and manual workers (M) over a three days period

(A)

Office worker		d-glucaric acid mmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
80	Office work	1.17	0.66	1.32	0.99	3.84	1.25	0.027
81	Office work	2.05	4.32	2.00	0.30	1.42	0.29	-0.048
82	Office work	2.07	1.71	1.58	1.94	1.65	1.83	-0.003
83	Office work	1.63	0.19	1.04	2.14	1.85	2.32	0.024
84	Sales Rep.	2.51	1.92	2.29	2.62	1.33	2.84	-0.001
86	Office work	1.05	0.67	2.78	0.37	1.39	1.38	0.006
	Mean	**1.75**	**1.58**	**1.84**	**1.39**	**1.91**	**1.65**	**0.001**
	SEM	*0.21*	*0.56*	*0.24*	*0.36*	*0.36*	*0.35*	*0.011*

n.s.: Sample not provided

(B)

Manual worker		d-glucaric acid mmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
10	Sewer Maintenance	2.12	1.63	1.50	1.26	6.62	0.58	0.023
14	Handling cement	0.82	1.66	1.67	3.26	10.13	0.52	0.070
15	Handling cement	2.41	2.57	3.21	2.52	2.23	10.37	0.089
16	Sewer Maintenance	4.36	3.80	3.47	3.39	5.41	1.16	-0.024
17	Sewer Maintenance	1.20	2.66	3.93	2.30	0.47	7.39	0.051
30	Site supervisor	0.65	2.61	3.17	4.82	1.59	5.8	0.054
32	Sewer Maintenance	2.81	7.1	4.24	4.53	3.73	2.06	-0.036
33	Site Supervisor	2.14	2.85	0.24	0.27	2.44	3.45	0.012
34	Site Supervisor	0.76	4.1	2.1	6.91	1.83	4.08	0.028
35	Slatter	2.05	2.86	n.s.	3.46	0.44	n.s.	-0.027
37	Laying Insulation	1.96	2.68	2.01	3.59	5.20	3.06	0.036
38	Painting Metal	n.s.	2.14	2.75	2.95	4.89	2.45	0.027
41	Painting Metal	n.s.	2.27	2.89	3.5	2.32	0.89	-0.026
45	Stripping Lead	5.44	1.99	3.42	5.63	1.22	3.00	-0.031
48	Laying Insulation	2.82	0.33	3.06	10.46	2.58	2.43	0.027
	Mean	**2.27**	**2.75**	**2.69**	**3.92**	**3.41**	**3.37**	**0.018**
	SEM	*0.39*	*0.39*	*0.29*	*0.63*	*0.68*	*0.75*	*0.010*

n.s.: No sample provided

M: Morning A: Afternoon

Table : 18.2 Daily morning and afternoon urinary output of d-glucaric acid and the rate of increase of excretion (i.e. slope "a") in groups of pavers (P1 & P2) over a three days period

(A)

Paver		d-glucaric acid mmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
7	Paver *	1.67	2.70	2.52	3.10	3.06	10.64	0.109
8	Paver *	1.93	2.39	1.92	3.90	3.31	10.00	0.105
11	Paver *	3.37	4.91	3.59	4.14	9.95	3.27	0.042
13	Paver *	0.65	4.91	2.34	6.44	3.10	1.19	-0.002
18	Paver *	2.37	2.18	3.62	2.82	6.69	1.64	0.028
19	Paver *	0.75	0.65	1.57	1.87	8.74	1.68	0.079
	Mean	1.79	2.96	2.59	3.71	6.35	4.74	0.060
	SEM	*0.42*	*0.68*	*0.35*	*0.64*	*1.39*	*1.79*	*0.018*

* Exact job description not specified

(B)

Paver		d-glucaric acid mmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
60	Raker	0.83	0.86	1.11	0.98	2.43	4.82	0.059
61	Raker	0.49	1.05	0.13	0.81	0.55	1.78	0.012
62	Raker	0.21	1.79	1.40	1.28	0.86	1.64	0.009
63	Tractor Driver	1.06	0.79	1.03	1.32	0.95	1.65	0.009
64	Screwman	0.47	0.80	1.36	0.33	0.05	2.51	0.016
65	Roller Driver	0.73	1.09	0.67	0.73	0.88	1.10	0.003
66	Foreman	0.62	0.68	1.81	2.02	2.51	1.37	0.024
67	Paver	1.69	0.44	3.33	1.50	3.06	1.16	0.013
68	Roller Driver	0.69	2.03	3.37	1.98	6.67	1.64	0.047
69	Raker	0.33	1.04	0.93	2.58	0.69	1.43	0.013
	Mean	0.71	1.06	1.51	1.35	1.87	1.91	0.020
	SEM	*0.13*	*0.16*	*0.34*	*0.22*	*0.62*	*0.35*	*0.006*

M: Morning

A: Afternoon

Table : 18.3 Daily morning and afternoon urinary output of d-glucaric acid and the rate of increase of excretion (i.e. slope "a") in groups of roofers (R1 & R2) over a three days period

(A)

Roofer		d-glucaric acid mmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
20	Roofer*	n.s.	n.s.	2.02	2.85	5.78	2.50	0.049
21	Roofer*	n.s.	n.s.	1.27	2.10	3.16	6.32	0.140
36	Roofer*	3.88	4.71	3.44	2.46	0.77	5.58	-0.015
39	Roofer*	1.02	1.37	2.05	3.66	5.18	1.25	0.037
40	Roofer*	1.61	5.53	1.45	2.70	2.22	2.53	-0.015
42	Roofer*	2.55	2.41	1.57	5.41	0.75	0.66	-0.029
43	Roofer*	0.78	2.60	2.18	8.35	5.04	4.98	0.080
44	Roofer*	2.56	2.01	2.45	7.37	1.72	8.52	0.075
46	Roofer*	3.29	0.46	1.00	4.69	4.45	3.58	0.043
	Mean	**2.24**	**2.73**	**1.78**	**4.40**	**3.23**	**3.99**	0.040
	SEM	*0.44*	*0.68*	*0.24*	*0.75*	*0.65*	*0.85*	*0.018*

* Exact job description not specified n.s.: Sample not provided

(B)

Roofer		d-glucaric acid mmol/mol creatinine						
		DAY 1		DAY 2		DAY 3		
Code	Job Description	M	A	M	A	M	A	*Slope "a"*
87	Mixer Operator	5.37	3.50	1.78	2.93	3.34	2.71	-0.030
88	Trowellcr	6.48	1.64	2.05	1.18	1.69	1.35	-0.061
89	Trowellcr	3.73	2.39	2.85	1.81	2.43	2.92	-0.011
90	Tipper	1.72	2.48	1.78	1.21	2.96	1.75	0.003
	Mean	**4.33**	**2.50**	**2.12**	**1.78**	**2.61**	**2.18**	-0.025
	SEM	*1.04*	*0.38*	*0.25*	*0.41*	*0.36*	*0.38*	*0.014*

M: Morning

A: Afternoon

CHAPTER 19

DISCUSSION OF

URINARY D-GLUCARIC ACID

19.1 INTRODUCTION

Polycyclic aromatic hydrocarbons are known to have the potential to induce hepatic enzyme activity. In order to evaluate the effect of exposure to PAH in bitumen fumes on liver function in pavers and roofers we chose to monitor UDGA excretion a frequently used biomarker of hepatic enzyme activity.

Several researchers have used UDGA as a biomarker of effect in studies assessing the risk of exposure to hepatoxic chemicals. Some of these studies have compared UDGA excretion between control and exposed group using a single pre-shift or post-shift spot urine sample (Edwards, 1990 and Ferriera, Jr. 1994). Others compared exposed groups with referents by examining the change between morning and afternoon UDGA excretion over a working shift (Colombi *et al.* 1987 and Franco, 1992). Pasquini *et al.* (1987) compared a bitumen fume exposed group with controls by observing the difference in UDGA excretion between morning samples and all day samples. All these studies with their different approaches for monitoring have found that this biomarker of effect is of limited use for monitoring environmental or occupational exposure to xenobiotics.

In a study assessing the effect of phetharbital on hepatic enzyme activity UDGA output was found to increase significantly over time and to return to normal after six days from cessation of drug dosing (Hunter *et al.* 1971). This suggested that it would be more sensible to monitor UDGA excretion over time rather than using the approaches followed by other investigators. This is purely because UDGA may not be sensitive enough to signal a small increase in hepatic enzyme activity that would take place over a working day.

19.2 Baseline Excretion of Urinary D-glucaric Acid

It was necessary prior to summarising the UDGA excretion over time to identify the baseline values for mean UDGA excretion in our population and relate them to levels reported in the literature. Knowledge of baseline values is essential for the interpretation of results of the

excretion of a biological marker over time since in certain circumstances high background output may indicate that excretion has approached steady state due to saturation of enzyme activity.

Knowing that the effect of potent hepatic enzyme inducers (phetharbital, 900 mg oral dose, daily for three weeks) on UDGA excretion in man was shown by Hunter and co-workers to be relatively rapidly reversible (Rubin, 1987) and suspecting that the effect of the measured occupational exposure to PAHs is going to be comparatively minimal; we assumed that it is sensible to consider that UDGA excretion at the start of a working week as baseline values for the control and occupationally PAH-exposed groups. This assumption is also supported by the observation of Maroni and co-investigators that the effect of occupational exposure to polychlorinated biphenyls were quite moderate when compared with that elicited by anti-convulsant drugs (Colombi, 1987).

There was no significant difference in the mean UDGA excretion observed in morning samples on day 1 among all groups. However, it was observed that group R2 had relatively higher values (4.33 UDGA mmol/mol creatinine) which might be attributed to a systematic error in the analysis; especially that their excretion in the all other samples was fairly constant (range; 1.78 - 2.61). The baseline UDGA excretion values measured for group C2, M, P1, P2 and R1 were within the range of reference values reported in by Colombi *et al.* (1987) for normal healthy men. In their study they found that UDGA output corrected for creatinine concentration in spot urine samples is normally distributed with approximately 70 % of the individuals falling within a range of (0.5 - 3.0 mmol/mol creatinine). Earlier studied examining UDGA excretion in normal individuals reported levels twice as high as those published by Colombi *et al.* (March J. *et al.* 1974). This may be attributed to differences in the analytical procedure whose performance has been improved through modifications over the years.

Pre-shift UDGA excretion concentration in control groups published by other scientists were on average slightly higher than the ones measured in this study (Morretto and Lotti, 1990; Edwards, 1990; Franco, *et al.* 1992 and Ferreira, Jr. 1994). There was no obvious reasons for these differences. It was difficult to compare the baseline values of UDGA in pre-

shift samples of the occupationally PAH-exposed groups (P1, P2 and R2) with those recorded in literature, since the day of sampling during the working week was either not specified or sampling was carried out on a mid-of or end-of-week day.

In summary, control (C2 and M) and exposed groups (P1, P2, R1 and R2) had comparable baseline values and the mean concentration of UDGA at the start of the working week indicated that none of the studied groups had excretion levels approaching steady state.

19.3 Rate of Increase of Excretion of Urinary D-glucaric Acid

Office workers (group C2): The lack of build up in DGA excretion in urine for control group C2 was expected. First, because their external dose was virtually none existing and secondly they had a zero rate of increase in U1-HP excretion suggesting that no bioaccumulation of PAHs was occurring that could have led to induction of liver enzyme activity.

Manual workers: The highest positive rate of increase of UDGA excretion was for group P1. The build up in this group of pavers was statistically significant when compared to that of control group C2 but not group M. The statistically significant build up of UDGA excretion in group M in relation to a zero rate of increase was the reason for this non-significant difference. The positive rate of increase in group M was partly expected since their external exposure to PAHs was not negligible and their U1-HP data showed that there was a build up in the internal dose of PAHs. This shows that the exposure of group M to background levels of PAHs and other hepatic enzyme inducers possibly present in their working environment should be acknowledged when interpreting the results of this risk assessment. These results also show that group M does not serve as a good control group this study where the effect of occupational exposure to PAHs on liver function are suspected to be small.

Group R1: The lowest rate of increase was that in group R1. It was not different from the null hypothesis (zero rate of increase). This was expected since their external exposure to PAHs was very close to background levels and more importantly their internal dose of PAHs

quantified by levels of U1-HP seemed not to increase over the working week.

Group P1: Group P1 had the highest rate of increase in UDGA excretion. We were not able to fully clarify this observation from the ambient monitoring assessment data which showed that this group did not have the highest external PAH-exposure. This was better explained by the selective biological monitoring program used. Their highest rate of increase of excretion of U1-HP suggest that a considerable amount of bioaccumulation of PAHs was occurring and therefore a build up in the internal dose of PAHs which might explain the induction of liver metabolism reflected in their highest rate of increase in UDGA excretion.

Group P2: The positive rate of increase of UDGA excretion in this group was significantly different from the null hypothesis but not from that of group C2. It was lower than that of group P1. The ambient monitoring assessment which showed that this group of pavers is the most highly PAH-exposed group does not agree with this observation. On the other hand, from the U1-HP results we might be able to explain this positive build up in UDGA excretion through the same argument as the one used for interpreting the results of group P1.

Group R1: There was no increase in the rate of UDGA excretion in this group. This was somehow expected since their internal dose of PAHs was no different from group C2. Therefore we speculate that their low level of external exposure to PAHs was not sufficient to lead to bioaccumulation of PAHs and thus an biologically effective dose that would induce hepatic enzyme activity enough to observe an increase in UDGA.

Group R2: The negative rate of increase seen in group R2 could not be explained. Excluding their mean UDGA excretion in pre-shift samples on day 1 from the data analysis, this group will have a positive rate of increase no different from zero. This latter observation could be explained by the U1-HP results, but not airborne PAH-exposure indices. Their external exposure to PAHs, quantified by the mean of the 3-day-GM of TWA concentrations of airborne PAH-exposure indices, was higher than all other group. Therefore, we would expect this group to have the

highest rate of increase of UDGA excretion. Obviously, this was not the case. Meanwhile, it appears from the U1-HP results that the internal dose of PAHs in this group was higher than group C2. The effect of that was mirrored in the higher values of UDGA. The zero rate of increase of U1-HP excretion in group R2 indicates an absence of bioaccumulation of PAHs which could explain the zero rate of increase of UDGA excretion. Therefore the PAH internal dose that group R2 have been subjected to might have led to an elevated baseline of UDGA excretion signalling some degree of induction of liver metabolism.

19.4 Summary of Discussion

The results of this study suggest that the rate of increase of UDGA is related to the build up in the internal dose of PAHs rather than the intensity of the external dose quantified using personal air sampling techniques. This is what we would expect since ambient monitoring does not take into consideration the dermal absorption of PAHs that can significantly contribute to the internal dose which is a better estimate of the biologically effective dose that would give rise to a toxicological response. It is important to mention here that these conclusion are drawn with reservation since the small number of individuals in each group did not allow us to statistically control for confounding factors whose effect are well documented.

It is concluded that UDGA is potentially a suitable biological effect marker of exposure to PAHs in bitumen fume if monitored along with a selective biomarker of exposure such as U1-HP. It is also suspected that an investigation examining its UDGA excretion over a full working week in a large number of occupationally PAH-exposed group would produce statistically significant results that would confirm our conclusions.

CHAPTER 20

RESULTS OF
SISTER CHROMATID EXCHANGE
AND
REPLICATIVE INDEX

20.1 INTRODUCTION

This chapter presents the results collated from the measurement of two cytogenetic endpoints (SCE frequency and RI - replicative index -) in three parts.

Part I: presents individual and mean data of SCE frequency in group C1, C2, M, P1, P2, R1, R2, PV and RF. The difference in the mean of SCE frequency among all group was tested for significance using ANOVA single-factor. The pooled standard deviation of all groups incorporated in an unpaired Student's t-test was used to test for statistically significant differences in the mean SCE frequency between selected groups.

Part II: presents individual and mean data of RI in group C1, C2, M, P1, P2, R1, R2, PV and RF. The difference in the mean of RI between all group will tested for significance using ANOVA single-factor. The pooled standard deviation of all groups incorporated in an unpaired Student's t-test will be used to test for statistically significant differences in the mean RI between selected groups.

Part III: Studies relationships existing between either of the cytogenetic endpoints and different airborne PAH-exposure indices in groups M, P1, P2, R1 and R2. It will also present the correlation between the two cytogenetic endpoints measured in all groups studied in part I and II.

20.2 PART I:

20.2.1 SCE Frequency

Three of individuals (C1 # 75, 76 and 77) out of eight in group C1 withdrew from the study because they stopped working in the department. In the manual workers group 2 subjects (M # 17 and 35) did not give a blood sample and another 2 subjects (M # 14 and 15) were not present on the day of blood sampling. One individual in group R2 (R2 # 46) did provide a blood sample. All individuals in group C2, P1 and P2 provided a

blood sample. The total number of individuals in each group are shown in figure 20.1. The highest mean SCE frequency measured in group C1 from all four surveys was used for data analysis.

The mean SCE frequency in group C1 shown in figure 20.1 (0.046 ± 0.001) was the lowest of all groups. That of group C2 was very similar (0.048 ± 0.002). The mean SCE frequency in group M (0.054 ± 0.003) was not significantly higher than both of group C1 and C2. Figure 20.1 illustrates that the mean SCE frequency in groups of pavers P1 or P2 was higher than group C1, C2 and M. The mean SCE frequency of group P1 (0.059 ± 0.001) was significantly higher than that of group C1 and C2 (unpaired Student's t-test; $p<0.05$) but not M (unpaired Student's t-test; $p>0.05$); while that of group P2 (0.063 ± 0.002) was significantly higher than all three (unpaired Student's t-test; $p<0.05$). In the groups of roofers the mean SCE frequency (group R1; 0.064 ± 0.003) and (group R2; 0.073 ± 0.007) was higher than all groups with statistical significance attained when compared to group C1, C2 and M (unpaired Student's t-test; $p<0.05$). That of group R2 was nearly 1.6 times that of group C1.

Figure 20.2 is showing the effect of pooling of group P1 and P2 as PV and R1 and R2 as RF. The mean SCE frequency of group PV (0.061 ± 0.001) was higher than that of group P1 but lower than that of group P2 and RF. The mean SCE frequency of group RF (0.067 ± 0.003) was lower than that of group R2 and higher than group R1. The difference between the mean SCE frequency of either of the pooled groups was significantly higher than those of group C1, C2 and M (unpaired Student's t-test; $p<0.05$).

20.3 PART II:

20.3.1 Replicative Index

The mean RI between all groups was lowest in group R2 (mean ± SEM; 189 ± 4) (fig. 20.3). They had the highest mean of 3-day GM of Σ4,5PAHs TWA concentration (see table 11.4a, b and c). The mean RI was highest in individual in group C1, who were virtually not exposed to any

of the 9 PAHs, followed by that of group M (198 ± 4) (fig. 20.3). The lowest mean RI was observed in group C2 (186 ± 4). Figure 20.3 shows that the mean RI for group P1 (193 ± 3), P2 (195 ± 6) and R1 (192 ± 4) were very similar.

There was no significant difference in the mean RI between all groups (ANOVA single-factor; $p>0.05$).

20.4 PART III

20.4.1 SCE Frequency and Airborne PAH-exposure Indices

The external exposure of 3 subjects in group M (M # 30, 32 and 48) was not quantified. They were excluded from the calculation of the correlation described in figure 20.4. The total number of individuals included was 36. A 2-day GM instead of a 3-day GM was used when only 2 samples out of three had detectable levels of phenanthrene. When one sample out of three had detectable levels of phenanthrene, the TWA concentration in that sample was used instead of the 3-day GM.

Figure 20.4 presents the relationship existing between a 3-day geometric mean of TWA concentration of phenanthrene in personal air samples and SCE frequency in peripheral blood lymphocytes in group M, P1, P2, R1 and R2. SCE frequency was positively correlated with the logarithmic transformation of the 3-day GM of TWA concentration of phenanthrene (regression slope = 0.006). The correlation coefficient was weak ($r = 0.278$) and not significant ($p>0.05$).

Since the 4,5 ringed PAHs were not frequently detectable in personal air samples of group M, P1 and P2 it was not feasible to identify a relationship between Σ4,5PAHs as an airborne PAH-exposure index and SCE frequency. An alternative way presented in figure 20.5 was used. In this figure, the relationship between the percentage of subjects with at least one personal air samples with detectable levels of Σ4,5PAHs and SCE frequency in peripheral blood lymphocytes is given. It is observed the two variables are highly ($r = 0.918$) and significantly ($p < 0.05$) correlated. It is

observed that the SCE frequency reached a plateau in the roofers groups at values 1.5 times the baseline SCE frequency observed in the combined group C1+C2.

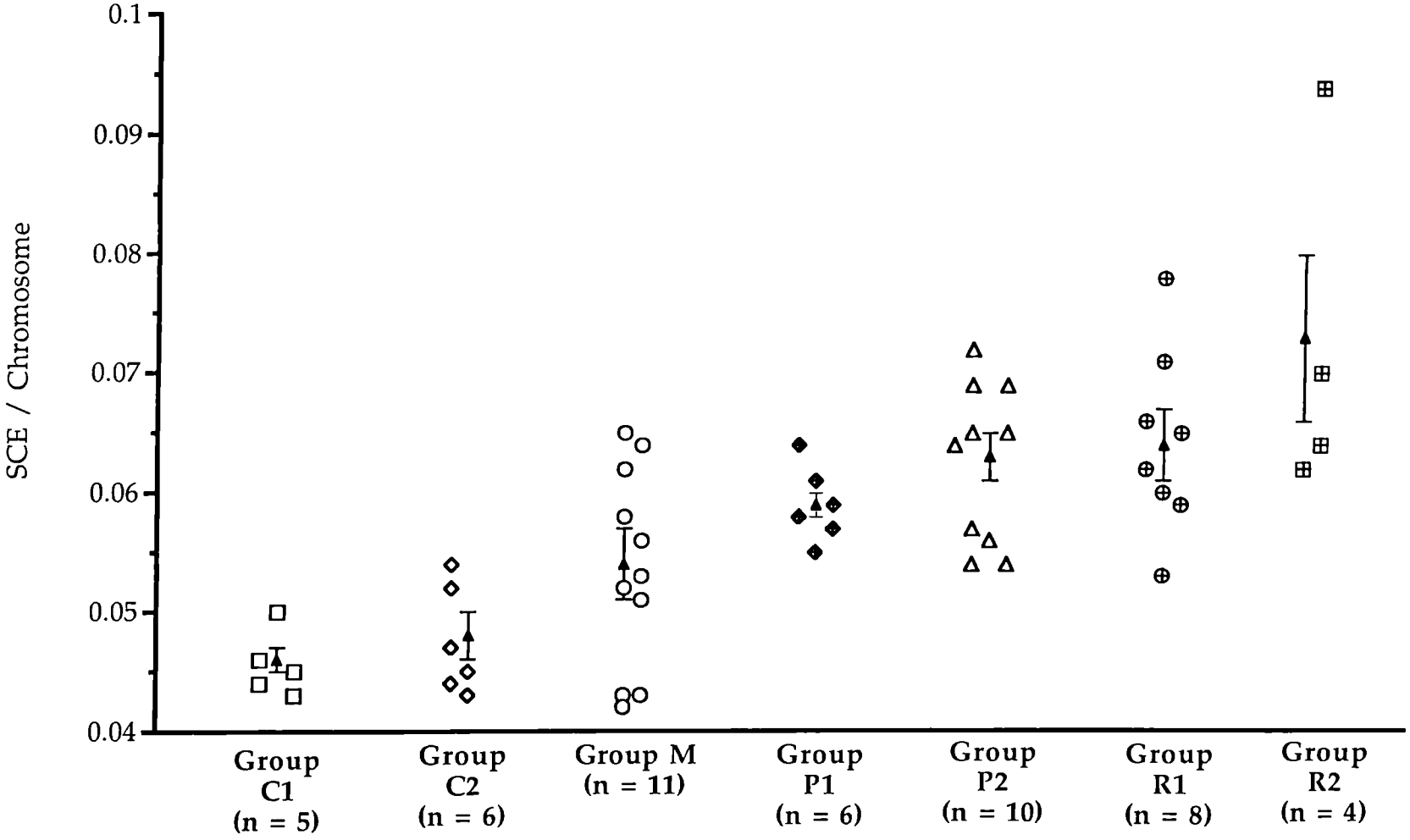
20.4.2 Replicative Index and Airborne PAH-exposure Indices

The same correlation as the one described in figure 20.4 was studied between the 3-day GM of TWA concentration of phenanthrene and RI and is presented in figure 20.6. RI was weakly ($r = 0.138$) and negatively (regression slope = -4.02) and non-significantly ($p > 0.05$) correlated with the logarithmic transformation of the 3-day GM of TWA concentration of phenanthrene.

20.4.2 SCE Frequency and Replicative Index

The correlation between the two cytogenetic endpoints is shown in figure 20.7. The two endpoints seem to be correlated in a fashion that when SCE frequency is increasing the RI is decreasing. This is indicated by the negative regression slope (-296.3). The correlation between these biological effect markers seem to be weak ($r = 0.185$) and not significant ($p>0.05$). Figure 20.8 is presenting the same correlation as in figure 20.7 however this time between the mean values of both the endpoints in group M, P1, P2 R1 and R2. As in the correlation between individual data points for both endpoints presented in figure 20.7 a negative (regression slope = -232.5) regression slope was also observed for the mean data. When mean data was tested the correlation coefficient was stronger ($r = 0.689$) than that of the individual data. Again, the negative regression slope in figure 20.8 suggests that as one of the endpoints is increasing the other is decreasing.

Figure : 20.1 Individual and mean ± SEM of SCE frequency in peripheral blood lymphocytes of group C1, C2, M, P1, P2, R1 and R2



Fiugure : 20.2 Individaul and mean ± SEM of SCE frequency in peripheral blood lymphocytes of group C1, C2, M, pavers (PV) and roofers (RF)

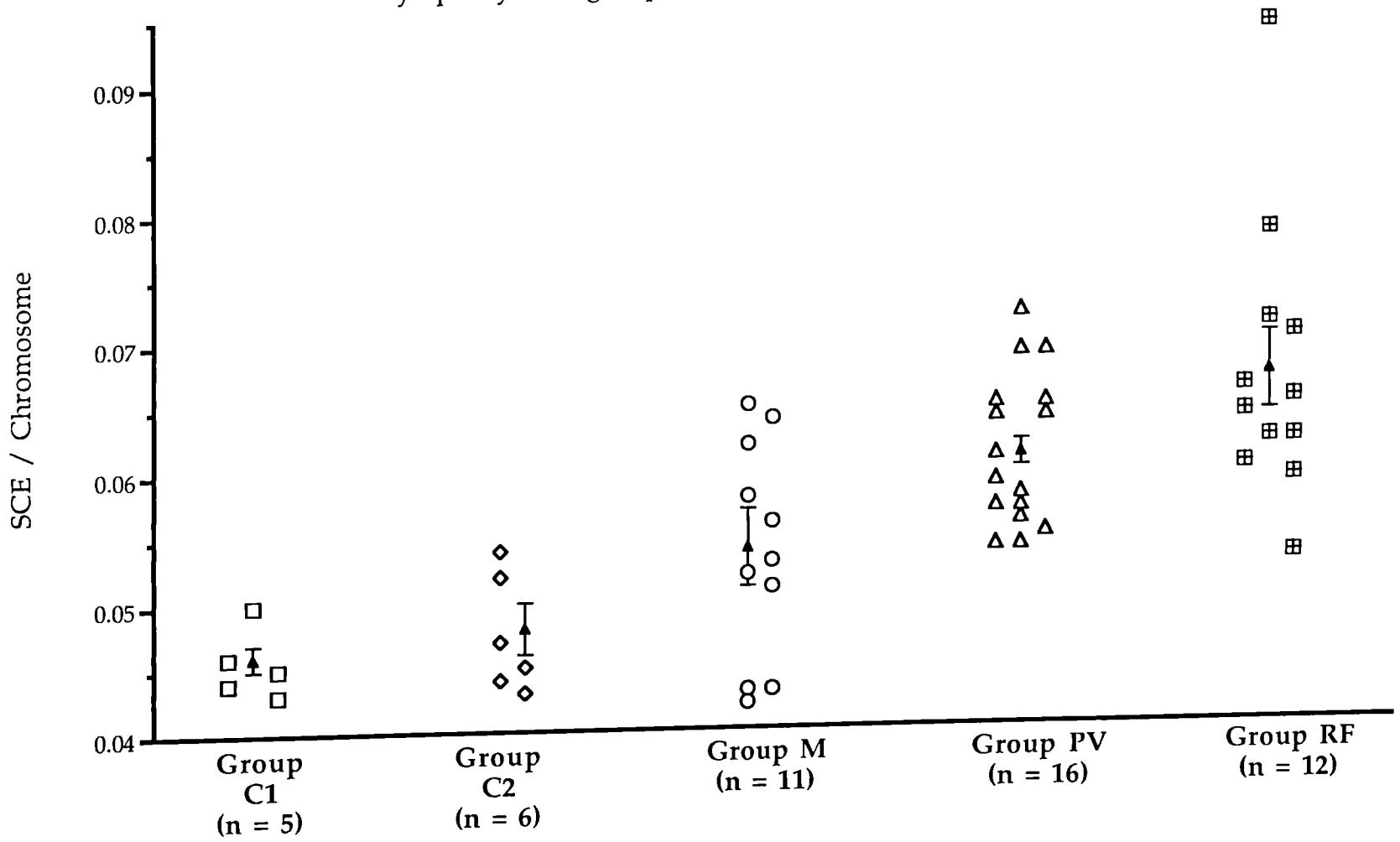


Figure : 20.3

Individual and mean ± SEM of replicative index in peripheral blood lymphocytes of group C1, C2, M, P1, P2, R1 and R2

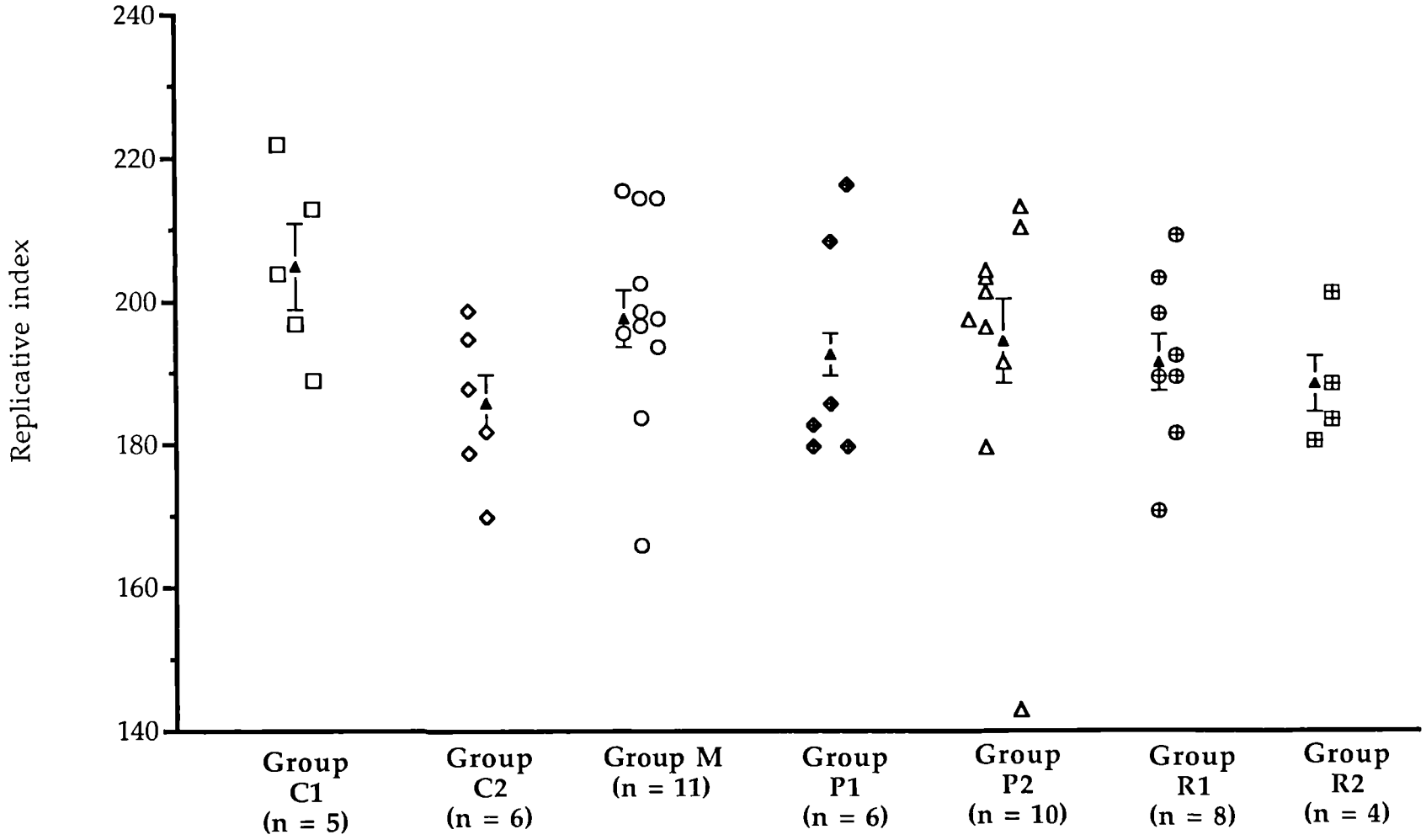


Figure : 20.4 Relationship between GM of TWA concentration of phenanthrene in personal air samples of days 1, 2 and 3 and SCE frequency in peripheral blood lymphocytes in group M, P1, P2, R1 and R2

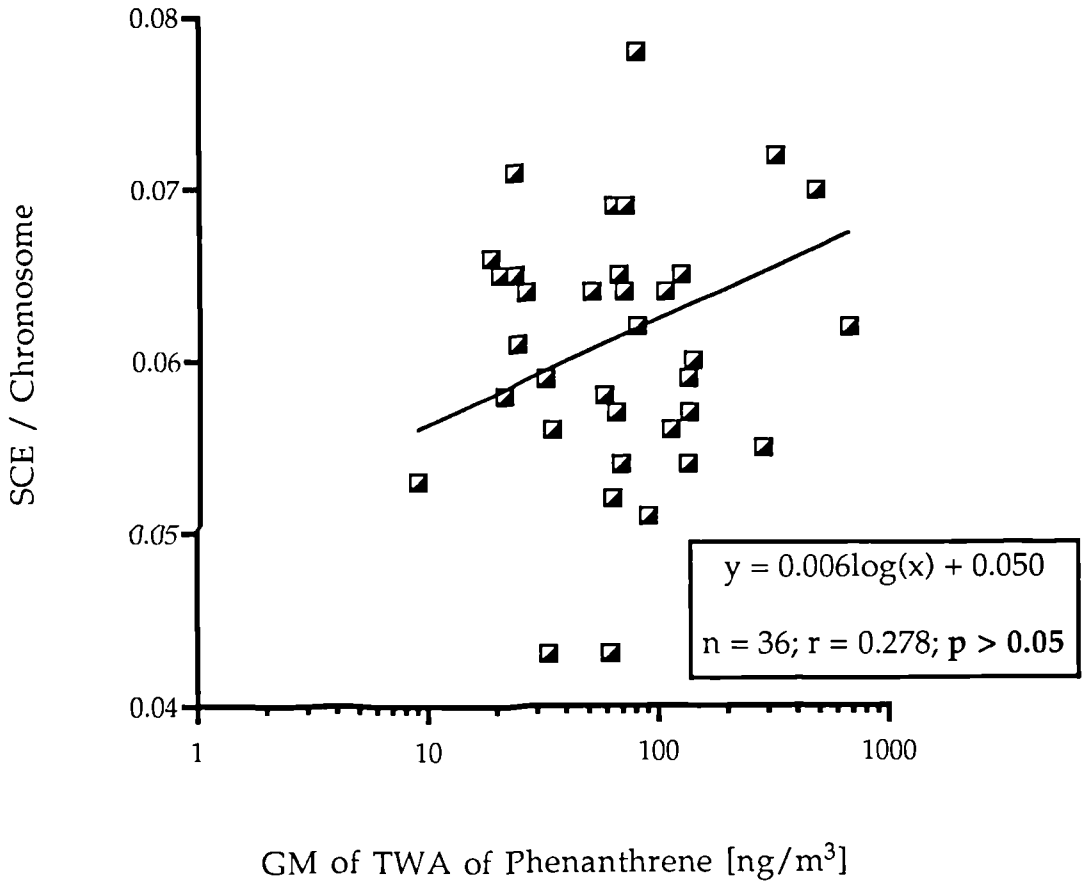
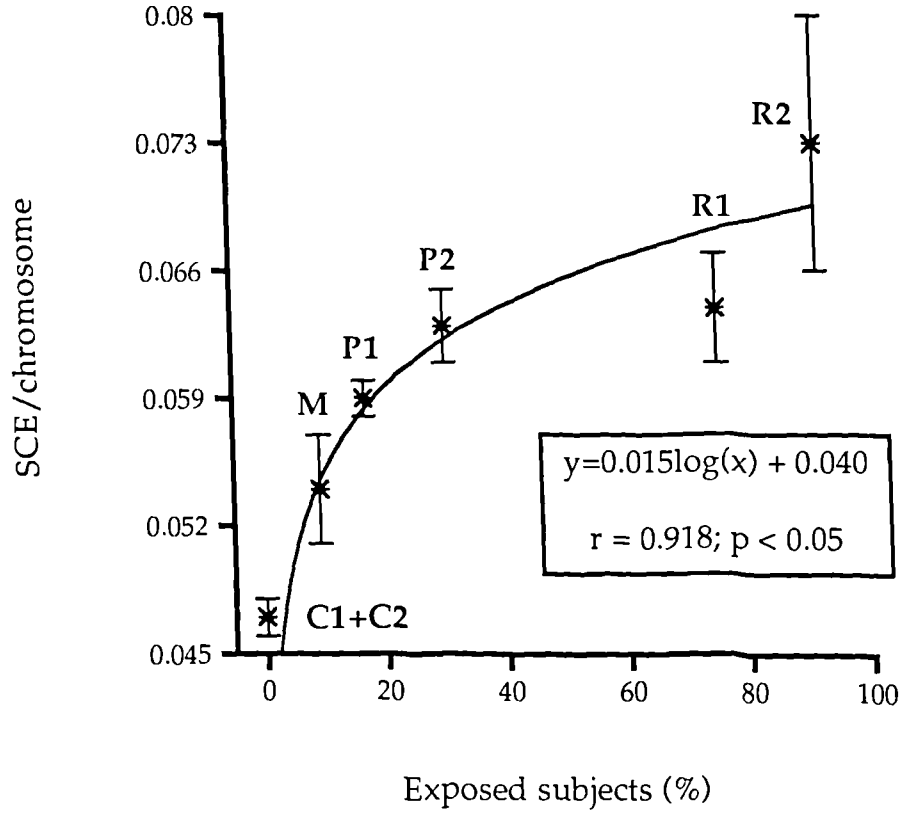


Figure : 20.5 Relationship between the percentage of subjects with personal air samples with detectable levels of Σ4,5ringed PAHs and mean SCE frequency in peripheral blood lymphocytes of group C1+C2, M, P1, P2, R1 and R2



Groups C1 + C2 (n = 11)
Group M (n=11)
Group P1 (n=6)
Group P2 (n=10)
Group R1 (n=8)
Group R2 (n=4)

Figure : 20.6 Relationship between GM of TWA concentration of phenanthrene in personal air samples of days 1, 2 and 3 and replicative index in peripheral blood lymphocytes of group M, P1, P2, R1 and R2

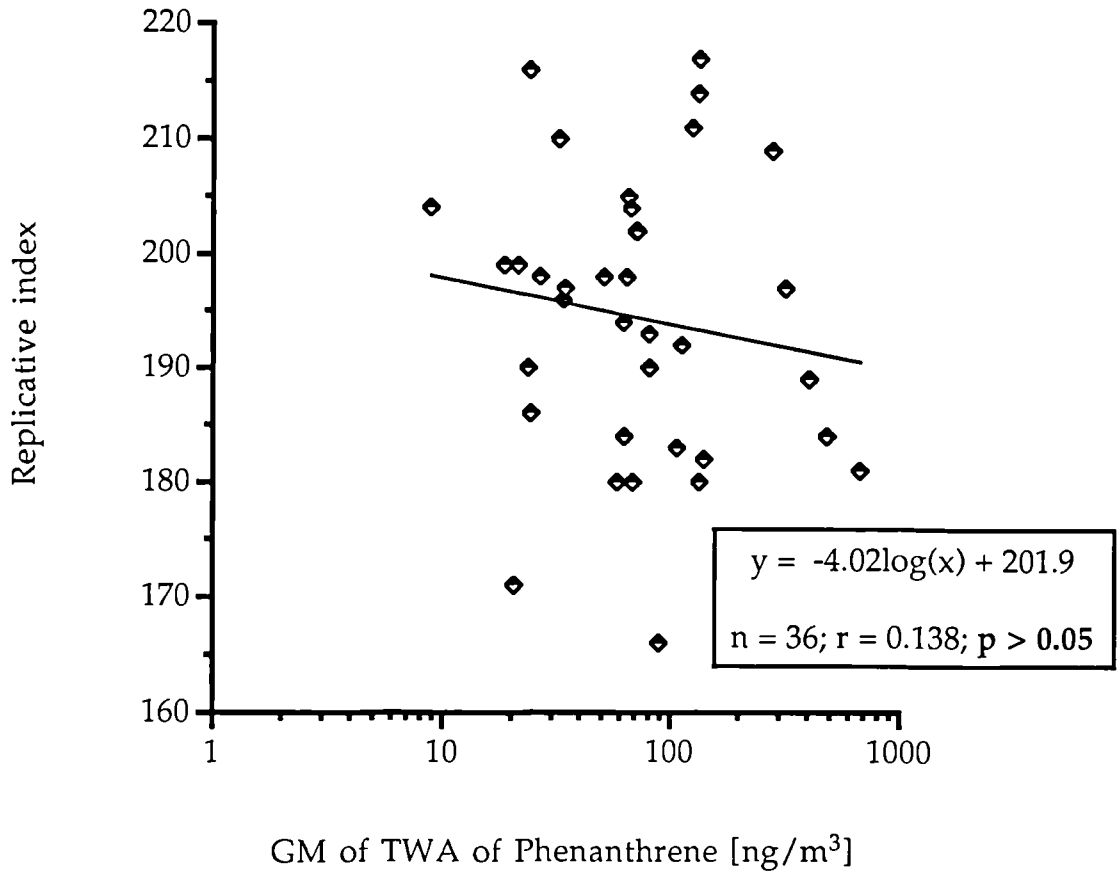


Figure : 20.7 Relationship between SCE frequency and replicative index in peripheral blood lymphocytes of individuals in group M, P1, P2, R1 and R2

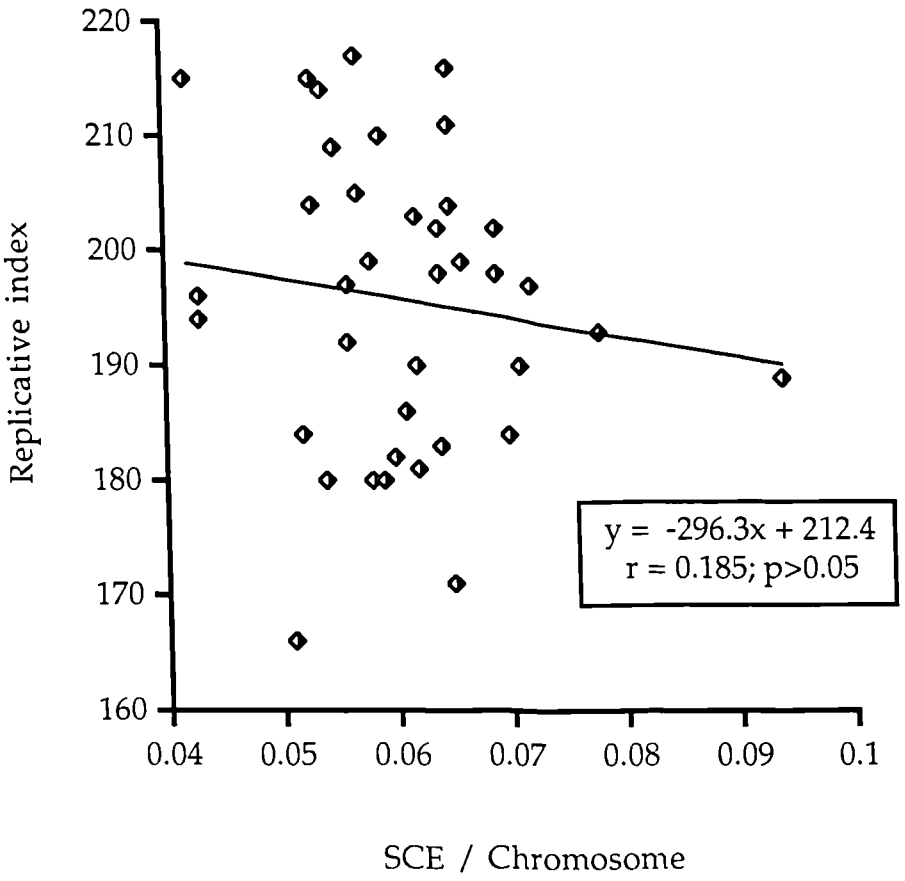
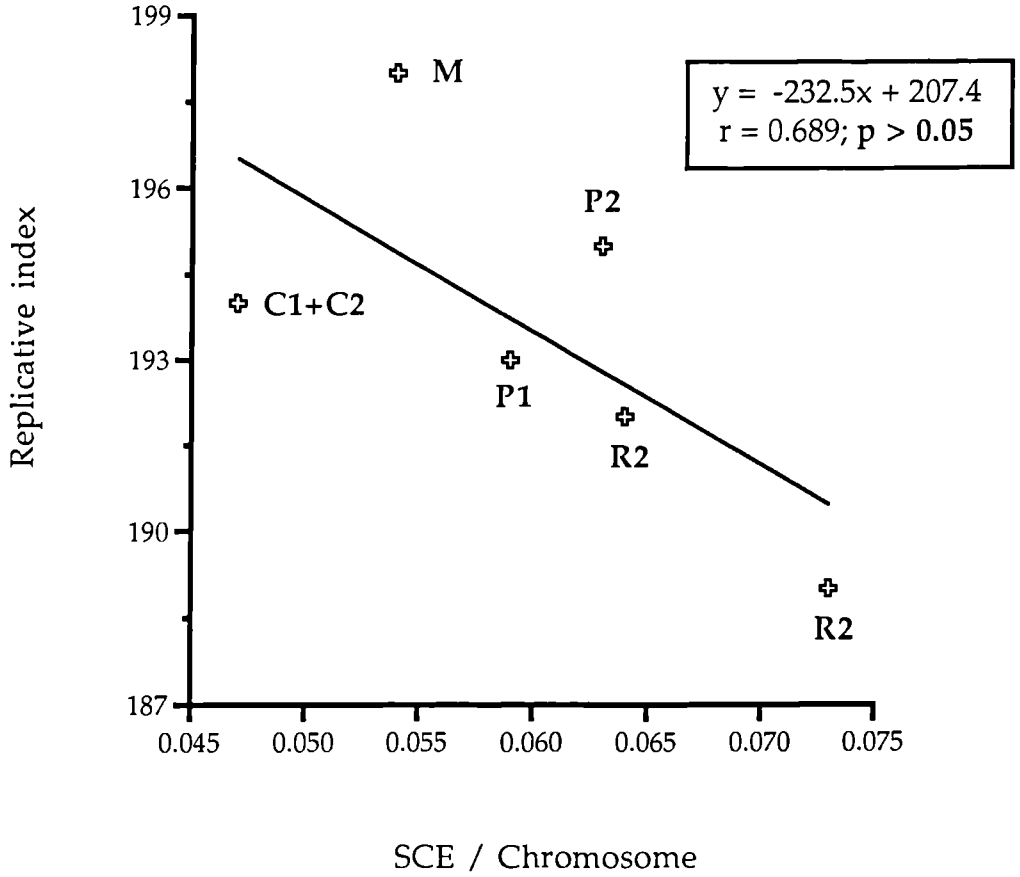


Figure : 20.8 Relationship between mean SCE frequency and mean replicative index in peripheral blood lymphocytes of group C1+C2, M, P1, P2, R1 and R2



CHAPTER 21

DISCUSSION OF
SISTER CHROMATID EXCHANGE
AND
REPLICATIVE INDEX

21.1 Sister Chromatid Exchanges in Peripheral Blood Lymphocytes

Sister chromatid exchange frequency and replicative index are the two cytogenetic endpoints studied in group C1, C2, M, P1, P2, R1 and R2.

The mean SCE frequency measured in group C1 was considered as baseline mean frequency and those of other groups were compared to it. To the best of our knowledge no studies have reported baseline values as low as the ones in this study. The closest was that reported by Edwards and Priestly (1993) which was twice as high as ours. We think that this was due to the exclusion of foetal calf serum from the culture medium. Some studies have shown that cell proliferation which is usually negatively correlated with SCE frequency was observed to increase with the exclusion foetal calf serum from the culture medium (Wolff *et al.* 1984). On the other hand another study have proved that the addition of foetal calf serum had no effect on cell kinetics (Mutchinik *et al.* 1980). The findings reported by different investigators do not show consistency. This implies that further work should be done on our culturing technique to confirm our suggestion.

The office workers had a mean SCE frequency no different from group C1. This was expected since the groups were fairly matched for confounding factors and their background exposure to PAHs as measured by the static air samples collected in their working environments was negligible.

Group M had an elevated mean SCE frequency. This could be due to their exposure to background environmental levels of PAHs. The effect of environmental exposure to pollutants on SCE induction have been addressed by researchers. Motykiewicz *et al.* (1992) found that mean SCE frequency in a population environmentally exposed to airborne pollutants are lower than that found in cokery workers; however significantly higher than what they measured in a rural population potentially exposed to lower levels of pollutants. Seemayer and co-workers have illustrated in an *in vitro* study that quantities of substances corresponding to airborne particulate collected from less than 1 m^3 of air where highly effective in inducing SCE formation in human lymphocytes (Seemayer *et al.* 1994). Our results together with those reported in the literature highlight the

genotoxic risk that group M has been subject to from exposure to background levels of PAHs as well as other pollutants.

All occupationally PAH-exposed groups had mean SCE levels significantly higher than group C1 and C2. This shows the effect of occupational PAH-exposure on SCE levels even at low levels. This observation may be argued since studies have failed to show any difference in SCE frequency between control and workers occupationally exposed to PAH levels an order of magnitude higher than those measured in this risk assessment (Brecher *et al.*, 1984 and Reuterwall *et al.*, 1991). Brecher and co-workers attributed the absence of significant differences in SCE levels, between control and aluminium plant workers, to the possible low bioavailability of the PAHs absorbed. In the study of Reuterwall *et al.* the lack of difference was linked with the strong confounding effect of smoking. These differences are plausible; however, the observed differences in this study at lower levels of PAH-exposure are probably due to the low baseline levels of the SCE method which could have provided it with greater resolution to detect small changes in SCE frequency.

The mean SCE frequency of groups of roofers (R1 and R2) was higher than those of group (P1 and P2). These differences did not reach statistical significance. This could have been attributed to the low number in the subgroups; however, pooling the subgroups into group PV and RF did not make any difference. These results did seem to agree with ambient monitoring assessment were it was observed that the roofers external dose to carcinogenic PAHs (i.e. Σ4,5PAHs) was much higher than that of the pavers.

In this study it was not possible to correlate SCE frequency with the TWA concentration of Σ4,5PAHs, which represents best the carcinogenic fraction of the external dose, since for most subjects the external exposure to these PAHs was none detectable. This is one of the reasons why the senisitivity of the method of PAH-species analysis should be improved. The second possible choice was pyrene since it occurred characteristically with the group of high molecular PAHs and correlated significantly with the sum of their concentrations. For the same reasons pyrene was not used. Phenanthrene was not correlated with SCE frequency. On the other hand the correlation of mean SCE frequency with

the percentage of exposed individuals among group C1+C2, M, P1, P2, R1 and R2 was highly significant. These observations show that SCE is related to external exposure however it is not purely dose dependant. The fact that mean SCE frequency appeared to plateau as the number of exposed individuals increased supports this conclusion. An *in vivo* study by Kligerman *et al.* (1985) on mouse showed that the relationship between the dose B(a)P and SCE frequency was non-linear. The slope was large at low doses and approached a plateau at high doses. They also reported, although non-significantly, a decrease in mitotic activity and a gradual slowing of cell cycle with increasing dose. The doses applied were through i.p. route and were much higher than what the pavers and roofers were exposed to. Although it is difficult to draw conclusions with all these differences between the two studies, our results seemed to be in agreement with their data. The reason for this curvilinear relationship observed in this study is unclear. SCE is correlated with cell killing (Deen *et al.* 1989). This relationship although non-causational may explain our observation; however, we do not suspect that cell killing would take place at such low levels of PAH-exposures. Nevertheless, cytotoxicity analysis of cultures could aid in revealing the possible reason behind the steady state levels of SCE observed in the mean frequencies of the roofers.

The highly significant correlation between mean SCE frequency and percentage of exposed workers show that at such low "noise" levels of PAH-exposure one should consider the possibility at assessing the genotoxic risk semi-quantitatively when obvious quantitative dose-response relationships are hard to define.

21.2 Replicative Index in Peripheral Blood Lymphocytes

Replicative index seemed to be less sensitive than SCE in showing significant differences between control and exposed groups. This deduction was based on the lack of correlation of replicative index with phenanthrene as well as the percentage of individual exposed to 4 and 5 ringed PAHs.

There was no correlation between SCE frequency and replicative index when all individuals were considered. When mean data of these

cytogenetic endpoints were considered we observed that RI values were progressively decreasing as SCE frequency increased. This phenomenon has been reported by other investigators (Sinués *et al.* 1991). Sinués and co-researchers wrote quoting Lindbald and Lambert that increased SCE response can accompany a depression in the rate of lymphocyte replication. Conversely, a depressed rate of DNA synthesis which can be manifested in culture as decline is cellular growth could result from increased levels of DNA damage. Moreover, a depressed RI in culture can give rise to a delay in mitogen responsiveness indicative of an immunological deficit. The difference theories confound our understanding of the relationship of RI depression and SCE induction. There is agreement between different scientists that RI is probably linked of immunocompetency (Tice *et al.* 1979; Crossen 1982 and Ortiz *et al.* 1994). Therefore the depression observed with decrease in SCE in the groups suggest that this matter is worth investigating with markers of immunotoxicity.

CHAPTER 22

GENERAL DISCUSSION AND CONCLUSIONS

22.1 GENERAL DISCUSSION AND CONCLUSIONS

Over the last two decades there has been an increased interest in the assessment of the health risks of populations exposed to occupational chemical carcinogens.

One important group of chemical carcinogens is the polycyclic aromatic hydrocarbons (PAHs) which are important in occupational health for several reasons: some are known to be potent carcinogens in man, there is strong epidemiological evidence that exposed groups have increased risks of lung, urinary tract, brain and skin cancers and many processes in a variety of workplaces are contaminated with PAHs.

Previous studies have looked at exposure to high levels of PAHs and the effects on human health (such as coke oven workers). However, the literature focusing on long-term exposure to moderate and low levels, such as those found in the roofing and road paving industries, is scarce.

PAHs are produced during roofing and paving processes using hot bitumen. Bitumen consists essentially of hydrocarbons and their derivatives and emissions are complex organic mixtures which contain PAHs. The emissions can be in the form of vapour or particulate matter and may gain entry into the human body through inhalation, skin absorption, and occasionally, ingestion. Airborne concentrations vary over a wide range depending on environmental conditions and working methods and risk may vary quite widely between exposed individuals.

Studies have been conducted to evaluate the risk of exposure to PAHs in bitumen fume; however none of these were designed to quantify the risk at more than two levels.

In this study, a novel strategy combining ambient, biological and biological effect monitoring has been used for estimating the adverse health risks due to PAHs-exposure from bitumen fumes. The study assessed: -

1) External exposure (external dose) as a measure of potential exposure to PAHs which was achieved by collecting samples of the air to

which workers are exposed and then quantifying the PAH profile.

2) Internal exposure (internal dose) the amount of PAHs absorbed into the body (body burden) which took into consideration exposure by all routes of entry and was assessed by biological monitoring of urinary 1-hydroxypyrene (1-HP) - a selective index of exposure to pyrene - and urinary thioethers (UTh.) - a non-selective index of exposure to a variety of chemicals - these were compared in urine samples of exposed and control groups.

3) Biological effect monitoring a measure of the biological response to PAHs exposure was achieved by measuring urinary output of d-glucaric acid (UDGA) - an index of hepatic enzyme activity - and cytogenetic analysis of sister chromatid exchanges (SCE) and Replicative Index (RI) in peripheral blood lymphocytes as well as micronuclei in exfoliated epithelial cells of the urinary system.

The ambient monitoring assessment carried out in the field was quantitative as well as qualitative. The quantitative part was acheived through measurement of PAHs in personal air samples while the qualitative depended on the subjective occupational hygiene assessment of the workplace. Both approaches were complimentary and revealed a number of interesting observations.

Exposure to PAHs from bitumen fume in the paving and roofing industry was very low in comparison to other industries such as steel works and foundries. In some cases the PAH level in personal air samples of pavers and roofers were similar to the levels found in personal air samples from occupationally non-exposed controls. It is assumed that a linear non-threshold model is the most appropriate model for any carcinogen and should be used as the primary basis for extrapolation of estimates of risk to very low levels of exposure to complex mixtures (Chong *et al.* 1989). Therefore, it is not valid to assume on the basis of these low PAH-exposure levels that the risk to health from exposure to bitumen fume is minimal.

PAH-profiles are as important as the absolute levels of PAHs with respect to evaluating the toxicity of the complex mixture. In other words,

the qualitative assessment of the "external dose" is essential for the risk evaluation. Identifying the PAH-profile can give us an indication on exposure toxicity by clearly identifying health risk factors that differ in various industrial environments. For example the quantification of the different PAH-species in vapour or particulate form can shed information on the bioavailability of the external dose. PAH in the vapour form are absorbed mainly through the pulmonary route; while PAH in particulate matter form could be equally absorbed through skin or inhalation. Information such particle size distribution is needed to estimate the contribution of the pulmonary route to the absorbed dose. Further information of dermal absorption of PAHs in vapour and particulate form is needed. This kind of information is of great use for interpreting results of biological monitoring assessments.

PAH-profiles offer a lot more than just information on the relative toxicity of the mixture. They could be used to identify the source of PAHs emission. In this study, a high naphthalene concentration signalled the presence of PAH-emission source in the working environment other than hot bitumen. The levels of 4 and 5 ringed PAHs in personal air samples were related to occupational bitumen exposure rather than the background environment. Accumulation of data on PAH-profiles could be used for the development of models for predicting the source of PAH-emissions and thus the evaluation of the working environment and the workers exposure to PAHs.

It was not until recently, that scientists acknowledged the importance of determining the PAH-profiles. In the past, external exposure to bitumen fume was assessed only by the measurement of total dust as Total Particulate Matter, Benzene Soluble Matter or Cyclohexane Soluble Matter. These indices tended to underestimate the potential toxicity of the exposure.

The current exposure limits applied to bitumen fume is the TLV recommended by the ACGIH of 5 mg/m^3. This exposure limit is not based on strong scientific evidence. Nonetheless, the bulk of the studies found in the scientific literature have measured exposure to bitumen fume by determining either TPM, BSM or CSM. Therefore it is unreasonable to abandon this approach and study exposure to PAHs only. The best strategy

is to use both measurements in parallel to allow the comparison of risk assessment between studies. It would be possible to derive an exposure limit for a selection of PAHs that could be used as a reference point to compare external exposure. Brandt *et al.* (1985) have found that PAHs in the breathing zone were directly related to the amounts of BSM. Brandt also showed that the mass of Σ14PAHs constitute 0.1 % of BSM (Brandt 1993). On the basis of Brandt's observations and the current TLV for bitumen fumes we proposed an empirical TLV-TWA for Σ14PAHs (4.3 $\mu g/m^3$) in the paving and roofing industries using bitumen based asphalt. Although TPM or BSM levels were not measured in personal air sample in this study it was still possible to compare our results semi-quantitatively to the current TLV for bitumen fume. The levels reported in our study were similar to those obtained in studies which have quantified bitumen exposure using the TPM approach. This shows that combining the two approaches allows the comparison between studies which could be used for risk assessment reviews and meta-analysis of epidemiological studies. For this research program to be effective there will be a need for an agreed method of measurement for airborne PAHs and more importantly the choice of PAHs analysed for. This program can also be applied in other industries where airborne PAHs are found.

The ambient monitoring assessment also showed that PAH levels in environmental air could contribute significantly to external exposure to PAHs. It also showed that there is considerable variability in background levels. Therefore it is important to quantify background levels in parallel with the personal air sampling when the aim is to assess the risk of occupational exposure to low PAH concentrations. This information will also assist the investigators in avoiding the problem of misclassification.

When ambient monitoring is not possible using job description for estimating external exposure is the only alternative. Occupational toxicology studies as well as case-control epidemiological studies have often used this approach. This approach appears to be acceptable if job descriptions are rigidly defined; however if jobs are shared according to need using this exposure estimation procedure can introduce bias into the risk estimation.

Ideally, no worker should be exposed to carcinogenic material at any level; this is not achievable, given the current levels of exposure to complex mixture of PAHs in the paving and roofing industry today. Moreover there are no occupational exposure limits which safeguards the health of the workers from exposure to these concentrations over a lifetime period. Therefore while scientists are trying to characterise the risk from exposure to PAHs regulators may choose to adopt the "as low as reasonably practicable" principle in order to minimise the risk as much as possible. To do so the hazard should be controlled.

Attempts should be made first at eliminating the hazard. This could be acheived through substitution. The use of cold asphalt for road paving eliminate the problem of exposure to bitumen fumes. However, it introduces another hazard. Solvents are used to clean the spades and shovels. This can lead to exposure to solvent fumes whose risk needs to be evaluated. Another way to reduce the hazard through reducing the fume emission is to use bitumen with low volatility (Brandt 1990). These control measure might be difficult to implement because of the strong resistance they might face from industry purely for economical reasons.

The second choice for minimising exposure is through engineering control measures such as process modification or segregation by using distance or process automation. Brandt *et al.* (1985) suggested that keeping bitumen temperature as low as reasonably practicable (i.e. at recommended application temperature) will reduce bitumen fume emission and therefore exposure. This however is rarely possible especially in small industries were the workers do not have the means for monitoring bitumen temperature (e.g. in the kettles) or even do not appreciate the significance of maintaining the temperatures at recommended application levels. The ambient monitoring assessment showed that the distance from the source of emission was related to the degree of exposure. For instance the kettleman/mixer operator was exposed to higher levels of PAHs than the other roofers. Similarly, the pavers working in the vicinity of the paving machine were more exposed. This problem could be avoided by automation of the process. Unfortunately, the current technology does not provide the facilities for such a measure.

It appears that at this point in time the engineering control measures for controlling exposure to bitumen fume during paving and roofing operations are not available. Therefore we turn to the last resort and that is the use of respiratory and personal protective equipment. Surprisingly, these were not used by either the pavers or the roofers in this study. The introduction of such control measures could be possible in the big industry since the implementation of such hazard control program is mainly the responsibility of the employer. But what about the workers who are self-employed ?

The occupational hygiene assessment showed that these industries have unsatisfactory industrial hygiene conditions. Therefore they should be covered by check-ups by industrial hygiene specialists for creating the conditions that are necessary for personal hygiene and for improvements in technology and hazard control.

Since the hazard of exposure to bitumen fume can not be eliminated therefore the risk assessment of exposure to PAHs is still of primary importance.

The ambient monitoring program provided quantitative and qualitative information on the external dose; however, it did not provide any knowledge about the internal dose or the route of exposure which in the case of PAHs was proven to be an important factor influencing both absorption (van Rooij *et al.* 1993) and toxicity.

To quantify the internal dose of PAHs appropriate biomarkers of exposure were monitored. It would be ideal to quantify the biological effective dose (i.e. the internal dose at site of manifestation of toxicity); this however in most occupational toxicology studies is not feasible due to unavailability of the target tissue. Therefore the closest estimation of that effective dose is the body burden (i.e. internal dose).

Assessing the risk of exposure to the complex mixture of compounds in bitumen fume a non-selective biomarker of exposure to electrophilic substances was applied. Urinary thioethers excretion was not influenced by occupational exposure to low levels of PAHs in bitumen fume, such as those in this risk assessment. The apparent limited use of

urinary thioethers in biomonitoring populations exposed to low levels of PAHs in bitumen fume does not mean that it should be excluded from such risk assessments since they may signal exposure and absorption of a number of electrophiles found in working environments, such as that of pavers and roofers, which selective biomarkers may not detect. Therefore the coupling of this non-specific biomarker with other selective biomarkers may turn out to be useful in assessing the overall risk from exposure to potentially genotoxic substances.

Selective biomonitoring of pavers and roofers using urinary 1-hydroxypyrene was a good marker of biological uptake of pyrene and by extrapolation PAHs. The profile of U1-HP excretion over time showed that there was bioaccumulation of pyrene in the pavers but not the roofers over the working week. Utilising this information in context with the ambient monitoring it was shown that the route of absorption of PAHs in the pavers was mainly by inhalation while that of the roofers was different to the pavers and was thought to be mainly through percutaneous absorption. This clearly shows that ambient and biological monitoring assessment are complementary approaches in risk evaluation and the simultaneous interpretation of data is a requirement.

In the light of the limited meaning of measurable internal dose with respect to its effect on the human body, there is a need to evaluate the adaptive response of the human body to different levels of exposure and try to relate them to disease.

Therefore, the final stage of the risk assessment was to evaluate the effect of exposure to low levels of PAHs on the workers' health. Human exposure to low levels of many environmental and occupational chemicals can result in adaptive biochemical changes in the liver that can serve as biological markers of the effect of exposure. We have measured excretion of d-glucaric acid in urine as a biomarker of hepatic enzyme activity. There was a difference in the profile of UDGA excretion over time between exposed and controls. The increase in the rate of excretion of UDGA was related to the internal dose of pyrene and therefore PAHs. It appears that liver metabolising activity has been induced by exposure to the low levels of PAHs. In contrast to other investigators (Pasquini *et al.* 1989 and Ferriera Jr. *et al.* 1994[a]) our results suggest that UDGA is a

biomarker of the effects of exposure to PAHs. The difference between our conclusion and what other researcher have reported are probably due to study design and the monitoring scheme followed.

D-Glucaric acid is a product of glucuronic acid metabolism in the liver. The glucuronic acid pathway involves enzymes other than those involved in xenobiotic metabolism. Hence we must take care in interpreting our results and suggesting that this association relationship between DGA and internal dose is a one of cause and effect nature. A better understanding of the mechanisms involved in the induction of hepatic enzymes leading to an increase in d-glucaric acid excretion are needed in order to evaluate its direct relevance to worker's health status. More studies are needed for collection of prospective data and extensive validation of this test in occupational toxicology studies, which up to date have disappointed the hopes raised by experimental studies. These studies will allow to elucidate the biological meaning of the biological change that UDGA is a marker of and eventually establish at what level an elevated value has prognostic significance for workers' health.

Bitumen fume contains human carcinogens such as B(a)P. The carcinogenic mechanism by which this compound manifests its effect appears to be related to the production of epoxides, such as (BaPDE), through biotransformation and its ability to covalently bind with macromolecules such as DNA forming adducts.

There is a close relationship between chromosome damage and cancer development (Sinues *et al.* 1991). Therefore it is probable that short-term cytogenetic tests, could be reasonably reliable for estimating the risk of exposure to potential mutagens/carcinogens and hence the prediction of DNA related effects and ultimately cancer (Hogstedt 1986).

In pavers and roofers SCE in peripheral blood lymphocytes was used for detecting exposure to chemical mutagens.

This assessment reports positive findings regarding SCE frequency. Roofers, who were exposed to the highest levels of 4,5 ringed PAHs "carcinogenic PAHs" had the highest SCE frequency. The pavers had a mean SCE frequency lower than the roofers however significantly

elevated when compared to controls. These findings suggest that SCE was a sensitive biomarker of effect of exposure to low levels of PAHs in bitumen fume.

A variety of confounding factors may affect SCE frequency and may lead to bias in the results. Moreover the mechanisms of SCE formation are still unknown. This makes it quite obvious that results of SCE in occupationally exposed groups of workers should be interpreted with great caution. The clinical significance of a positive finding is unknown and there are no basis available to relate them to an increased risk for any disease. In addition, the use of SCE as a cytogenetic endpoint to determine "safe" levels of exposure is questionable. The limited understanding of the relevance of the SCE signal to health states that more studies of this type are needed inorder to improve our knowledge about SCE induction in humans arising from occupational exposure to genotoxic xenobiotics.

With all these limitations, it is still possible to consider that cytogenetic changes such as SCE can be considered as only predictive of disease on a probabilistic basis, that is, there is a probability that a particular population with excess of cytogenetic effects will manifest some mutational or carcinogenic disease end point some time in the future (Goyer and Rogan, 1987). On this basis it is worthwhile noting that a meta-analysis of epidemiologic studies have demonstrated that the aggregate relative risk for lung, stomach and skin cancer in roofers was higher than in the pavers (Partanen and Boffetta, 1994). The authors attributed these elevated risks to exposure to coal-tar whose exposure to involves a higher risk than bitumen fume. With reservation we could say that our SCE data points in the same direction as those of the reported by epidemiologic studies.

Peripheral blood lymphocytes are usually used as a surrogate tissue for biomonitoring exposure to xenobiotics. Determining the effect of exposure in target tissue would probably give a better estimate of an identified risk. Elevated incidence of bladder cancer has been reported in workers exposed to bitumen fume (Hansen 1989). Therefore biomonitoring effects such as micronuclei in exfoliated epithelial cells of the urinary system might have given a closer evaluation of the damage

caused by the biologically effective at the site of manifestation of toxicity (i.e. bladder).

Attempts were made to monitor micronuclei in exfoliated epithelial cells in pavers and roofers; however they were unsuccessful due to the low number of cells collected in the urine of workers. Cell collection over a longer period (e.g. a working week) might increase the sensitivity of the assay. The relative easiness of this assay and its significant value in the assessment of risk require that efforts are made to study its applicability in occupational toxicology studies.

In summary, pavers and roofers are occupationally exposed to low levels of PAHs in bitumen fumes. These exposures seem to be sufficient in causing change in biomarkers of exposure and effect.

Currently, the reduction of the exposure levels is not feasible through control measures other than the use of respiratory protective equipment and personal protective equipment especially in small industry. These control measures do not provide absolute protection. This means that assessment of these low levels of exposure should be carried out to evaluate their risk to health.

Finally, the ultimate aim of ambient and biological monitoring of exposure to genotoxicants is the prevention of occupationally induced cancer and the global eradication of such a disease. Nothing less should satisfy those involved in the prevention of occupational disease.

CHAPTER 23

FUTURE WORK

23.1 FUTURE WORK

A number of questions and ideas have risen from the work done in this thesis. The ideas for future work suggested here is targeted at answering these question as well as giving new dimensions to the risk assessment.

1) to study the PAH-profile in bitumen and bitumen fume and to compare these profiles to those found in personal air samples collected in parallel. This will provide information for confirming the source of PAHs in personal air samples.

2) to determine both benzene soluble material and the PAH-species in personal air samples from bitumen workers. This will aid the understanding of the toxicity of the bitumen fume exposure, arriving at an OEL for PAHs and relate current risk assessment conducted PAH-exposure in bitumen fume to those in the literature where only benzene soluble material was measured.

3) to determine the size of airborne particles to which PAHs are absorbed. This will provide important information for determining the route of exposure to PAHs.

4) to assess the significance of the dermal absorption in PAHs exposure. This could be acheived in several ways: 1. through controlling exposure by use of respiratory protection and then determining 1-hydroxypyrene excretion. 2. using patch deposition studies and skin swab determinations to assess dermal exposure and dermal absorption

5) Investigating the role of dermal absorption in PAH-exposure by using *in vitro* techniques.

6) to parallel these studies in workers exposed to bitumen fumes with studies in the flooring industry or underground asphalt workers who are potentially exposed to higher levels of PAHs.

7) to further elucidate the effect of exposure to PAHs by studies of biomarkers of exposure in target tissues (e.g. DNA adducts in exfoliated cells of the urinary system) and correlate them to biomarkers of effect such as frequency of micronuclei in the same target tissue. This however requires increased sensitivity of the micronucleus test.

8) there is a need to inform the concerned industry of such findings and try to join efforts in minimising the risk of exposure to bitumen fume first through attempting to control the hazard at source and failing that through RPE and PPE. More work need to be done so judgements can be based on strong scientific evidence.

APPENDIX I

CONSENT FORM, QUESTIONNAIRE

AND

STUDY PROTOCOL

DEPARTMENT OF ENVIRONMENTAL & OCCUPATIONAL MEDICINE

THE UNIVERSITY OF NEWCASTLE UPON TYNE

Biological Monitoring Unit

Occupational Chemical Study-Consent Form

I, ..., acknowledge that Dr. Edwards and his PhD. student Mr. Hatjian have explained to me the purpose of this occupational chemical exposure study. I have been given an information sheet summarising this and the procedures to be followed. My signature on this consent form indicates my agreement to participate.

I understand that I am NOT being asked to modify my work practices in any way that may increase my exposure to chemicals in my occupational environment.

I agree to provide urine samples and, if requested, a blood sample, as specified on the information sheet, for the purpose of determining whether the work place chemicals have been absorbed into my body.

I understand that I am free to withdraw from this study at any time or to refuse to divulge any information requested on the forms.

I understand that the data obtained from this study remains the property of Dr. Edwards and his PhD. student Mr. Hatjian, but that they are willing to disclose to me the results obtained from my own samples. I acknowledge their right to report the results of the study provided that I retain my anonymity in any such report.

Signed .....

Witness .....

Date .....

DEPARTMENT OF ENVIRONMENTAL & OCCUPATIONAL MEDICINE

THE UNIVERSITY OF NEWCASTLE UPON TYNE

Biological Monitoring Unit

Occupational Chemical Study-Questionnaire

Name: .....

Sex: M F Age: .....

Date(s) and time(s) at which
urine samples were collected .....

Place of Work .....

Briefly describe what you did at work on the day(s) samples were collected.

.....

.....

Do you know what types of chemicals (e.g. solvents, fumes, dusts, pesticides etc.) you may have been exposed to during the past week:

(a) at work?

.....

(b) at home?

.....

Do you drink alcoholic drinks? Yes No If Yes, how many (e.g. pints/day) .....

Do you smoke NO/CIGARETTES/CIGARS/PIPE

If so, approximately how many per day? (Circle one response)

1-5 6-10 11-20 more than 20

If No, have you ever smoked? Yes No

Do you eat during working hours? Yes No

Did you eat or drink anything on the day(s) on which samples were obtained which you consider to be unusual compared to your usual dietary habits? If so, please specify

.....

Do you eat food like cabbage, radish, or any similar type of vegetables?

Yes No

If Yes, did you eat any before or on the day(s) samples were collected.

Yes No

Do you have any hobbies that you think might include hazardous chemicals: (e.g. painting, gardening)? Yes No

If Yes, please specify .....

Do you drink coffee? Yes No

If Yes, approximately how many cups/day .....

Are you currently taking any medicine? If so please specify:

(a) prescribed by your doctor

.....

(b) bought from the chemist

.....

DEPARTMENT OF ENVIRONMENTAL AND OCCUPATIONAL MEDICINE

THE UNIVERSITY OF NEWCASTLE UPON TYNE

Biological Monitoring Unit

Occupational Chemical Study

Thank you for agreeing to participate in this study. its purpose is to determine whether urine and blood samples can be useful as indicators of whether certain chemicals have been absorbed into your body as a result of working in an environment where chemical exposure may take place.

It is emphasised that you are NOT being asked to deliberately expose yourself to chemicals nor to modify your work practices so as to increase your exposure to chemicals. We are interested in the potential for chemical exposure to occur during the course of your normal working day.

We would like to measure the amount of chemicals in the air which you breathe at work. You will be asked to wear an apparatus designed to do this. It is a small battery-operated air pump - about the size of a "Walkman" personal cassette player - which is worn on a belt attached to the body. This is connected by a narrow flexible hose to a filter unit which traps chemicals from the air. The filter apparatus is attached to the clothing - usually at the lapel - in a position where it can sample air from the "breathing zone". This sampling apparatus will collect air samples for all or a portion of the working day.

One of the ways in which the body deals with chemicals absorbed into the system is to convert them into metabolites which are more readily cleared from the body in urine. The differences between the amounts of these metabolites found in your urine collected before and after work should indicate the extent of exposure to workplace chemicals. It would be very helpful if you would also agree to providing morning and evening urine samples on a non-working day, or over several days where your normal work procedures vary on a day-to-day basis.

If the chemicals have reacted, in a specific way, with the chromosomes which regulate cell function, it may be possible to detect these effects by testing some of the cells present in a blood sample.

In addition to providing samples of urine and/or blood as detailed below, we ask you to provide us with additional information which will enable us to determine whether dietary factors or social habits may influence the results.

ALL INFORMATION GIVEN WILL BE TREATED AS CONFIDENTIAL AND YOU WILL NOT BE IDENTIFIED BY NAME IN ANY REPORT OF THE RESULTS.

URINE SPECIMENS

Morning sample - In the morning, collect the whole of the first voiding in the bottle provided. Record the time and date and ensure the bottle has your name or initials on the label. Repeat for all four days of the survey.

Evening sample - Soon just before finishing work or immediately following work empty the bladder completely and record the time and date and ensure the bottle has your name or initials on the label. Repeat for the first three days of the survey.

Make sure both bottle is sealed and place it, together with a completed copy of the questionnaire, in a paper or plastic bag.

BLOOD SAMPLES

You may be asked to provide a blood sample of 10 ml volume, which will be drawn from an arm vein by an occupational health nurse or physician associated with this project on the morning of the fourth day of the survey.

If you have any queries about sampling procedures or the project please telephone: Dr. J. W. Edwards, Mr. B. Hatjian or Dr. J. Harrison at this department (091) 222 7195.

APPENDIX II

RESULTS OF

PERSONAL AIR SAMPLES

Code/Job Description : 10 / Sewer Maintenance

Sample									
Volume m3	0.97			P.F.			0.65		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	1340	1340	P.F.	P.F.	P.F.	n d	106	106
Acenaphthene	n.d.	47.6	47.6	P.F.	P.F.	P.F.	n.d.	17.9	17.9
Phenanthrene	5.5	28.3	33.8	P.F.	P.F.	P.F.	n.d.	n.d.	n d.
Pyrene	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.	n.d.	n.d.	n d
B(b)F & B(k)F	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.	n.d.	n.d.	n.d.
∑8PAHs	5.5	76.0		P.F.	P.F.		n.d.	17.9	
∑9PAHs	5.5	1416		P.F.	P.F.		n.d.	124	
∑8PAHs (F +O)	81.5			P.F.			17.9		
∑9PAHs (F +O)	1420			P.F.			124		

Code/Job Description : 14 / Handling Cement

Sample									
Volume m3	0.91			0.59			0.59		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n d	411	411	n.d.	111	111	n.d.	n.d.	n.d.
Acenaphthene	n d.	22.1	22.1	n.d.	7.2	7.2	n.d.	n.d.	n.d.
Phenanthrene	5.7	8 5	14.2	n.d.	n.d.	n.d.	n d.	n.d.	n.d.
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	5.7	30.6		n.d.	7.2		n.d.	n.d.	
∑9PAHs	5.7	442		n.d.	118		n.d.	n.d.	
∑8PAHs (F +O)	36.2			7.2			n.d.		
∑9PAHs (F +O)	448			118			n.d.		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

P.F.: Pump failure

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 15 / Handling Cement

Sample									
Volume m3	0.40			0.51			0.56		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	842	842	n.d.	738	738	n.d.	243	243
Acenaphthene	n.d.	53.7	53.7	n.d.	78 7	78.7	n d.	149	149
Phenanthrene	13.2	41.8	55.0	17.4	41.3	58.7	25.9	22.4	48 3
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	13.2	95.5		17.4	120		25.9	171	
Σ9PAHs	13.2	937		17.4	858		25.9	414	
Σ8PAHs (F +O)	109			137			197		
Σ9PAHs (F +O)	951			875			440		

Code/Job Description : 16 / Sewer Maintenance

Sample									
Volume m3	0.91			0.59			0.60		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d	697	697	n.d.	3070	3070	n.d.	33.4	33.4
Acenaphthene	n d	62.9	62.9	n.d.	422	422	n.d.	n.d.	n.d.
Phenanthrene	9 5	36.7	46.2	17.4	185	202	25.9	n.d.	25.9
Pyrene	5.5	n.d.	5.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	15.0	99.6		17.4	607		25.9	n.d.	
Σ9PAHs	15.0	796		17.4	3690		25.9	33.4	
Σ8PAHs (F +O)	115			624			25.9		
Σ9PAHs (F +O)	811			3700			59.3		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

P.F.: Pump failure

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 17 / Sewer Maintenance

Sample Volume m3	0.66			0.59			0.62		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	2440	2440	n.d.	133	133	n.d	328	328
Acenaphthene	n.d.	151	151	n.d.	19.3	19.3	n d.	36.2	36.2
Phenanthrene	38.3	143	181	17.4	n.d.	17.4	25.9	8.5	34.5
Pyrene	56.2	n.d.	56.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	42.9	42.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	166	166	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n d.
∑8PAHs	94.6	503		17.4	19.3		25.9	44.7	
∑9PAHs	94.6	2940		17.4	152		25.9	373	
∑8PAHs (F +O)	598			36.7			70.7		
∑9PAHs (F +O)	3030			170			399		

Code/Job Description : 33 / Site Supervisor

Sample Volume m3	0.97			0.81			1.01		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	988	988	n.d.	42.3	42.3	n.d.	72.3	72.3
Acenaphthene	n.d.	21.9	21.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenanthrene	7.6	26.9	34.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	7.6	48.9		n.d.	n.d.		n.d.	n.d.	
∑9PAHs	7.6	1040		n.d.	42.3		n.d.	72.3	
∑8PAHs (F +O)	56.4			n.d.			n.d.		
∑9PAHs (F +O)	1050			42.3			72.3		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

P.F.: Pump failure

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 34 / Site Supervisor

Sample Volume m3	0.81			0.92			0.95		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	6.6	762	769	n.d.	23.7	23 7	n.d.	50.2	50.2
Acenaphthene	n.d.	23.7	23.7	n.d.	n.d.	n.d.	n d.	6.0	6 0
Phenanthrene	n.d.	24.0	24.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	n.d.	47.7		n.d.	n.d.		n.d.	6.0	
∑9PAHs	6.6	810		n.d.	23.7		n.d.	56.2	
∑8PAHs (F +O)	47.7			n.d.			6.0		
∑9PAHs (F +O)	816			23.7			56.2		

Code/Job Description : 35 / Slatter

Sample Volume m3	1.01			0.81			n.s.		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	1380	1380	n.d.	59.2	59.2	n.s.	n.s.	n.s.
Acenaphthene	n.d.	33.1	33.1	n.d.	n.d.	n.d.	n.s.	n.s.	n.s.
Phenanthrene	5.6	24.7	30.3	n.d.	n.d.	n.d.	n.s.	n.s.	n.s.
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.s.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.s.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.s.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.s.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.s.
∑8PAHs	5.6	57.8		n.d.	n.d.		n.s.	n.s.	
∑9PAHs	5.6	1440		n.d.	59.2		n.s.	n.s.	
∑8PAHs (F +O)	63.4			n.d.			n.s.		
∑9PAHs (F +O)	1450			59.2			n.s.		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

n.s.: No sample

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 37 / Laying Insulation

Sample									
Volume m3	1.01			1.01			1.00		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	1630	1630	n.d.	172	172	n.d.	57.3	57.3
Acenaphthene	n.d.	102	102	n.d.	7.1	7.1	n.d.	6.5	6.5
Phenanthrene	n.d.	62.8	62.8	n.d.	n.d.	n.d.	n.d.	n.d.	n d.
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	n.d.	165		n.d.	7.1		n.d.	6.5	
Σ9PAHs	n.d.	1800		n.d.	179		n.d.	63.8	
Σ8PAHs (F +O)	165			7.1			6.5		
Σ9PAHs (F +O)	1800			179			63.8		

Code/Job Description : 38 / Painting Metal

Sample									
Volume m3	1.01			0.97			P.F.		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.s.	1690	1690	n.d.	89.5	89.5	P.F.	P.F.	P.F.
Acenaphthene	n.s.	49.4	49.4	n.d.	5.3	5.3	P.F.	P.F.	P.F.
Phenanthrene	n.s.	125	125	5.7	n.d.	5.7	P.F.	P.F.	P.F.
Pyrene	n.s.	9.4	9.4	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.
B(a)A	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.
B(b)F & B(k)F	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.
B(a)P	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.
DiB(a,h)A	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	P.F.	P.F.	P.F.
Σ8PAHs	n.s.	184		5.7	5.3		P.F.	P.F.	
Σ9PAHs	n.s.	1870		5.7	94.8		P.F.	P.F.	
Σ8PAHs (F +O)	184			11.0			n.d.		
Σ9PAHs (F +O)	1870			101			n.d.		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

P.F.: Pump failure

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 41 / Painting Metal

Sample Volume m3	0.97			1.03			1.01		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	2430	2430	45.4	55 6	101	n d.	97 4	97 4
Acenaphthene	n.d.	213	213	n.d.	n.d.	n.d.	n.d.	74 3	74.3
Phenanthrene	10.8	210	221	9.6	n.d.	9.6	58.5	279	338
Pyrene	5.9	5.7	11.6	n.d.	n.d.	n.d.	55.6	108	164
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	261	450	711
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.7	32.3	45.0
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	130	n.d.	130
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.2	n.d.	11.2
∑8PAHs	16.7	430		9.6	0.0		528	944	
∑9PAHs	16.7	2860		55.0	55.6		528	1040	
∑8PAHs (F +O)	446			9.6			1470		
∑9PAHs (F +O)	2880			111			1570		

Code/Job Description : 45 / Stripping Lead

Sample Volume m3	1.05			0.97			1.01		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	9070	9070	n.d.	55 3	55.3	n.d.	n.d.	n.d.
Acenaphthene	n.d.	50.9	50.9	n d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenanthrene	11.4	47.2	58.6	7.9	n.d.	7.9	n.d.	n.d.	n.d.
Pyrene	6.4	5.9	12.4	8.4	n.d.	8.4	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	17.9	104		16.2	n.d.		n.d.	n.d	
∑9PAHs	17.9	9170		16.2	55.3		n.d.	n.d.	
∑8PAHs (F +O)	122			16.2			n.d.		
∑9PAHs (F +O)	9190			71.6			n.d.		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 7 / Paver (Not Specified)

Sample Volume m3	0.90			0.92			0.92		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	6.5	4020	4030	8.5	1620	1630	n d	3020	3020
Acenaphthene	n.d.	355	355	n.d.	177	177	n.d.	451	451
Phenanthrene	98.7	435	534	6.6	91.5	98.0	205	230	435
Pyrene	47.7	6.9	54.5	n.d.	n.d.	n.d.	12.9	n.d.	12.9
B(a)A	12.5	n.d.	12.5	n.d.	n.d.	n.d.	n d.	n.d.	n.d.
B(b)F & B(k)F	12.1	n.d.	12.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	11.9	n.d.	11.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	183	797		6.6	268		218	681	
∑9PAHs	189	4820		15.1	1890		218	3700	
∑8PAHs (F +O)	980			275			899		
∑9PAHs (F +O)	5010			1910			3920		

Code/Job Description : 8 / Paver (Not Specified)

Sample Volume m3	0.93			0.95			0.95		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	2480	2480	n.d.	1250	1250	n.d.	994	994
Acenaphthene	n.d.	232	232	n d.	163	163	n.d.	124	124
Phenanthrene	43.2	154	197	n.d.	78.3	78.3	12.4	67.8	80.2
Pyrene	32.0	n.d.	32.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	75.2	386		n.d.	242		12.4	192	
∑9PAHs	75.2	2866		n.d.	1490		12.4	1190	
∑8PAHs (F +O)	461			242			204		
∑9PAHs (F +O)	2950			1490			1200		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 11 / Paver (Not Specified)

Sample Volume m3	0.87			0.89			0.92		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	2180	2180	n d.	78 8	78.8	n d	205	205
Acenaphthene	n.d.	152	152	n.d.	77.9	77.9	n d	445	445
Phenanthrene	46.9	101	148	6.6	34.9	41	122	279	401
Pyrene	15.6	n.d.	15.6	n.d.	n.d.	n.d.	13.3	n.d.	13.3
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.	n.d.
Σ8PAHs	62.5	253		6.6	113		136	723	
Σ9PAHs	62.5	2430		6.6	192		136	928	
Σ8PAHs (F +O)	315			119			859		
Σ9PAHs (F +O)	2490			198			1060		

Code/Job Description : 13 / Paver (Not Specified)

Sample Volume m3	0.87			0.58			0.94		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	1440	1440	n.d.	4110	4110	n.d.	1290	1290
Acenaphthene	n.d.	14.8	14.8	n d.	505	505	n d.	135	135
Phenanthrene	33.8	54.8	88.6	45.4	298	343	7.0	74.9	81.8
Pyrene	9.7	n.d.	9.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n d.	n d.	n.d.	n.d.	n.d.	n d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	43.6	69.6		45.4	803		7.0	210	
Σ9PAHs	43.6	1510		45.4	4910		7.0	1500	
Σ8PAHs (F+O)	113			848			217		
Σ9PAHs (F+O)	1550			4960			1500		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 18 / Paver (Not Specified)

Sample Volume m3	0.71			0.96			0.96		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	4270	4270	n.d.	202	202	n d	894	894
Acenaphthene	7.2	376	383	n.d.	16 4	16 4	n.d	145	145
Phenanthrene	91.2	70.2	161	13.1	n.d.	13.1	11.7	81.9	93.6
Pyrene	31.6	n.d.	31.6	n.d.	n.d.	n d.	7.2	n.d.	7.2
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	130	446		13.1	16.4		18.9	227	
∑9PAHs	130	4720		13.1	219		18.9	1121	
∑8PAHs (F+O)	576			29.5			246		
∑9PAHs (F+O)	4850			232			1140		

Code/Job Description : 19 / Paver (Not Specified)

Sample Volume m3	0.67			0.98			0.94		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	197	197	n.d.	69.1	69.1	n.d.	693	693
Acenaphthene	n.d.	15.7	15.7	n.d.	6.5	6.5	n.d.	66.8	66.8
Phenanthrene	18.5	32.5	51.0	8.5	n.d.	8.5	n.d.	33.7	33.7
Pyrene	7.1	n.d.	7.1	n.d.	n.d.	0.0	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	25.7	48.2		8.5	6.5		n.d.	100	
∑9PAHs	25.7	245		8.5	75.6		n.d.	794	
∑8PAHs (F+O)	73.9			15.0			100		
∑9PAHs (F+O)	271			84.0			794		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 60 / Raker

Sample									
Volume m3	0.84			0.82			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	652	652	n d.	2050	2050	n.d.	3210	3210
Acenaphthene	n.d.	24.1	24.1	n.d.	56.6	56.6	n d.	168	168
Phenanthrene	10.3	27.7	38.0	24.7	57.8	82.6	94.9	363	458
Pyrene	6.2	n.d.	6.2	8.8	n.d.	8 8	20.1	n.d.	20.1
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	16.6	51.8		33.5	114		115	531	
∑9PAHs	16.6	704		33.5	2160		115	3740	
∑8PAHs (F+O)	68.4			148			646		
∑9PAHs (F+O)	720			2190			3860		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 61 / Raker

Sample									
Volume m3	0.78			0.84			0.82		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	318	318	n.d.	855	855	n.d.	1810	1810
Acenaphthene	n d	14 0	14.0	n.d.	32.9	32.9	n.d.	87.1	87.1
Phenanthrene	12.1	17.1	29.2	27.1	27.6	54.7	29.4	161	191
Pyrene	7.1	n.d.	7.1	12.1	n.d.	12.1	27.7	n.d.	27.7
B(a)A	n d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	19.3	31.1		39.2	60.5		57.1	249	
∑9PAHs	19.3	349		39.2	916		57.1	2060	
∑8PAHs (F+O)	50.4			99.7			306		
∑9PAHs (F+O)	369			955			2120		

Code/Job Description : 62 / Raker

Sample Volume m3	0.84			0.84			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	496	496	n.d.	1650	1650	n d.	1960	1960
Acenaphthene	n.d.	17.8	17.8	n.d.	55.4	55.4	n d.	119	119
Phenanthrene	6.4	12.0	18.5	20.1	50.2	70.3	74.1	143	217
Pyrene	n.d.	n.d.	n.d.	n.d.	n d.	n.d.	30.1	n.d.	30.1
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.5	n.d.	12.5
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	6.4	29.8		20.1	106		117	262	
Σ9PAHs	6.4	526		20.1	1760		117	2220	
Σ8PAHs (F+O)	36.2			126			379		
Σ9PAHs (F+O)	533			1780			2340		

Code/Job Description : 63 / Tractor driver

Sample Volume m3	0.84			0.84			0.80		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	601	601	n.d.	1560	1560	6.2	2310	2320
Acenaphthene	n.d.	34.1	34.1	n.d.	68.1	68.1	n.d.	138	138
Phenanthrene	30.6	45.5	76.1	43.2	76.7	120	45.1	173	218
Pyrene	14.0	n.d.	14.0	8.6	n.d.	8.6	15.7	n.d.	15.7
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.5	n.d.	12.5
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	44.6	79.6		51.8	145		73.3	311	
Σ9PAHs	44.6	681		51.8	1710		79.6	2620	
Σ8PAHs (F+O)	124			197			385		
Σ9PAHs (F+O)	725			1760			2700		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 64 / Screwman

Sample									
Volume m3	0.84			0.84			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	417	417	9.8	7080	7090	n.d.	7220	7220
Acenaphthene	n.d.	25.6	25.6	n.d.	221	221	n.d.	338	338
Phenanthrene	9.0	8.6	17.6	81.0	135	216	141	487	628
Pyrene	n.d.	n.d.	n.d.	7.6	n.d.	7.6	26.6	6.0	32.6
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	9.0	34.2		88.6	356		168	831	
Σ9PAHs	9.0	451		98.4	7440		168	8050	
Σ8PAHs (F+O)	43.2			444			999		
Σ9PAHs (F+O)	460			7530			8220		

Code/Job Description : 65 / Steel-Tyre Roller Driver

Sample									
Volume m3	0.84			0.82			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	n.d.	0.0	n.d.	1344	1344	n.d.	1261	1261
Acenaphthene	n.d.	22.4	22.4	n.d.	65.5	65.5	n.d.	81.3	81.3
Phenanthrene	n.d.	23.5	23.5	8.1	48.5	56.6	17.3	83.8	101.1
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.0	n.d.	6.0
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	0.0	45.9		8.1	114		23.3	165	
Σ9PAHs	0.0	45.9		8.1	1460		23.3	1430	
Σ8PAHs (F+O)	45.9			122			188		
Σ9PAHs (F+O)	45.9			1470			1450		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 66 / Foreman

Sample Volume m3	0.84			0.84			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	686	686	n.d.	965	965	n d	1560	1560
Acenaphthene	n.d.	24.8	24.8	n.d.	49.7	49.7	n d.	78 1	78.1
Phenanthrene	13.9	25.8	39.7	14.7	37.7	52.4	21 0	107	128
Pyrene	n.d.	841	841	n.d.	n d.	n.d.	6.5	n.d.	6.5
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	13.9	892		14.7	87.4		27.5	185	
Σ9PAHs	13.9	1580		14.7	1050		27.5	1750	
Σ8PAHs (F+O)	905			102			212		
Σ9PAHs (F+O)	1590			1070			1780		

Code/Job Description : 67 / Paving Machine Driver

Sample Volume m3	0.84			0.84			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n d.	342	342	7.8	12100	12100	25.2	28600	28600
Acenaphthene	n d.	53.4	53.4	n.d.	204	204	36.0	666	702
Phenanthrene	28.2	37.9	66.1	109	86.8	196	2110	484	2600
Pyrene	6.8	n.d.	6.8	5.8	n.d.	5.8	302	n.d.	302
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	69.7	n.d.	69.7
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	32.8	n.d.	32.8
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	24.3	n.d.	24.3
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	35.0	91.3		115	291		2580	1150	
Σ9PAHs	35.0	433		123	12400		2600	29700	
Σ8PAHs (F+O)	126			406			3730		
Σ9PAHs (F+O)	468			12500			32300		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 68 / Steel-Tyre Roller Driver

Sample									
Volume m3	0.84			0.82			0.78		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	343	343	n.d.	1210	1210	n d.	1750	1750
Acenaphthene	n.d.	23.9	23.9	n.d.	64.2	64 2	36 0	114	150
Phenanthrene	7.4	20.2	27.6	12.0	48.0	60.0	39.9	154	194
Pyrene	n.d.	n.d.	n.d.	n.d.	822	822	17	n.d.	17 5
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d	n d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	7.4	44.1		12.0	934		93.3	268	
Σ9PAHs	7.4	387		12.0	2144		93.3	2020	
Σ8PAHs (F+O)	51.5			946			362		
Σ9PAHs (F+O)	395			2160			2110		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 69 / Raker

Sample									
Volume m3	0.84			0.80			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n d	11000	11000	6 2	10100	10100	n.d.	355	355
Acenaphthene	n.d.	43.4	43 4	n.d.	184	184	n.d.	12.0	12.0
Phenanthrene	19 9	49.5	69.4	122	73.0	195	19.7	7.7	27.4
Pyrene	6 7	n.d.	6.7	7.3	n.d.	7.3	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n d	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n d.	n.d.	n.d.	n.d.	n d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	26.6	92.9		129	257		19.7	19.7	
Σ9PAHs	26.6	11100		136	10300		19.7	375	
Σ8PAHs (F+O)	120			386			39.4		
Σ9PAHs (F+O)	11100			10400			395		

Code/Job Description : 20 / Roofer (Not Specified)

Sample Volume m3	n.s.			0.84			0.80		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.s.	n.s.	n.s.	n.d.	127	127	n.d.	59.4	59.4
Acenaphthene	n.s.	n.s.	n.s.	n.d.	10.3	10.3	n.d.	8.6	8.6
Phenanthrene	n.s.	n.s.	n.s.	n.d.	9.1	9.1	n.d.	8.8	8.8
Pyrene	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	n.s.	n.s.		n.d.	19.4		n.d.	17.4	
Σ9PAHs	n.s.	n.s.		n.d.	146		n.d.	76.8	
Σ8PAHs (F+O)	n.s.			19.4			17.4		
Σ9PAHs (F+O)	n.s.			146			76.8		

Code/Job Description : 21 / Roofer (Not Specified)

Sample Volume m3	n.s.			0.38			0.63		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.s.	n.s.	n.s.	n.d.	3130	3130	n.d.	258	258
Acenaphthene	n.s.	n.s.	n.s.	n.d.	356	356	n.d.	15.3	15.3
Phenanthrene	n.s.	n.s.	n.s.	25.1	210	235	10.1	17.9	28.0
Pyrene	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	8.8	n.d.	8.8
B(a)A	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	13.8	n.d.	13.8
B(b)F & B(k)F	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	n.s.	n.s.		25.1	565		32.6	33.3	
Σ9PAHs	n.s.	n.s.		25.1	3700		32.6	291	
Σ8PAHs (F+O)	n.s.			590			65.9		
Σ9PAHs (F+O)	n.s.			3730			324		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

n.s.: No sample

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 36 / Roofer (Not Specified)

Sample Volume m3	1.05			1.02			1.01		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	672	672	n d.	2570	2570	n d.	81.6	81.6
Acenaphthene	n.d.	32.3	32.3	n.d.	223	223	n.d.	7.4	7.4
Phenanthrene	8.3	85.1	93.4	20.6	568	588	8.8	42.8	51.5
Pyrene	9.4	7.8	17.1	17.3	n.d.	17.3	10.2	n.d.	10.2
B(a)A	n.d.	n.d.	n.d.	11.1	n.d.	11.1	25.2	n.d.	25.2
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	10.2	n.d.	10.2	n.d	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	17.6	125		59.3	790		44.2	50.2	
Σ9PAHs	17.6	798		59.3	3360		44.2	132	
Σ8PAHs (F+O)	143			850			94.4		
Σ9PAHs (F+O)	815			3420			176		

Code/Job Description : 39 / Roofer (Not Specified)

Sample Volume m3	1.08			1.02			0.88		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	2070	2070	n.d.	237	237	n.d.	62.3	62.3
Acenaphthene	n.d.	122	122	n d.	11.2	11.2	n.d.	9.7	9.7
Phenanthrene	5.4	66.2	71.6	n.d.	4.9	4.9	n.d.	n.d.	n.d.
Pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(b)F & B(k)F	n.d.	n.d.	n.d.	n d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	5.4	189		n.d.	16.1		n.d.	9.7	
Σ9PAHs	5.4	2260		n.d.	253		n.d.	71.9	
Σ8PAHs (F+O)	194			16.1			9.7		
Σ9PAHs (F+O)	2270			253			71.9		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 40 / Roofer (Not Specified)

Sample Volume m3	1.05			1.02			1.01		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	712	712	n.d.	104	104	n d.	551	551
Acenaphthene	n.d.	49.8	49.8	n.d.	8.1	8.1	n d.	8 5	8.5
Phenanthrene	21.7	90.0	112	6.7	15.2	22.0	15.7	201	216
Pyrene	27.6	n.d.	27.6	11.0	n.d.	11.0	11.2	n.d.	11.2
B(a)A	12.6	n.d.	12.6	9.8	n.d.	9.8	37.6	n.d.	37.6
B(b)F & B(k)F	11.9	n.d.	11.9	n.d.	n.d.	n.d.	n d.	n.d.	n.d.
B(a)P	12.8	n.d.	12.8	10.2	n.d.	10.2	11.0	n.d.	11.0
DiB(a,h)A	45.1	n.d.	45.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	132	140		37.7	23.4		75.5	209	
∑9PAHs	132	852		37.7	128		75.5	761	
∑8PAHs (F+O)	271			61.1			285		
∑9PAHs (F+O)	983			165			836		

Code/Job Description : 42 / Roofer (Not Specified)

Sample Volume m3	1.05			0.92			0.95		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n d.	1470	1470	n.d.	346	346	n.d.	5.4	5.4
Acenaphthene	n.d	n.d.	0.0	n d.	14.1	14.1	n.d	n.d.	n.d.
Phenanthrene	105	25.0	130	n.d.	8.1	8.1	n.d.	n.d.	n.d.
Pyrene	86.7	205	292	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)A	50.7	n.d.	50.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
B(b)F & B(k)F	13 5	n.d.	13.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	7.9	n.d.	7.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	263	231		n.d.	22.2		n.d.	n.d.	
∑9PAHs	263	1700		n.d.	369		n.d.	5.4	
∑8PAHs (F+O)	494			22.2			n.d.		
∑9PAHs (F+O)	1960			369			5.4		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 43 / Roofer (Not Specified)

Sample Volume m3	1.03			0.92			1.01		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	731	731	n.d.	163	163	n.d.	n.d.	n.d.
Acenaphthene	n.d.	45.4	45.4	n.d.	5.2	5.2	n.d.	5.1	5.1
Phenanthrene	21.6	131	152	n.d.	5.5	5.5	10.3	5.7	16.0
Pyrene	44.9	n.d.	44.9	n.d.	n.d.	n.d.	10.1	n.d.	10.1
B(a)A	14.1	n.d.	14.1	n.d.	n.d.	n.d.	29.4	n.d.	29.4
B(b)F & B(k)F	11.7	n.d.	11.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	10.2	n.d.	10.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	102	176		n.d.	10.7		49.8	10.8	
∑9PAHs	102	907		n.d.	174		49.8	10.8	
∑8PAHs (F+O)	279			10.7			60.6		
∑9PAHs (F+O)	1010			174			60.6		

Code/Job Description : 44 / Roofer (Not Specified)

Sample Volume m3	1.01			0.91			0.95		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	2120	2120	n.d.	338	338	n.d.	89.6	89.6
Acenaphthene	n.d.	n.d.	0.0	n.d.	13.9	13.9	n.d.	5.9	5.9
Phenanthrene	n.d.	94.9	94.9	n.d.	8.9	8.9	10.3	n.d.	10.3
Pyrene	n.d.	127	127	n.d.	n.d.	n.d.	10.1	n.d.	10.1
B(a)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29.4	n.d.	29.4
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	n.d.	222		n.d.	22.8		49.8	5.9	
∑9PAHs	n.d.	2340		n.d.	361		49.8	95.5	
∑8PAHs (F+O)	222			22.8			55.6		
∑9PAHs (F+O)	2340			361			145		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 46 / Roofer (Not Specified)

Sample									
Volume m3	1.02			1.01			1.00		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	1020	1020	n.d.	145	145	n.d.	n.d.	n.d.
Acenaphthene	n.d.	93.0	93.0	n.d.	15.9	15.9	n.d.	6.5	6.5
Phenanthrene	14.8	269	284	6.2	12.9	19.0	8.2	10.2	18.4
Pyrene	7.3	58.9	66.2	n.d.	n.d.	n.d.	8.1	n.d.	8.1
B(a)A	20.7	n.d.	20.7	n.d.	n.d.	n.d.	25.0	n.d.	25.0
B(b)F & B(k)F	11.1	n.d.	11.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	9.7	n.d.	9.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	63.5	421		6.2	28.8		41.3	16.7	
Σ9PAHs	63.5	1440		6.2	174		41.3	16.7	
Σ8PAHs (F+O)	484			35.0			58.0		
Σ9PAHs (F+O)	1500			180			58.0		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAI ls less naphthalene

m3: meter cubed

Code/Job Description : 87 / Kettleman, Mixer Operator

Sample Volume m3	0.83			0.84			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	6170	6170	n.d.	2570	2570	46.4	972	1020
Acenaphthene	n.d.	530	530	n.d.	223	223	29.3	102	131
Phenanthrene	112	505	617	251	568	819	446	177	623
Pyrene	95.8	23.6	119	465	n.d.	465	173	12.4	186
B(a)A	25.5	n.d.	25.5	105	n.d.	105	64.0	n.d.	64.0
B(b)F & B(k)F	n.d.	n.d.	n.d.	53.3	n.d.	53.3	12.2	n.d.	12.2
B(a)P	n.d.	n.d.	n.d.	71.0	n.d.	71.0	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	233	1059		945	790		725	291	
∑9PAHs	233	7230		945	3360		771	1260	
∑8PAHs (F+O)	1290			1740			1020		
∑9PAHs (F+O)	7460			4310			2030		

Code/Job Description : 88 / Troweller

Sample Volume m3	0.78			0.81			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	770	770	n.d.	951	951	n.d.	556	556
Acenaphthene	n.d.	38.0	38.0	n.d.	15.6	15.6	n.d.	15.6	15.6
Phenanthrene	83.0	41.0	124	7.1	23.2	30.3	65.6	29.1	94.7
Pyrene	42.2	n.d.	42.2	n.d.	n.d.	n.d.	26.1	n.d.	26.1
B(a)A	16.4	n.d.	16.4	n.d.	n.d.	n.d.	7.4	n.d.	7.4
B(b)F & B(k)F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑8PAHs	142	79.0		7.1	38.8		99.0	44.7	
∑9PAHs	142	849		7.1	990		99.0	600	
∑8PAHs (F+O)	221			45.9			144		
∑9PAHs (F+O)	991			997			699		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

∑8PAHs: All PAHs less naphthalene

m3: meter cubed

Code/Job Description : 89 / Troweller

Sample Volume m3	0.78			0.81			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	n.d.	770	770	n.d.	925	925	n.d	515	515
Acenaphthene	n.d.	38.0	38.0	12.1	106	118	n.d.	16.3	16.3
Phenanthrene	414	41.0	455	1151	460	1610	58.6	34.0	92.6
Pyrene	190	n.d.	190	409	71.5	480	27.1	n.d.	27.1
B(a)A	55.7	n.d.	55.7	173	16.9	189	6.7	n.d.	6.7
B(b)F & B(k)F	13.7	n.d.	13.7	44.7	n.d.	44.7	n.d.	n.d.	n.d.
B(a)P	n.d.	n.d.	n.d.	10.2	13.9	24.1	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	674	79.0		1800	668		92.4	50.3	
Σ9PAHs	674	849		1800	1590		92.4	566	
Σ8PAHs (F+O)	753			2470			143		
Σ9PAHs (F+O)	1520			3390			658		

Code/Job Description : 90 / Tipper

Sample Volume m3	0.87			0.81			0.84		
	DAY 1			DAY 2			DAY 3		
	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)	Filter	ORBO	(F+O)
PAH-Species	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3	ng/m3
Naphthalene	8.5	2010	2020	n.d.	1100	1100	n.d.	590	590
Acenaphthene	26.5	280	307	n.d.	103	103	n.d.	57.2	57.2
Phenanthrene	890	104.5	994	227	140	367	164	154	318
Pyrene	374	n.d.	374	112	12.0	124	66.8	8.8	75.6
B(a)A	550	n.d.	550	102	n.d.	102	22.7	n.d.	22.7
B(b)F & B(k)F	67.5	n.d.	67.5	12.7	n.d.	12.7	n.d.	n.d.	n.d.
B(a)P	230	n.d.	230	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiB(a,h)A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ8PAHs	2140	385		453	255		254	220	
Σ9PAHs	2150	2395		453	1360		254	810	
Σ8PAHs (F+O)	2530			709			474		
Σ9PAHs (F+O)	4550			1810			1060		

(F+O): Sum of TWA concentrations on filter and ORBO tube

n.d.: Not detectable

Σ8PAHs: All PAHs less naphthalene

m3: meter cubed

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