AN EXAMINATION OF EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE IN PUBLIC PLACES AND RISK OF LUNG CANCER AND HEART DISEASE

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The author, whose name appears on the title page of this work, has obtained, for the research described in this work, either:

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ABSTRACT

A three phase study examined environmental tobacco smoke (ETS) exposure and subsequent long-term risk of lung cancer and heart disease. Nicotine and cotinine were markers for the chemicals in ETS. Comparisons were made between airborne nicotine concentrations in casinos with differing levels of smoking restrictions. Measurement of nicotine using ceiling and breathing zone monitors was also conducted. Nicotine and cotinine concentrations in saliva and urine of exposed volunteers were measured concurrently. Concentrations of nicotine and cotinine were then used to calculate risk of lung cancer and heart disease. Results demonstrated that 1) levels of airborne nicotine were lower where smoking was restricted, 2) airborne nicotine measurements should be conducted in the breathing zone, and 3) conclusions based on salivary cotinine concentrations should be based on measurements averaged from groups of exposed individuals. Calculated risk levels for lung cancer ranged from 3.9 per million exposed to 1 per 10 persons exposed.

DEDICATION

This work is dedicated to my wife Heather and my sons Alec and Jonathan. While the responsibilities of a family did present some challenges with respect to the completion of this work, having the three of you as a part of my life, makes the completion of the work all the more meaningful.

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entered the MET program. Her continued encouragement throughout my studies kept me going right up to the end.

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LIST OF ABBREVIATIONS AND ACRONYMS

CFVR = Coronary flow velocity reserve

CO = Carbon monoxide

EPA = Environmental Protection Agency

ETS = Environmental tobacco smoke

GC/MS = Gas chromatography with mass spectrometry

GSTM1 = Glutathione S-transferase mu

GSTM1*2 = Glutathione S-transferase mu null

HCl = Hydrochloric acid

IARC = International Agency for Research on Cancer

IRIS = Integrated Risk Information System

MeOH = Methanol

NaOH = Sodium hydroxide

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol

NNAL-Gluc = glucuronides of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol

NRS = Normal rabbit serum

OSHA = Occupational Safety and Health Administration

RSP= Respirable suspended particulate

SFU = Simon Fraser University

TWA = Time weighted average

US = United States

INTRODUCTION

Cigarette smoking is generally accepted as a cause of lung cancer as well as several other cancers, cardiovascular disease, respiratory illnesses and various other diseases. While the case for active smoking as the causative agent for a host of diseases is very strong, environmental tobacco smoke (ETS), also known as second-hand smoke or passive smoking, is still the subject of some degree of controversy with respect to its potential as a causative agent for the variety of diseases it has been linked with. On one hand there is a growing body of evidence, both epidemiological and biochemical, that exposure to ETS, causes lung cancer, cardiovascular disease and various other diseases as well. It is recognised by Health Canada (2003) as being a cause of death and disease and is classed by the US EPA as a "Group A" carcinogen (i.e. there is sufficient evidence that the substance causes cancer in humans) (US EPA, 1994). The US EPA (1994) goes even further and states that ETS is the only Group A carcinogen that causes cancer at concentrations normally encountered in environmental settings. On the other hand, despite Health Canada, the US EPA, and many other government and non-government agencies around the world reaching this conclusion, and the continued mounting evidence against ETS, there remains members of the scientific community that feel that the conclusions reached by health and government agencies are incorrect. As would be expected, the tobacco companies also take this stance – although their opinion may be driven less by scientific uncertainty and more by the bottom line.

In a study examining restrictive smoking legislation and cigarette consumption

between 1961 and 1982, the Tobacco Institute (undated but *circa* 1985) concluded that 21% of the variation in cigarette consumption was due to restrictive legislation and, in their words, those who say they work under smoking restrictions said they smoked about 1.25 fewer cigarettes per day which translates into 350 million fewer packs of cigarettes a year in the US. Because the tobacco industry regards policy actions related to ETS exposure as a serious threat to its viability, the industry has a strategy of funding and promoting its own reviews on the subject that, in general, call into question the scientific case against ETS (Chapman, 1997; Bero, Galbraith, and Rennie 1994). While the tobacco industry, in a conscious effort, has manufactured much of the controversy, some of the controversy arises from the methodology used to calculate the human health risks. A variety of methods are presently used to estimate the human health risk of ETS exposures. Because of the degree of uncertainty involved in the use of these methodologies, there is consequently a fair degree of uncertainty surrounding the risks from this contaminant of indoor environments.

One of the problems with trying to calculate risk from exposure to ETS stems from the fact that cigarette smoke contains over 4000 chemicals, more than 50 of which are known or suspected human carcinogens (Health Canada, 2003). As summarized by Daisey (1999), of the 4000 chemicals in second hand smoke, only about 400 have been quantified. When people are exposed to ETS, they are exposed to a toxic "soup" for which the information regarding its makeup is incomplete. In order to calculate risk from exposure to ETS, it is important to be able to assess exposure. Given the number of chemicals involved, accurately discerning the level of exposure and hence the degree of risk related to that exposure becomes a daunting task. One of the methods used to quantify exposure is to use a marker for ETS. Using a substance present either in the air where tobacco products have been smoked or in the physiological fluids of individuals exposed to ETS is one method used to accomplish this.

The National Research Council's (1986), criteria for a valid marker of ETS dictate that it should be unique or nearly unique for ETS so that other sources are minor in comparison, easily detectable, emitted at similar rates for a variety of tobacco products, and have a fairly constant ratio to other ETS components of interest under a broad range of environmental conditions. In general, nicotine and its main metabolite, cotinine, meet these criteria and are commonly used markers for ETS exposure. Concentration of nicotine in air is used to represent the amount of ETS in air, while both nicotine and cotinine concentrations in physiological fluids or hair are used as evidence of exposure to ETS. Nicotine is a chemical that is present in all tobacco products (Benowitz, 1999) and nicotine in air has only one source, that being the burning of tobacco (Leaderer, 1990). Similarly, cotinine in the plasma, urine or saliva of non-tobacco users has as its only practical source the metabolism of nicotine from ETS exposure (Brown, 1999). Both nicotine and cotinine are reported as being relatively easily quantified by a variety of methods (LaKind et al., 1999). Consequently, these compounds have gained widespread use for the exposure assessment required for the calculation of risk to non-smokers from ETS exposure.

While these markers may have gained widespread use in assessing exposure to ETS and the subsequent calculation of risk due to that exposure, there are several aspects with respect to their use as surrogates for ETS exposure, or how that exposure translates to risk, that may call into question the validity of the resultant calculations of risk. Questions centre on how representative nicotine concentrations in air (or the resulting cotinine concentration in physiological fluids) are of the myriad of chemicals contained in ETS, and in particular those chemicals that are suspected of causing disease, and additionally, is it reasonable to use these markers to estimate the exposure to ETS? There is also the question of biological plausibility and further, whether the epidemiological evidence supports that ETS is causing lung cancer and heart disease at

the levels that these markers are actually encountered? Is it biologically plausible that ETS will cause lung cancer and heart disease at the levels that these markers that are actually encountered, and is there epidemiological evidence that this is occurring? With respect to the use of nicotine and cotinine concentrations to assess risk, questions have been raised regarding the derivation of the concentrations of these markers used to determine unacceptable levels of risk for exposed individuals and the methodology used to perform risk assessments. Additionally, questions relate to problems with the use of nicotine and cotinine as markers and whether there may be better methods that could be used. This project focuses on the use concentrations of nicotine in air and/or the subsequent concentrations of nicotine or cotinine in the physiological fluids of exposed non-smokers to calculate their risk of lung cancer and cardiovascular disease.

The first question that must be answered in considering the use of nicotine or cotinine concentrations in the calculation of risk, is whether it is reasonable to use nicotine and cotinine concentrations as markers representing ETS exposure. Are these two chemicals adequate to represent ETS as a whole and in particular the chemicals of concern? Given the number of different chemicals found in ETS it is unlikely that there is any one marker that will be adequately representative of all of them. Learderer and Hammond (1991) found that nicotine concentrations were related to respirable suspended particulate matter (RSP) concentrations with RSP concentrations about 9.8 times those of nicotine. Particulate matter is where much of the focus on the disease causing potential of ETS exposure has been (Daisey, 1999). LaKind et al. (1999) contend that while several of nicotine's properties make it ideally suited as a marker for exposure to ETS (uniqueness to tobacco, abundance in sidestream smoke, and relative ease of measurement), the fact that nicotine doesn't follow first order decay kinetics (and thus ages differently from other ETS substances) indicates that it should not be used as a surrogate for other substances in ETS. These authors present arguments that 3-

ethenyl pyridine (3-EP) may be a better tracer for ETS gas phase and particulate phase components than nicotine due to its first order decay kinetics (LaKind et al., 1999). In spite of the different decay kinetics of nicotine and 3-EP, Jenkins et al. (1996), found that nicotine and 3-EP concentrations were highly correlated. In addition, these authors confirmed that nicotine concentration was in fact highly correlated with concentration of RSP - with RSP having a concentration 10.9 times that of nicotine. While nicotine does appear to be correlated with RSP, a large portion of the organic mass, including many biologically active components, is found in fractions of ETS other than particulate matter (Daisey, 1999). Daisey (1999) suggests that nicotine, in addition to being a good tracer for particulate matter, may be useful as tracer for other ETS components provided smoking occurs regularly in the environment, the system is at quasi-steady state, and the sampling time is significantly longer than the characteristic times for removal processes. Daisey (1999) further suggests that in order to ultimately ascertain its usefulness as a tracer for components other than particulate matter, the ratios of these components to nicotine needs to be determined in realistic environments (Daisey, 1999). This would also be the case for any other tracer that may be proposed for use as a marker for ETS exposure. Benowitz (1999) in an extensive review of the use of biomarkers for ETS exposure concluded that when a person is exposed to ETS, the intake of nicotine is reflective of exposure to the other components of ETS.

Cotinine in physiological fluids has also been used as a marker for the amount of ETS exposure one has been subjected to. Since cotinine is the main metabolite of nicotine, measuring the concentration of cotinine in body fluids will give a direct indication of the amount of nicotine that was absorbed and if absorption efficiency is known, the amount of nicotine in the air. Jenkins and Counts (1999), contend however, that salivary cotinine is not a good quantitative indicator of airborne nicotine exposure for individual subjects as the results of their study indicated that there was a substantial

amount of variation between airborne nicotine concentrations and salivary cotinine levels. The results of their study did however indicate that for larger groups of subjects, there was a strong correlation between the levels of nicotine in air and salivary cotinine concentrations (Jenkins and Counts, 1999). Benowitz (1999) concludes that while inter-individual variability may limit the value of predictions based on measurements in individuals, the variability is compensated for in studies of large numbers of subjects, as is the case with epidemiological studies.

Another factor requiring consideration when trying to link levels of cotinine and nicotine, as surrogates for ETS, to the risk of lung cancer and cardiovascular disease, is whether or not ETS is toxic at the levels being considered. Is it biologically plausible and is there epidemiological evidence that ETS, at real levels of exposure, causes lung cancer and cardiovascular disease in non-smokers passively exposed? With respect to lung cancer, there is substantial evidence regarding the carcinogenic potential of ETS and thus the biological plausibility of it as a carcinogenic agent. According to the US EPA (1994), it is indisputable that smoking causes lung cancer in humans and there is no evidence that there will be a threshold below which smoking will not cause cancer. Thus even low dose exposures would increase risk of lung cancer to some degree. In addition, although it is a dilute mixture of mainstream and sidestream smoke, ETS is chemically similar to the smoke inhaled by smokers (containing a number of carcinogenic compounds) and large numbers of people who do not smoke have been demonstrated as having been exposed to, absorbing and metabolizing significant amounts of second hand smoke (US EPA, 1994). In one study using 5 non-smokers exposed to ETS for 3 hours, urine concentrations of a metabolite (NNAL) of a tobacco-specific lung carcinogen (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) were found to be significantly higher (almost 10 times) after exposure as compared with pre-exposure levels (Hecht et al., 1993). Additionally, levels of NNAL and its glucuronides (NNAL-

Gluc) in ETS exposed non-smokers compared with smokers (~1.6%) are consistent with the level of excess risk for ETS exposed non-smoking women compared to smokers (~1-2%) giving further biochemical support for ETS as a lung carcinogen in non-smokers (Hecht, 2002).

While the increased levels of carcinogen metabolites lend support to the biological plausibility of ETS as a lung carcinogen, there is also evidence from laboratory studies on animals demonstrating the ability of secondhand smoke to both cause cancer and damage DNA, further supporting the biological plausibility of ETS as a carcinogen (US EPA, 1994). It is one thing to find evidence of a link between ETS and cancer in animal studies, Husgafvel-Pursiainen et al. (2000) found a significant 3-4 fold increased risk of mutation of the p53 tumour suppressor gene in passive smoking lung cancer cases over never-smoker lung cancer cases lending credence to the suggestion that the mechanisms of lung carcinogenesis in ETS-exposed never-smokers include mutations in the p53 gene similar to that seen in smokers.

In addition to the evidence of biological plausibility of ETS causing lung cancer, there is also ample epidemiological evidence for ETS as a causative agent for lung cancer. Of the 16 pollutants that have been designated by the US EPA as "Group A" carcinogens, only second hand smoke has been demonstrated in studies to cause cancer at typical environmental levels (US EPA, 1994). Numerous studies have found an increase in the risk of cancer for those exposed to secondhand smoke vs. those that are not exposed and, according to the US EPA (1994), there is remarkable consistency in the studies that support a causal association between secondhand smoke and lung cancer. In their review of the epidemiological studies on ETS the US EPA (1994), found that in all 14 of the studies that examined the relationship between exposure and effect, there was a trend of increasing response with increasing exposure, that the trend was statistically significant in 10 of the 14 and that there was less than a one in a billion

probability finding a statistically significant trend in this many of the studies by chance. More recently, Zhong, Goldberg, Gao, and Jin (1999) found that long-term occupational exposure to ETS, either alone or in combination with home exposure, conferred an increased risk of lung cancer among women who never smoked and that the risk increased with increasing number of hours of daily exposure to ETS in the workplace. Risk was also observed to increase with increasing number of smoking co-workers. Along this same line, for the highest level of ETS exposure in the workplace and in vehicles, there was a significant increased risk of lung cancer in a study by Kreuzer, Krauss, Kreienbrock, Jockel, and Wichmann, (2000). Lee et al. (2000) found that ETS exposure occurring in childhood potentiates the effect of high doses of exposure in adult life in determining the development of lung cancer. Furthering the epidemiological case for ETS causing lung cancer, cessation of exposure has been observed to reduce risk of lung cancer. Boffetta et al. (1998) and Boffetta et al. (1999) observed an association between cessation of ETS exposure and the suggestion of a decrease in the risk of lung cancer.

In addition to lung cancer, second hand smoke exposure has been linked to various other cancers. Villeneuve, Johnson, Mao, Hanley, and Canadian Cancer Registries Research Group (2004) found a weak association between pancreatic cancer and ETS. In addition to this association with pancreatic cancer, their results suggest that ETS smoking exposure may confound the risk of pancreatic cancer associated with active smoking measures commonly used in epidemiologic studies. Kropp and Chang-Claude (2002) concluded that the hypothesis of a causal relationship between active and passive smoke exposures and breast cancer was strengthened with the results of their study. With respect to ETS exposure and breast cancer, Gammon et al. (2004) found an increased odds ratio among non-smokers who had lived with a smoking spouse for over 27 years.

While the case for ETS as a causative agent for lung cancer (and possibly other cancers) is strong, can the same be said for ETS as a causative agent for cardiovascular disease? Similar arguments can be made for the links between ETS exposure and cardiovascular disease as were made for ETS and lung cancer. As is the case for lung cancer, active smoking has been demonstrated to cause cardiovascular disease, with an estimated 180,000 deaths in the US from cardiovascular disease due to active smoking (Howard and Thun, 1999). Lower doses of tobacco smoke, as are experienced in passive smoking, would also be expected to cause an effect, although unlike as is the case for lung cancer, there is the possibility of a threshold below which there is no effect.

Howard and Thun (1999), reviewed several human and animal studies examining the sequence of events involved in the development of cardiovascular disease and concluded that the studies, which measured effects on platelets, thrombosis, vascular endothelium and exercise tolerance, were particularly informative in demonstrating the mechanism, dose response relationship, and biologic plausibility of ETS as a causative agent. Otsuka et al. (2001) found that passive smoking substantially reduced coronary flow velocity reserve (CFVR) in healthy non-smokers, and provided, in their opinion, direct evidence that passive smoking may cause endothelial dysfunction of the coronary circulation, an early process of atherosclerosis. Further, this change may be one reason why passive smoking is a risk factor for cardiac disease morbidity and mortality in non-smokers. Moffatt, Chelland, Pecott, and Stamford (2004) observed a significant negative impact of ETS on blood lipids adding evidence that supports a role of enhanced atherosclerosis via alterations in lipid profiles. Moffatt et al. (2004) also found that during ETS exposure respiratory CO levels were significantly increased, as compared to baseline and post-exposure, by almost 100% and the authors concluded that CO in circulation may contribute to cardio-vascular disease via promotion of atherosclerosis, hypoxia on the myocardium and increased stress due to the elevated levels of

carboxyhemoglobin.

In addition to being biologically plausible for ETS to cause cardiovascular disease, epidemiological studies have linked ETS exposure with increased risk of coronary heart disease. In a review of epidemiological studies of the association of ischemic heart disease risk and ETS and a subsequent meta-analysis, Thun, Henley, and Apicella (1999) found that the relative risk (RR) for fatal and nonfatal coronary events among never smokers married to smokers, compared to those with spouses who did not smoke was 1.25 (95% confidence intervals of 1.17 - 1.33). Of further interest, the authors observed in the three studies that presented data separately for non-smokers married to current or former smokers, the association was stronger when the spouses continued to smoke (RR=1.16, 95% confidence intervals 1.06 - 1.28) than with former smokers (RR=0.98, 95% confidence intervals 0.89 - 1.08).

Law, Morris, and Wald (1997) did an evaluation of the evidence of ETS and ischaemic heart disease in an attempt to explain why the associated risk of ischaemic heart disease caused by exposure to ETS is almost half that of smoking 20 cigarettes per day while the level of exposure is only about 1% of smoking. They found that the effect is explained mostly by a non-linear dose-response relation between exposure to tobacco smoke and risk of heart disease and also found that the excess risk of smoking one cigarette per day of 39% is similar to the risk in a non-smoker living with a smoker. Howard and Thun (1999), report that based on pooled analyses of epidemiological studies, non-smokers exposed to ETS in the workplace have an increased risk of cardiovascular disease 1.35 times that of those not exposed and that the risk is 1.23 times greater for those exposed at home. Based on the relationship between ETS exposure and lung cancer and cardiovascular disease it seems likely that ETS is a causative agent for these two diseases at levels normally encountered in the environment.

Nicotine and cotinine appear to be acceptable markers for exposure to ETS and as it is biologically plausible that ETS can cause both lung cancer and cardiovascular disease, and there is substantial epidemiological evidence that a causative relationship for both of these diseases is likely, it leads to an examination of how the observed concentrations of nicotine and cotinine are used in the calculation of risk. One of the ways that nicotine and cotinine concentrations in physiological fluids are used in risk calculation is to first utilize them to calculate exposure based on cigarette equivalents. Once calculated, cigarette equivalents can then be used to compare how the passive smoke exposure would compare to the exposure one would receive if it was as a result of active smoking. Using the dose response curve for smokers (dose of cotinine vs. response in number of lung cancers or cardiovascular disease), one can extrapolate down to a low dose exposure level equivalent to that of passive smoke exposures. In pooling the results from several studies, Hackshaw (1998), found that passive smokers had, on average, about 1% of the concentration of cotinine or nicotine in urine or saliva of that of active smokers.

A problem with using cigarette equivalents based on nicotine or cotinine concentrations is that it may subsequently underestimate the risk of disease. Hammond, Sorensen, Youngstrom, and Ockene (1995), suggest that if cigarette equivalents were based on some of the carcinogens in ETS rather than nicotine, the cigarette equivalents would be much higher. If the 4-aminobiphenyl adduct of haemoglobin were used, exposure to ETS would be estimated as close to 10% of that of active smoking (Dockery and Trichopoulos, 1997; Hammond et al., 1995). Similarly, using urinary mutagenicity would result in an estimate of 4% of active smoking while using N-nitrosodimethylamine could result in an estimate between 33 – 50% of active smoking (Hammond et al., 1995). These numbers are clearly much different than the levels of exposure that are calculated when nicotine or cotinine are used to estimate cigarette equivalents. Based on these

other measures for calculating cigarette equivalents, it would seem that the use of linear extrapolation of cotinine or nicotine concentrations based on cigarette equivalents would thus underestimate the true risk from ETS.

Conversely Rosenbaum, Sterling, and Weinkam (1998), using RSP and factoring in the differences in retention of particulates in the lungs of non-smokers from ETS in comparison to the retention of particles in the lungs of smokers from active smoking, came up with a dose for passive smokers about .001% of active smokers. These authors contend that using nicotine for cigarette equivalents overestimates risk. In either case, it is difficult to estimate the risk to non-smokers of lung cancer based on these results. These estimates could be gross underestimates of risk of lung cancer when compared to the risk using carcinogens to calculate cigarette exposure, or over estimates if using the amount of respirable suspended particulates.

When comparing the extrapolation based on nicotine concentration produced cigarette equivalents one gets a better picture of whether this approach is acceptable or not. Using linear extrapolation Hackshaw (1998) estimated that passive smokers would have about 1% of the risk of lung cancer in active smokers, which is an estimated relative risk of 1.19 or 19% excess risk. This level of risk is in general agreement with the level of risk of lung cancer that was calculated by Hackshaw (1998), 1.24 or 24% excess risk, in pooling 37 epidemiological studies of non-smoking women living with smoking husbands. In contrast, the risk of lung cancer among never smokers calculated by Rosenbaum et al. (1998), based on extrapolation from smokers to non-smokers using the cigarette equivalents from retained dose of RSP would lead to 1 death per 4.7 million never smokers. The epidemiological evidence does not support the level of risk being this small.

Cotinine is the short term marker of choice for epidemiological studies to assess risk with levels in body fluids possibly elevated 10 or more times in the most heavily

exposed groups of non-smokers (Repace and Lowrey, 1990). While questionnaires are frequently used for epidemiological studies, the use of cotinine or nicotine concentrations can greatly improve the accuracy of the assessment. By using cotinine or nicotine, it is possible to confirm the exposure groupings that participants have placed themselves in. Confounding by non-smoker misclassification, and in particular spousal non-smoker misclassification, is an issue that is frequently raised as a source of error to explain the causative relationships that are seen in epidemiological studies (Lee, 1998). The use of cotinine levels in physiological fluids to confirm smoking status can reduce this misclassification. In general most smokers have a urine cotinine level of >100ng/ml whereas non-smokers have a level that is usually below 15 ng/ml (Jenkins and Counts, 1999). These levels can thus be used to confirm the non-smoking status of those claiming to be non-smokers.

In addition to reducing non-smoker misclassification, the use of cotinine levels can help to get a better understanding of the true risk, as for most people who claim to have no exposure, their actual exposure when measured using cotinine is not zero. As discussed by Repace and Lowrey (1990), few non-smokers have been found to have an absence of cotinine in their body fluids. This may be of significant importance because according to Hackshaw (1998), the inclusion of non-smoking women in the unexposed group that are assumed to have zero risk but in fact are actually exposed to ETS from people other than their spouse, both inside and outside the house, will dilute the true risk to non-smokers from exposure to ETS in general. Additional support for the use of cotinine or nicotine concentrations in epidemiological studies for the calculation of risk comes from the fact that the mean levels of urinary nicotine and cotinine in body fluids increase, both with an increasing self-reported ETS exposure, and with an increasing number of cigarettes smoked per day by active smokers (Repace and Lowrey, 1990). The fact that the levels go up with increasing exposure and that this is consistent with

what is observed in active smokers is indicative that the use of these markers in epidemiological studies of ETS to measure exposure is reasonable, and to reduce misclassification desirable.

In addition to using nicotine or continine concentrations to calculate cigarette equivalents or for confirming the level of exposure in epidemiological studies and ultimately, with either of these methods, to estimate risk of lung cancer or cardiovascular disease from ETS exposure, these concentrations can also be used to build models to predict the risk of these outcomes. These models may be used to either predict the risk from a given level of exposure or to set levels of exposure that will ensure levels of risk that are not exceeded. Repace and Lowrey (1993) built a model to predict the environmental concentrations of nicotine that would lead to *de minimis* risk (acceptable) and *de manifestis* risk (of obvious or evident concern). This sort of work may be of great value to regulatory bodies in setting standards protective of worker health.

While their model initially used RSP concentrations in air, they later used the ratio of RSP to nicotine in ETS, demonstrated by Leaderer and Hammond (1991), to be about 10 to 1, to convert the exposure concentrations to nicotine in air (Repace and Lowrey, 1993). Steady state cotinine levels in plasma were then related to the estimated nicotine exposure by pharmacokinetic modelling in order to be able to assess the accuracy of the exposure estimates (Repace and Lowrey, 1993). After validating the model and finding that the model predicted cotinine levels to within 10-15% of the available data for median and peak levels of cotinine measured in the body fluids of non-smokers, Repace and Lowrey (1993) then modelled risk of lung cancer from nicotine and cotinine concentrations. The model predicts a 1e-6 (*de minimis*) risk of lung cancer for nicotine in air concentration of 7.5 ng/m^3 (8-hr TWA), which yields a corresponding steady state cotinine value of 2.6 e-3 ng/ml urine and conversely a 3e-4 (*de manifestis*) risk of 2.3 µg/m^3 (air nicotine, 8-hr TWA), yielding a corresponding steady state cotinine

concentration of 0.8 ng/ml urine is also predicted (Repace and Lowrey, 1993). These levels can then be used to set regulatory requirements for indoor air quality. The risk model on which these values were based was successful in predicting the risk ratio observed in the American Cancer Society Cohort study on passive smoking and lung cancer and predicted exactly the misclassification adjusted odds ratio for spousal passive smoking and lung cancer derived by the US EPA in its meta-analysis of 11 US epidemiological studies (Repace and Lowrey, 1993).

Models have also been used to predict the risks to the population as a whole based on levels of air nicotine or cotinine in body fluids representing ETS exposure. In a recent study, by modelling the nicotine from ETS in office air and salivary cotinine in non-smoking US workers, Repace, Jinot, Bayard, Emmons, and Hammond (1998), estimated the percentage of ETS exposed office workers that exceed the US Occupational Safety and Health Administration's (OSHA's) significant risk level for heart disease and lung cancer (1/1000). They found that more than 95% of office workers exceed OSHA's significant risk level for heart disease mortality and 60% exceed OSHA's significant risk for lung cancer mortality (Repace et al., 1998). The results of this study indicate that 4000 heart disease deaths and 400 lung cancer deaths occur annually among US office workers at the 28% prevalence of unrestricted smoking in office workplaces at the time of the study (Repace et al., 1998).

Given the ways in which concentrations of nicotine and cotinine are used in the calculation of risk of disease from exposure to ETS, there are potential problems that may be encountered. Certainly one problem could be the use of one measurement at one point in time to determine the concentration of either nicotine in air or of nicotine or cotinine in a physiological fluid. While the concentration in air may not be an issue if the previously discussed criteria suggested by Daisey (1999), are met, if they are not, particularly if the room is not at quasi steady state, it may be impossible to determine

how biased the measured concentration is with respect to the actual level of ETS in the air. A similar problem can be said for measurements of nicotine or cotinine in physiological fluids. If the levels of these compounds are not at steady state, they may not be very reflective of the true level of ETS exposure. Using nicotine in plasma (or the corresponding levels in saliva or urine) this may be even more problematic as the half-life of nicotine is approximately 2 hours so the timing of the sample is crucial to determining exposure levels (Dockery and Trichopoulos, 1997). Using the levels of nicotine or cotinine to assess cigarette equivalents may also pose some problems particularly in trying to convey the level of risk to others. According to Repace and Lowrey (1990), when using cigarette equivalents as a measure of risk, significant risks from ETS exposure as compared to a *de minimis* level of risk might be concealed by statements that relate non-smokers exposures as being orders of magnitude lower than the exposures represented by smoking a single cigarette.

Despite the potential problems with the use of nicotine and cotinine concentrations as markers for ETS exposure in the calculation of risk, it does appear that their use is reasonable. It is clearly biologically plausible that ETS causes lung cancer and cardiovascular disease at the levels of nicotine and cotinine that are measured. In addition, it appears that despite the host of chemicals in ETS for which ratios to nicotine are not known, and that nicotine's decay kinetics differs from many of the chemicals for which ratios are known, provided smoking occurs regularly in the environment, the system is at quasi-steady state and the sampling time is significantly longer than the characteristic times for removal processes, nicotine does represent ETS in general. Cotinine levels also appear to be representative of ETS exposure as long as the measurements are not used individually, but rather are used as an average value from a group of measurements. The risk assessments conducted to date appear to be

using the measurements of nicotine and cotinine in a manner that is consistent with what is known about ETS from field measurements. One of the key pieces of evidence in favour of the use nicotine and cotinine concentrations for risk calculation is that the resulting calculations of the level of risk of lung cancer and heart disease related to a concentration of nicotine or cotinine are in strong agreement with the level of excess risk of lung cancer and heart disease that has bee observed in epidemiological studies. This is the case both for models that predict risk as well as models using cigarette equivalents to predict risk by linear extrapolation. In both of these cases, the risk predictions agree with the levels of risk that were found in the epidemiological studies that have been conducted. If levels of nicotine currently observed in cigarettes are reduced (which is an approach suggested in order to reduce their addictiveness) the use of nicotine and cotinine to assess exposure to ETS may have to be re-assessed. Until such time, and until a better marker for ETS exposure is determined, nicotine and cotinine should continue to be used for the measurement of ETS exposure allowing for the subsequent calculation of risk of lung cancer and cardiovascular disease.

If an increase in the risk of disease is predicted from exposure to ETS, then conversely one would expect to observe a reduction in that risk upon termination of the exposure. Supporting this expectation, Boffetta et al. (1999) found an indication of a protective effect after cessation of ETS exposure. Similarly the International Agency for Research on Cancer (IARC) in a large case control study in Europe found a 16% excess risk of lung cancer due to spousal exposure to ETS, a 15% excess risk due to workplace exposure to ETS and a decrease in excess risk in both cases after a cessation of exposure (Boffetta et al., 1998). Additionally, Kiyohara et al. (2003) postulate that variability in susceptibility to carcinogens may be particularly important at low degrees of environmental exposure. They feel that the effect of phenotype or genotype is likely to

be much more evident at a low dose of exposure than at high-dose exposure because at high dose exposures saturation of the enzyme activity occurs among those with both phenotypes of high and low activity, but does not at low-level exposures. This link has been demonstrated with active smokers with a stronger association between cancer risk and metabolic genotypes among light smokers than among heavy smokers (Kiyohara et al., 2003). Similarly, Malats et al. (2000) found that GSTM1*2 genotype (determining a lack of the phase II detoxifying enzyme GSTM1) is associated with an increased risk for lung cancer in non-smokers. ETS with its corresponding very low-level exposure to the carcinogens it contains may particularly affect the susceptible population.

Repace et al. (1998) used estimations of the uptake and metabolism of nicotine to calculate saliva cotinine levels, which were subsequently used to estimate the risk of lung cancer and heart disease. Similarly, the studies by Repace and Lowery (1993) and Repace and Lowery (1990) involved estimations of various aspects of exposure or dose in calculation of risk from ETS exposure. In other models, the route of exposure of nicotine was through intravenous infusion. Benowitz and Jacob (1993) used intravenous dosing of nicotine to describe nicotine and cotinine elimination pharmacokinetics in smokers and non-smokers. Similarly, Curvall, Vala, Enzell, and Wahren (1990) used intravenous infusions of nicotine and cotinine measurements in body fluids to simulate and evaluate nicotine intake during passive smoking. In the present study, the estimates are replaced with actual measurements of air nicotine exposure and corresponding measurements of the levels of nicotine and cotinine in urine and saliva to ascertain dose. The use of actual measurements of these levels will ultimately allow for a more accurate prediction of the risk of lung cancer and heart disease than what can be expected will be obtained using the current models.

Past risk analyses like that of Repace et al. (1998) have used previously reported measurements of the level of nicotine in air in various workplaces and compares them to levels of cotinine in blood of workers in similar work environments from other studies in order to calculate the theoretical risk of heart disease and lung cancer posed by exposure to these environments. While the risk analysis done by Repace et al. (1998) does use actual measurements of air nicotine and blood cotinine levels for the calculation of risk, these measurements were taken from separate studies. By using measurements from two different studies Repace and Lowery (1998) must make the assumption that the air nicotine level in the study from which the blood cotinine levels were taken was the same as in the study that the air nicotine levels were taken from. In addition, the measurements of the cotinine in blood were taken at only one time point. The use of a single point in time for the measurement of cotinine in blood has a significant inherent assumption. By using a single time point one must assume that the level of cotinine was at steady state at the time the sample was taken. This assumption may have a significant impact on the resulting calculation of risk. The present study looks at the measurement of nicotine in air to calculate risk as well as attempts to compare the measurement of nicotine in air with subsequent cotinine levels measured in physiological fluids at different time points to gain a better understanding of how much nicotine is absorbed during exposure to second hand smoke. With the improved understanding of nicotine absorption more accurate estimates of risk of lung cancer and heart disease should be possible.

MATERIALS AND METHODS

Passive Air Monitoring for Nicotine in Greater Vancouver Casinos:

In the first phase of the project, the amount of nicotine in air was measured and used to determine the risk of lung cancer and heart disease due to passive smoke exposure. This portion of the study was conducted in casinos representing three different exposure scenarios. The first exposure scenario was in a casino where smoking was not permitted anywhere inside the facility. The second exposure scenario was a casino where smoking was permitted only in a separately ventilated smoking room. The third exposure scenario was a casino where smoking was permitted throughout the facility with no restrictions other than smokers were not permitted to smoke at designated non-smoking tables within in the facility but these tables were still within the general area of the facility. In order to calculate risk of lung cancer and heart disease from second hand smoke exposure in each of the facilities, nicotine levels were measured in each of the facilities and used to represent the multitude of chemicals in ETS.

Construction of Passive Air Monitors

Measurement of nicotine was accomplished by use of specially designed passive monitors. The monitors were built as described by Hammond and Learderer (1987). Briefly, a Teflon-coated glass fibre filter (Emfab TX4Hi20ww, Pallflex Corp., Putnam, CT) was treated with a 5% sodium bisulfate, 4% ethanol solution and allowed to dry. This treatment had previously been found to be effective in trapping nicotine on the filter

paper (Hammond and Learderer, 1987). The treated filter paper was placed on top of a 37 mm support pad (Pall Gelman Laboratory cellulose support pad) and both were placed in the bottom of a 37 mm diameter styrene acrylonitrile cassette (Pall Gelman 37 mm three piece air monitoring cassette catalogue number 4339). The filter paper and support pad were held in place by a windscreen. The windscreen was constructed from the styrene acrylonitrile spacer (the middle piece of the three piece cassette) onto which a 10 µm pore 47 mm Poretics polycarbonate filter paper (catalogue number K99CP04700) had been glued and the overlapping portion of the filter trimmed off. The spacer had previously been machined in the Simon Fraser University machine shop so that the distance from the windscreen to the filter paper was 1.17 cm, this distance was consistent with the passive monitors used by Hammond, Leaderer, Roche, and Schenker (1987). This would ensure a sampling rate in agreement with the 25mL/min as had been calculated as the theoretical rate and subsequently validated by Hammond et al. (1987). Figure 1 shows the components of the monitor laid out prior to construction and figure 2 shows a monitor as constructed. The monitors were transported to and from the casinos with a closed cassette half replacing the windscreen and plugs inserted in the openings in either side of the monitor assembly to ensure an airtight seal of the unit until they were to be put into service for sampling nicotine. Figure 3 illustrates the monitor as transported.

Figure 1. Components of passive monitors used for nicotine sampling.

From left to right: windscreen, sodium bisulfate/ethanol-treated filter, support pad, closed cassette bottom

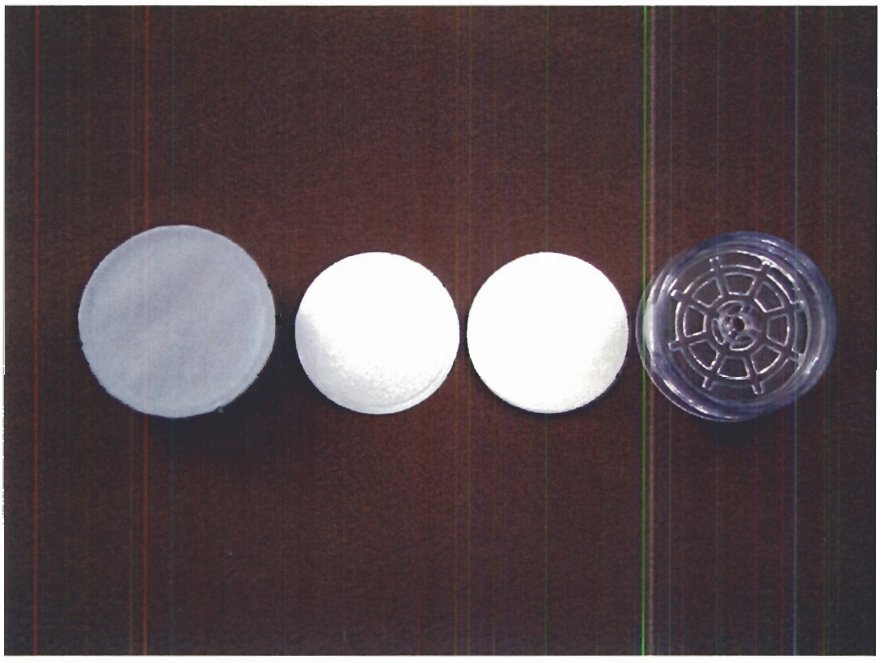


Figure 2. Passive monitors for nicotine sampling as constructed.

Figure 3. Passive monitor as sealed for transport and storage

Location and Set Up of Passive Monitors

Upon arrival and entry into the casino, as the monitors were secured in place using 3M command adhesive strips, the closed cassette half was removed from the monitor to be replaced by the windscreen and the plug was removed from the remaining closed half of the cassette. 15-20 passive monitors were affixed to the ceiling, or to the wall at or near ceiling height, at each of the casinos. During the process of securing the monitors to the ceiling or wall, the monitors were oriented so as to be perpendicular to the apparent direction of airflow (based on the location of the air intake and outlets) with the windscreen facing into the direction of wind flow. The monitors were left in place in the casinos for 1 week. The approximate locations the monitors were placed in each of the casinos are illustrated in Figures 4, 5 and 6. Each casino was revisited at the same time one week after the placement of the monitors for removal and collection of the monitors. At the time of collection, the windscreen was removed, a closed cassette half was put back on and the plugs were reinserted into either end of the monitor assembly, once

again rendering the monitor airtight. The monitor assemblies from each casino were put into a zip-loc bag and transported to the lab at SFU and placed in a freezer at -18°C for storage until analysis.

Figure 4. Location of passive monitors in the casino with no smoking permitted.

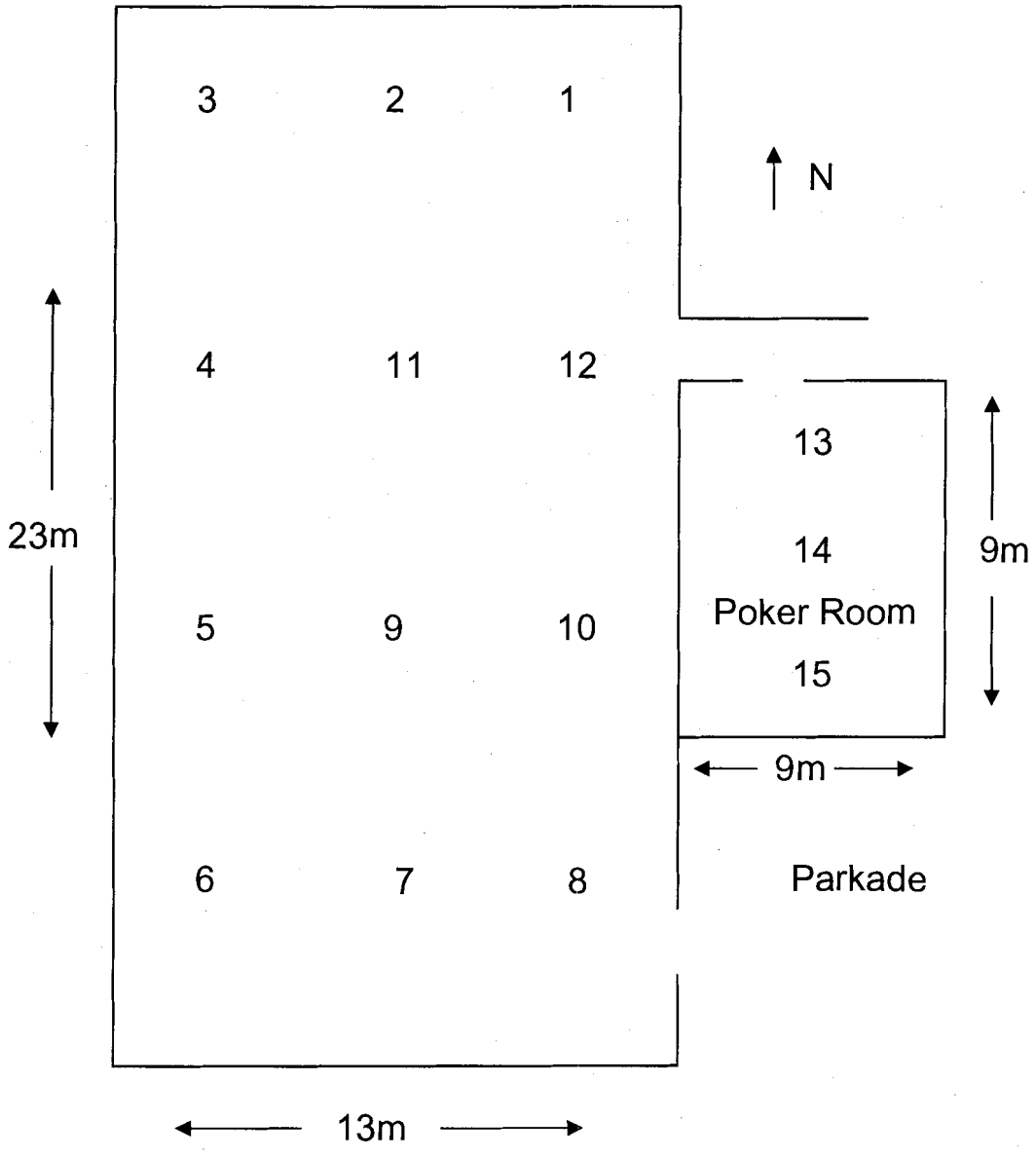


Figure 5. Location of passive monitors in casino with smoking room

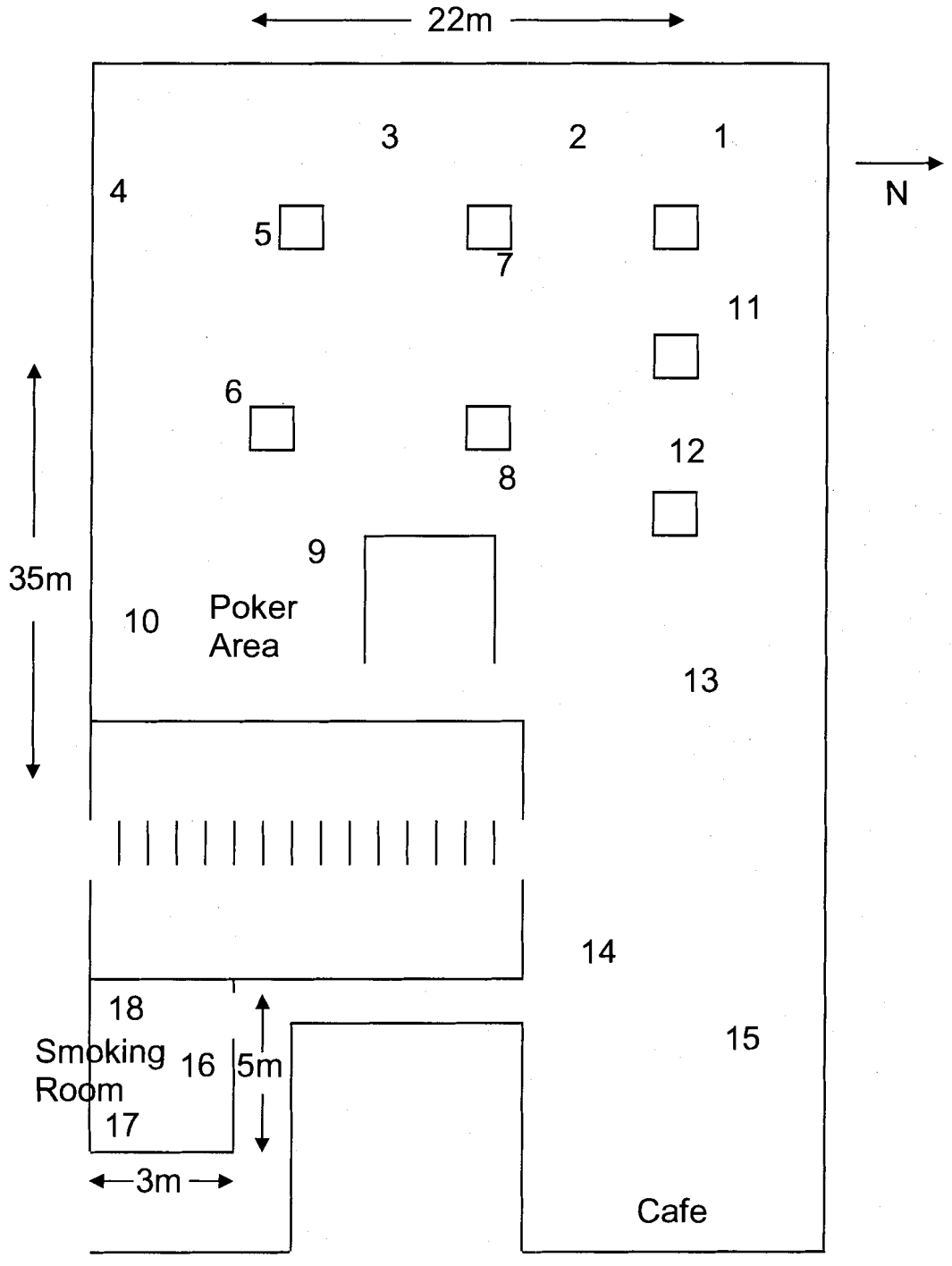
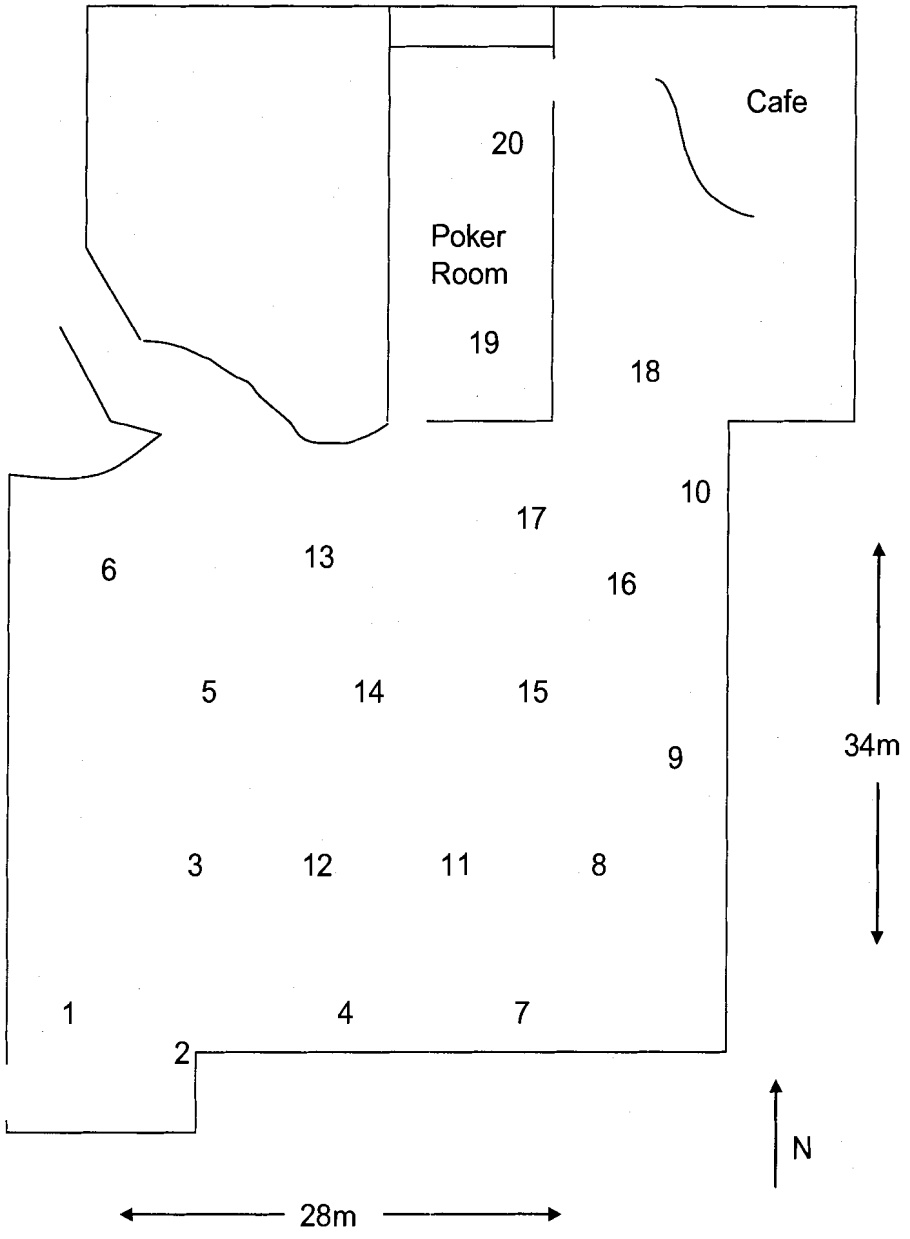


Figure 6. Location of passive monitors in casino with no smoking restrictions



Filter Analysis

At the time of analysis, the monitor assemblies were removed from the freezer and disassembled. Analysis of the treated filters using gas chromatographic analysis with mass spectrometry (GC/MS) was performed by Dr. L. Vinnakota and Dr. G. Gao following the methodology reported by Hammond and Learderer (1987). In order to analyse the amount of nicotine on the filters from the passive monitors, a standard curve using the sodium bisulfate and ethanol treated filters was first prepared. A nicotine standard solution was prepared by pipetting 1 µl of nicotine into a 100-ml volumetric flask and made up to volume with toluene/butanol (90:10). 100 µl of this solution was diluted with toluene/butanol (90:10) to 10 ml to produce a 1ng/µl nicotine standard solution. Treated filters were then spotted with 1, 5, 10, 50, 100 and 200 µl of solution to produce filters with 1, 5, 10, 50, 100 and 200 ng treatments for the standard curve. The treated papers were each cut into small pieces and placed in centrifuge tubes containing 2 ml of water and 100 µl of ethanol and then vortexed. In between cutting each filter, the scissors were washed with MeOH. Next, 50 ng of nicotine-d_3 in 0.01N HCl was added to the tube and the mixture was vortexed for 1 minute. To this solution, 2 ml of 10N NaOH containing 0.2 M ammonia was added and the mixture was again vortexed. In order to extract the nicotine into the solvent phase, 2 ml of ammoniated heptane was added to the centrifuge tube and the tube was capped and mixed on a mechanical shaker for 15 minutes. After shaking, the tubes were centrifuged at 2500 x g for 15 minutes. The organic layer containing the nicotine was then removed into a clean conical centrifuge tube. A thin layer of the organic layer was left in the centrifuge tube in order to avoid getting any aqueous solution into the new centrifuge tube. The volume of the organic layer was reduced at room temperature under a gentle stream of nitrogen. The residual liquid was transferred from the centrifuge tubes into Gas Chromatograph with Mass Spectrometry (GC/MS) glass inserts and toluene/butanol (90:10) solution was then used

to rinse the centrifuge tubes into the GC/MS inserts. The GC/MS inserts were placed into the automatic sampler of a Hewlett-Packard GC/MS model number 5890 for analysis. The GC/MS was connected to and controlled by a personal computer equipped with Hewlett-Packard Chemstation software for data storage and processing. The GC/MS column in use had a length of 30 m and an internal diameter of .53 mm. Helium was used as the carrier gas. The GC/MS was programmed using the selective ion method to analyse for ions 84 (nicotine) and 87 (nicotine-d_3) after a 4.5 minute solvent delay using a 8 minute time window. The oven temperature was programmed to 50 °C. After a one minute start up, the oven was programmed to go from an oven temperature of 70 °C to 250 °C at a rate of 25 °C/minute with the maximum oven temperature set to 300 °C and run time set at 9.2 minutes. The injector temperature was programmed at 250 °C and the detector temperature was set to 280 °C. The auto sampler was programmed to perform two solvent A washes, two solvent B washes, two sample washes, and two sample pumps.

Passive Air Monitoring with a Simultaneous Human ETS Exposure Trial in a Casino Lacking Smoking Restrictions

In this phase of the study, 2 human volunteers were exposed to environmental tobacco smoke by having them spend time in the general area of a casino where smoking was permitted. Additionally, overlapping the exposure of the human volunteers, another trial was conducted with the passive monitors affixed to the ceiling of the casino for one week, in approximately the same locations as during the initial phase of the study. The purpose of this phase of the study was to attempt to quantify the extent of exposure to environmental tobacco smoke given a fixed period of exposure and to validate the initial measurement of the concentration of nicotine in this casino as measured in the first phase of the study.

Ethical Approval and Volunteer Recruitment

Prior to conducting this portion of the experiment, details of the study and a proposed volunteer consent form and volunteer information form had been submitted to the SFU ethics approval committee. The volunteer consent and volunteer information forms are attached in Appendices A & B. Two non-smoking volunteers were recruited for participation in this phase of the study. A non-smoker was defined as a healthy individual between the ages of 20-40, who had not smoked for the past year any tobacco products including cigarettes, cigars or pipe tobacco, did not use chewing tobacco of any kind, did not live with a person who smoked any tobacco products, and was not using any form of nicotine replacement therapy. Each volunteer was required to read the information sheet and sign the consent form. In addition each volunteer was given a Subject Feedback form. A copy of the Subject Feedback form is located in Appendix C.

Sample Collection

In order to determine the amount of environmental tobacco smoke to which the volunteers were exposed, passive nicotine monitors were worn by the volunteers for the duration of the time they were inside the casino. The passive monitors worn by the volunteers were constructed in the same fashion as those used in the first phase of the study with the addition of an alligator clip fastened to the bottom piece of the polystyrene cassette to allow the monitor to be affixed to the shirt collars of the volunteers. Figure 7 illustrates the passive monitors as worn by the volunteers.

In addition to the passive monitors worn by the volunteers, in order to accommodate the determination of the actual dose of environmental tobacco smoke each volunteer received, samples of urine and saliva were collected from each of the volunteers. The fluids were then analysed for nicotine and its main metabolite, cotinine.

Figure 7. Passive monitors as worn by human volunteers in phase two of study

Prior to entry into the casino, each volunteer had two passive nicotine monitors affixed to the collar of their shirt in the breathing zone. The volunteers were required to submit a urine and saliva sample prior to ETS exposure. These samples were collected to allow for the calculation of baseline levels of nicotine and cotinine. In addition to their use for calculation of baseline levels of nicotine and cotinine, the pre-exposure urine and saliva samples served to allow for objective confirmation of the non-smoking status of the volunteers, and that the volunteers had not had recent ETS exposure. The duration of the exposure period was 5 hours during which time the volunteers were free to move about the general public area of the casino. At predetermined times during the ETS exposure, and for 96 hours from the initiation of the exposure, saliva and urine samples were collected for analysis of nicotine and cotinine levels. Saliva was sampled by having the volunteer collect the saliva in their mouth and spit as much as possible into a 16 ml VWR Scientific Products glass sample vial with molded screw cap (product number 66011-121). The vials were closed and labelled with the time and date of sample collection and a volunteer number (assigned to each volunteer for all of their

samples prior to sample collection). The volunteers were instructed to collect the total volume of urine they voided during the predetermined time period in a 24 hour urine sample container, and pour off an aliquot of the urine collected for that time period into a urine specimen container and appropriately label it with the volunteer number, the time period of the sample and the total volume of urine collected for that time period. To aid in the consistency of sample collection, the volunteers were each given a sample collection guide sheet with the suggested times and date for saliva sample collection and another for suggested time periods for urine sample collection. In addition to aiding in timeliness of sample collection, these sheets also allowed for the actual time/time period of sample collection to be recorded. A copy of the sample collection sheets provided to the volunteers can be found in appendix D. The volunteers were directed to try to adhere to the sample collection period but more importantly, that actual sample times were to be accurately recorded. Samples collected before and during the exposure period were transported to the lab for storage in the freezer immediately after the completions of the sampling period. Samples collected for the remainder of the sample collection period were stored frozen at the volunteers' homes until delivery to the lab for frozen storage until analysis.

Sample Analysis

Saliva and urine were analysed for nicotine and cotinine by Dr. L. Vinnakota and Dr. G. Gao using a modified version of the method used for analysis of the filter papers. First a standard curve was prepared by pipetting 1 ml of saliva (or 1:4 urine) into five different centrifuge tubes. Next, into the tubes, 25, 50, 100, 250 or 500 ng of cotinine (dissolved in 0.01 M HCl) was added. 5, 10, 20, 50 or 100 ng of nicotine (also dissolved in 0.01 M HCl) was also added to the tubes. Into each tube 200 ng of cotinine-d_4 in 0.01 N HCl and 20 ng nicotne-d_3 was added. The tubes were vortexed and then 0.5 ml of 2M

NaOH containing 0.2 M ammonia was added and the tubes were then vortexed again. Next 3 ml of a mixture of toluene and 1-butanol (70:30) was added and the tubes were capped and mixed on a shaker for 10 minutes. After shaking, the tubes were centrifuged at 2500 x g for 15 minutes. The top organic layer was then removed to clean, conical centrifuge tubes containing 0.5 ml of 0.5 M sulfuric acid. Care was taken to leave a small amount of the organic layer to avoid any chance of getting water in the new centrifuge tube. The new tubes were capped and mixed on a shaker and then centrifuged to separate the layers. The top organic layer was discarded using vacuum suction. Aqueous potassium carbonate (0.5 ml of 50% w/v containing 0.2 M ammonia) and 200 µl of 90:10 toluene-butanol were added to the centrifuge tube. The tubes were capped and mixed on a shaker, centrifuged and placed in a dry-ice acetone bath. The top organic layer was transferred into a GC/MS glass insert using a small amount of 90:10 toluene butanol to rinse the centrifuge tube as necessary. The tubes were then analysed on the GC/MS to make the standard curve for the extraction. The saliva and urine samples were then analysed using this method (other than the addition of the nicotine or cotinine standards). For this phase of the study, the set up of the GC/MS was the same as in phase one except for the analysis of the urine and saliva, the instrument was programmed to detect additional ions 176 (cotinine) and 180 (cotinine-d_4).

Human ETS Exposure Trial in a Bingo Smoking Room

In this phase of the study, 6 healthy non-smoker volunteers (three males and three females) were exposed to ETS during a 4 hour stay in the smoking room of a bingo hall.

Volunteer Recruitment

Six healthy non-smoker volunteers (three males and three females) were recruited for this phase of the study. Appendices E and F contain copies of the volunteer consent form and volunteer information sheet used for this phase of the study. The same Subject Feedback form used in the second phase was used in the third phase. Again all volunteers were required to complete the consent form prior to participation in the study. In addition to the consent form and information form, a week prior to participating in the study, volunteers were given a protocol for the experiment that they were to follow. A copy of the protocol can be found in appendix G.

Sample Collection

Similar to the exposure of volunteers in the casino in the second trial, the volunteers were required to wear a passive monitor to sample the air for ETS nicotine and to provide a saliva and urine samples at pre-determined times prior to, during and after the exposure period. The schedule for collection of the samples in this phase of the study is included in Appendix H. Urine collection was done in the same fashion as the second phase of the study. Saliva was collected by first rinsing the mouth with water and swallowing the water. After waiting 10 minutes, a cotton salivette (Sarstedt catalogue number 51.1534) from the top portion of the 25 plastic tube was inserted into the mouth. The salivette was chewed for 2-3 minutes while moving it around the mouth and allowing it to soak up as much saliva as possible. Again without touching it, the salivette was deposited back into the top portion of the plastic tube and the cap placed back on. The tubes were labelled with the volunteer's pre-assigned letter and the time of the sample from the start of exposure. As was done in the trial in the casino using volunteers, the pre-exposure urine and saliva samples as well as the urine and saliva samples collected during the exposure period, along with the passive monitors that were

worn during the exposure period, were transported after the exposure period to the lab at SFU for storage in the freezer. The volunteers were instructed to keep their post exposure samples in their freezers until the end of the sampling period at which time they were to be transported to the lab at SFU for storage in the freezer until analysis.

Physiological Fluid Analysis

The urine and saliva samples were analysed for cotinine and nicotine using the radioimmunoassay procedures that had been developed in the lab of Dr. Helen Van Vunakis. The procedures used follow those described in Van Vunakis, Gjika, and Langone (1993). A separate radioimmunoassay kit was purchased for analysis of both nicotine and cotinine from the Van Vunakis lab at Brandeis University. As per the instructions on the kits, the kits arrived to the lab frozen, packed on dry ice and they were subsequently stored frozen until use. The analysis of the urine and saliva samples for cotinine was done as follows. First all the necessary solutions were prepared. Isogeltris buffer was prepared to contain 0.14 M NaCl, 0.01 M tris-HCl and 0.1% gelatine and then adjusted to a pH of 7.4 using 10N and 1N NaOH. The Isogeltris buffer was used for dilution in the preparation of all other solutions used in the radioimmunoassay. The cotinine standards were prepared by diluting the 50 µg/ml standard solution from the kit to first prepare a 50,000 pg/ml solution. An aliquot from this solution was then diluted to prepare a 5,000 pg/ml solution. The ^{3}H-cotinine solution from the kit was diluted to yield approximately 10000 cpm/ml. The 1:10 diluted Normal Rabbit Serum (NRS) in the kit, for background binding of the radioimmunoassay, was further diluted 1:100. The Ra. 495D (20-41) Anti-Cotinine-CDI-Thyroglobulin in the kit was diluted 1:100. The undiluted NRS was diluted 1:25 and the goat serum was diluted 1:4. All dilutions for preparation of solutions for the analysis were done using the isogeltris buffer pH 7.4.

After preparation of the reagent solutions for the assay, the analysis was carried out. Plastic tubes (Sarstedt Inc., No. 55,535 3.5 ml with caps) were placed in a tube holder and labelled with the appropriate sample number. Each sample being analysed in duplicate (including those tubes used for preparation of the standard curve). To each tube 0.5 ml of Isogeltris buffer was added. Next the inhibitor was added to each tube with the exception of those tubes used in the standard curve to determine total binding and no binding. The inhibitor was either a known amount of cotinine (1000, 500, 200, 100, 50, 20 or 10 pg) using the appropriate amount of one of the standard cotinine solutions to deliver the desired amount of cotinine diluted to 0.1ml with isogeltris buffer or 0.01 ml of a test sample of urine or saliva diluted to 0.1 ml with isogeltris buffer. The tubes not receiving inhibitor received instead 0.1 ml of isogeltris buffer. Next 0.1ml of the radio-labelled cotinine was added to each tube. After adding the radiolabelled cotinine, 0.1 ml of antibody was added to each tube with the exception of the first two and last two tubes which received 0.1ml of NRS 1:1000 as a non-specific background control. All tubes were then vortexed and subsequently incubated at 37 °C for 1 hour. After incubation, 0.1 ml of the 1:25 diluted NRS was added to each tube and the tubes were again vortexed. Next 0.1ml of the goat serum was added to each tube and the tubes were again vortexed. At this point the tubes were incubated at 4 °C overnight. After the overnight incubation, the tubes were centrifuged for 45 minutes at 1000 x g at 4 °C. The supernatant was decanted from each tube and the sides of the tube were wiped out using a cotton swab, taking care not to touch the pellet. The pellet was then dissolved by adding 0.1 ml of 0.1 M NaOH and then 2 ml of scintillation fluid was added to each tube and the tubes were vortexed. The tubes were placed in scintillation vials, and the vials were capped. Prior to analysis, the tubes were left to sit in the dark overnight to ensure that the pellet was completely dissolved. The tubes were run through the scintillation counter and the results for the tubes containing known amounts

of cotinine were used to plot the standard curve and the standard curve was subsequently used to determine the cotinine levels in the test samples.

The sample analysis for nicotine in urine and saliva was similar to that used for cotinine. A nicotine radioimmunoassay kit was obtained from Dr. Helen Van Vunakis at Brandeis University. Again, as was done with the cotinine analysis, the procedure for nicotine analysis followed that specified by Van Vunakis et al. (1993). First all solutions used in the analysis were prepared. The Isogeltris buffer solution was prepared to contain 0.14 M NaCl, 0.01 M Tris-HCl and 0.1% gelatine and adjusted to pH 7.4 using 10 N and 1 N NaOH. Again the Isogeltris solution was used for the dilution to prepare all subsequent solutions used in this radioimmunoassay. The nicotine standards were prepared by diluting the 50 µg/ml standard from the kit to first prepare a 50,000 pg/ml solution and then further diluting this solution to prepare a 5000 pg/ml solution. The radio labelled nicotine solution was prepared by diluting the nicotine, L-(-)-[N-Methyl ^{3}H] solution from the kit 1:20 to approximately ~10000 cpm/0.1ml. The antibody was prepared, to ensure binding of 35%-50% of the total counts added, by diluting the Ra 526 D-22-38 Anti Nicotine-CDI-BSA 1:500. A non-specific background control was also prepared by further diluting a 1:10 dilution of Normal Rabbit Serum (N.R.S.) to 1:500. Undiluted N.R.S was prepared to a dilution of 1:25. Goat Anti-Rabbit-Gamma Globulins was prepared by diluting previously undiluted Goat serum (lot # DESP-III) Anti-Rabbit-Gamma Globulins 1:4.

After preparation of the reagent solutions for the assay, the analysis was carried out. Plastic tubes (Sarstedt Inc., No. 55,535 3.5 ml with caps) were placed in a tube holder and labelled with the appropriate sample number. Again each sample was analysed in duplicate (including those used for the standard curve). First, 0.5 ml of Isogeltris buffer, pH 7.4, was added to each tube. Next the inhibitor was added to each

tube with the exception of those tubes that were used in the standard curve to determine total binding and no binding. The inhibitor was either a known amount of nicotine (5000, 2500, 1250, 500, 250, 125 or 50 pg) using the appropriate one of the standard nicotine solution diluted to 0.1ml with isogeltris buffer or 0.01 ml of a test sample of urine or saliva diluted to 0.1 ml with isogeltris buffer. The tubes not receiving inhibitor, received 0.1 ml of isogeltris buffer. Next, all tubes received 0.1 ml of ^{3}H Nicotine solution and, with the exception of the first two and last two tubes, 0.1 ml of the antibody. The first two and last two tubes received 0.1 ml of NRS 1/500 as a non-specific background control. Each tube was then vortexed and then all tubes were incubated at 37 °C for 60 minutes. After incubation, 0.1 ml of the 1:25 dilution of NRS was added to each tube and they were vortexed again. Next 0.1 ml of 1:4 dilution of Goat Anti-Rabbit-Gamma Globulins was added to each tube and all tubes were again vortexed before being incubated at 4°C overnight. The next morning, the tubes were centrifuged cold (4°C) at 1000 x g for 45 minutes. After centrifuging the tubes, the supernatant was decanted and the sides of the tubes were wiped off with a Q-tip taking care not to touch the pellet. The pellet remaining in each tube was then dissolved with 0.1ml of 0.1M NaOH and 2ml of scintillation fluid was then added to each tube and the tubes were vortexed. The tubes were placed in scintillation vials, the vials capped and, prior to analysis, the tubes were then left to sit overnight in the dark to ensure that the pellet was completely dissolved. The tubes were run through the scintillation counter and the results for the known amounts of nicotine were used to plot a standard curve and the standard curve was in turn used to determine the nicotine levels in the test samples.

Passive Air Monitor Analysis

As the pure nicotine and d3-nicotine that had been used in the first two phases of the study were no longer usable, the analysis of the filters in this phase was first

attempted using a modification of the radioimmunoassay protocol that had been used for analysis of the saliva. The initial attempt used the procedures used for nicotine analysis of saliva. In this case, instead of analysing urine or saliva, the aqueous solution from the extraction of the filters was used. This was done at the point when there was an aqueous solution to analyse (i.e. once the filters had gone through the extraction of the nicotine into aqueous solution). The extraction of the nicotine to aqueous solution was done using the same protocol as had been used in the first two phases for the analysis of the filters from the passive monitors. The one difference was that the treatment of the filters with nicotine for preparation of a standard curve (to verify that the radioimmunoassay method could be used to analyse the filters from the passive monitors) was done using the buffered standards prepared for the radioimmunoassay method to spot known amounts of nicotine on the filter papers instead of using a nicotine standard prepared from pure crystalline nicotine. This method proved unsuccessful. Based on a discussion with Dr. Helen Van Vunakis (the developer of the radioimmunoassay methodology for nicotine and cotinine) to determine if the radioimmunoassay for nicotine had ever been used to analyse for nicotine captured from air monitoring for nicotine previously it was decided that another approach was warranted. The indication was that there was no reference in the literature of the assay having been used in this fashion but that it had been used to analyse hair for nicotine (Klein and Koren, 1999). The suggestion was that the methods used in that experiment may be able to be adapted for the analysis of the filter papers in the present study. It was also suggested that the problem could be that the buffer in the nicotine standard solution may have been interfering with the release of the nicotine from the treated filter papers. In an attempt to determine if the buffer in the nicotine standard was interfering with the release of nicotine from the filter paper after spotting it on the filter paper or if the method for analysing hair for nicotine could be modified for the analysis of the filters

from the passive monitors could be used, a trial using three methods simultaneously was done.

In this trial of the variations in methods, the first method, for comparison sake, again used known amounts of the buffered nicotine standard spotted on the filter paper prior to starting the extraction process. The results of this were compared with trials where known amounts of the buffered nicotine standard was put directly into the test tube before carrying on with the extraction process and another trial where known amounts of the buffered nicotine standard was put into the test tube along with 50 ul of 1N HCl. Again all three of these methods were unable to produce a consistent recovery of nicotine.

Another trial was attempted using the full extraction of the nicotine into the organic solvent and then extracting back into aqueous solution. This alteration of methodology again proved unable to produce a consistent recovery of nicotine. At this point the radioimmunoassay was abandoned as a method to analyse the filters and a return to the use of the original methodology used to analyse the filters in the first two phases of the study was undertaken. As there were only 6 filters to be analysed from the exposure in the Bingo smoking room, the cost of using pure methyl-d_3 nicotine was prohibitive. So for this phase, rather than preparing the internal standard using pure methyl-d_3 nicotine as was the case in the initial two phases, nicotine-d_3 salicylate salt was dissolved in double distilled water to prepare the internal standard. All other procedures used in the initial phase filter analysis remained the same for analysis of the filters from the personal passive monitors worn in the Bingo smoking room.

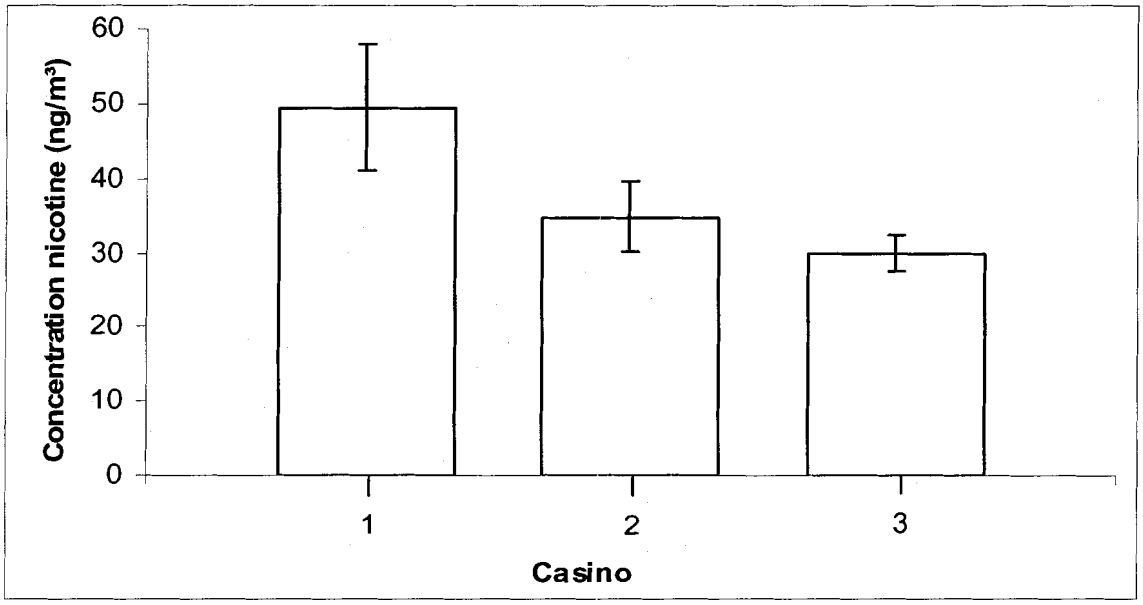
RESULTS AND DISCUSSION

Initial Passive Air Monitoring Trial

The passive monitors from the three different casinos in the initial phase of the study were analysed to determine the amount of nicotine they had trapped. The results of the analysis for mass of nicotine indicated that the concentration of nicotine in air did not appear to be normally distributed, and so a log conversion of the data was performed prior to averaging in order to obtain an average mass of nicotine on the filter papers. The average concentration of nicotine in the air was then calculated using the average mass of nicotine found on the filters, a total sampling time of 1 week=10080 minutes, and an air sampling rate for the passive monitors of 24 mL/min. The air sampling rate of the passive monitors used for this calculation had been previously validated by Hammond and Leaderer (1987) and subsequently by Kuusimaki et al. (1999) and found to be consistent with the theoretical sampling rate for the passive monitors as constructed. For calculation of the concentration of nicotine in air, the passive monitors in poker rooms were not included in the calculation, as the casino where smoking was restricted to a separate smoking room did not have a separate poker room provided.

Figure 8 illustrates the average nicotine in air with the 95% confidence intervals. As can be seen in this figure, the amount of nicotine found on the filters from passive monitors that had been placed in the casino where smoking was permitted was significantly greater than those in both the casino where smoking was confined to a smoking room and the casino where smoking was not permitted anywhere inside the facility.

Figure 8. Concentration of nicotine in air in casinos with three levels of smoking restrictions (Where: casino 1 = casino with smoking permitted, casino 2 = casino with no smoking permitted, and casino 3 = casino with smoking permitted only in approved smoking room)



While the amount of nicotine in air was found to be greater in the casino where smoking was not restricted than in either the casino with smoking restricted to a separately constructed smoking room or the casino with smoking not permitted, it was almost three orders of magnitude less than expected based on the levels that have been reported in previous studies with sampling done using personal breathing zone monitors (Trout, Decker, Mueller, Bernert, and Pirkle, 1998; Hammond et al., 1995), or the inferred concentration of nicotine in air based on measurements of cotinine in saliva (Jarvis, Foulds, and Feyerabend, 1992). This can likely be explained, at least in part, by the location chosen for placement of the filters in the casinos. While placement in the breathing zone is ideal as it best represents the air that occupants of the room are exposed to, the need to prevent tampering with the filters combined with a lack of suitable surfaces in the casino at breathing zone height on which to fasten the monitors necessitated placing the monitors on the ceiling or high up on walls and pillars. The passive monitors were oriented so as to be perpendicular to the direction of air flow in an

attempt to ensure that an adequate amount of would be sampled. It appears however that the locations used did not suffice for accurately measuring the concentration of nicotine in air based on what could reasonably be expected to be present. That nicotine was measured in the casinos with smoking restricted was consistent with the findings of Hammond et al. (1995) and the smell of smoke was noticed inside the entrance to the casino with no smoking permitted.

Although the passive monitors appeared to substantially under represent the amount of nicotine in the air, for comparison sake, the average ETS nicotine in the air was still used to calculate a theoretical level of risk of lung cancer and heart disease from exposure to the air in each of the casino settings. The calculations were done using the methodology used previously by Repace et al. (1998) and Repace and Lowrey (1993) for the calculation of risk of lung cancer and heart disease due to ETS exposure. Risk was measured by substituting the average air nicotine found in this study into the formulae previously developed by Repace et al. (1998) for the calculation of salivary cotinine levels and then comparing with the *de minimis* (1/1000000) and *de manifestis* (3/10000) risk levels for risk of lung cancer and heart disease from ETS exposure from Repace and Lowrey (1993).

First the average daily dose of ETS nicotine absorbed by the lung into plasma was calculated in units of µg/ day using equation 1.

Equation 1: Average daily dose of nicotine

$$D = \alpha \rho H N_{avg}$$

Where **α** is the absorption efficiency for inhaled nicotine (0.71), **ρ** is the non-smoker's respiration rate during exposure in m^3/hr (0.9.), **H** is the duration of exposure in hrs/day

(7), and **Navg** is the daily average nicotine concentration (in this case taken from the average found in the present study) (Repace et al., 1998)

These values were then used to calculate the mean plasma cotinine concentration (**P**) in units of µg/ml using equation 2.

Equation 2: Mean plasma cotinine concentration

$$P = \Phi D / \delta \tau$$

Where Φ is the nicotine to cotinine conversion efficiency (0.78), δ is the non-smokers' plasma cotinine clearance in ml/min (61) and τ is the length of a day in minutes (1440) (Repace et al., 1998)

From the mean plasma cotinine concentrations it is then possible to calculate the steady-state saliva cotinine concentration (**S**), in units of µg/ml using equation 3

Equation 3: Steady-state saliva cotinine concentration

$$S = \gamma P$$

Where γ is the salivary-to-plasma cotinine ratio (1.16) (Repace et al., 1998)

The results of the calculations of salivary cotinine levels are listed in table 1.

Table 1: Predicted steady state saliva cotinine levels based on average concentration of nicotine in air

Casino's smoking restrictions	Daily average dose of ETS nicotine (µg/day)	Mean plasma cotinine concentration (µg/ml)	Steady state saliva cotinine concentration (µg/ml)
smoking permitted	0.221	1.96e-6	2.28e-6
smoking room only	0.156	1.38e-6	1.60e-6
no smoking	0.134	1.19 e-6	1.38e-6

The salivary cotinine level in response to, and thus the risk posed by, the air in the casino where smoking is not restricted to a separate room nor to outside the building, as would be expected, was greater than either the casino where no smoking was permitted or the casino where smoking was restricted to a separately ventilated smoking room. Repace and Lowrey (1993) found that a *de minimis* risk of lung cancer for a 45 year working lifetime is associated with salivary cotinine levels above 0.4 pg/ml and that the *de manifestis* risk level occurs at salivary cotinine levels above 0.14 ng/ml. While none of the steady state salivary cotinine levels calculated from the air nicotine concentrations in the three casinos exceeds the *de manifestis* risk level, all exceed the *de minimis* risk level. The fact that all exceed the *de minimis* risk level is in agreement with the findings of Repace et al. (1998) who suggest that nearly all non-smoking workers in the United States exceed the *de minimis* risk level. The risk of lung cancer from exposure to the air in the casino with no smoking restrictions would have been expected to exceed the de manifestis level but measured levels were less than would be expected for reasons discussed previously and thus the corresponding risk levels were also much reduced.

Repace and Lowrey (1998) estimate the risk of death from heart disease from exposure to ETS to be tenfold that of the risk of lung cancer death giving cotinine levels

1 tenth those for lung cancer death. From this they also suggest salivary cotinine levels above 0.04 ng/ml exceed the significant risk level, 1/1000, used by the US OSHA as a legally defensible significant risk or regulatory action level. Again all of the salivary cotinine levels calculated from the air nicotine concentrations in the three casinos exceed the *de minimis* risk level for heart disease death, but do not exceed the *de manifestis* risk level for heart disease death or the OSHA significant risk level. This is contrary to the findings of Repace and Lowrey (1998) who claim that more than 95% of non-smoking workers exceed not only the *de manifestis* risk level for death from heart disease, but also the OSHA significant risk level.

While calculating the salivary cotinine levels from the average ETS nicotine in air concentrations can be used to confirm through simple comparison whether the levels are above or below the *de minimis* or *de manifestis* risk levels for reported salivary cotinine levels, it is possible to calculate the actual risk level for each of the ETS nicotine levels from the casinos.

Risk of lung cancer was calculated by first using equation 4 (USEPA, 1989) to calculate the intake rate.

Equation 4: Intake rate

$$\mathbf{I = \frac{C \times CR \times EF \times ED}{BW \times AT}}$$

Where: **I** = Intake (mg/kg•day), **C** = Chemical Concentration (e.g. mg/m^3 from the study), **CR** = Contact Rate (15.2 m^3/day), **EF** = Exposure Frequency (250 days/ year), **ED** = Exposure Duration (45 years), **BW** = Body Weight (70 kg) and **AT** = Averaging Time (25,500 days) (USEPA, 1989)

The intake rate was then used to calculate risk of lung cancer using equation 5 (USEPA, 1989)

Equation 5: Risk of lung cancer

Risk = CDI x SF

Where: **Risk** = a unitless probability of an individual developing cancer, **CDI** = chronic daily intake averaged over 70 years (mg/kg•day), and **SF** = slope factor (kg•day/mg) (USEPA, 1989)

As there was no slope factor for ETS on the EPA's website IRIS nor was one reported in any of the literature reviewed, a slope factor was calculated by substituting the *de minimis* concentration of nicotine in air from Repace and Lowrey (1993) into equation 4 to solve for the chronic daily intake and then using that value in equation 5 to solve for the slope factor. The slope factor was calculated to be SF = 4.175 (kg•day/mg). Intake rates for the 3 sampling periods and the resulting risk levels are listed in table 2. As would be expected, given the air nicotine concentrations that the risk levels were calculated from and the fact that all of the levels were in excess of the *de manifestis* concentrations, the risk of lung cancer death was found to be approximately 3.9 -6.6 lung cancer deaths per million exposed individuals.

Table 2: Intake rates and risk levels for lung cancer for three casino types

Location	Intake rates (mg/kg•day)	Risk of lung cancer
Casino with no restrictions on smoking	1.58 E-6	6.6 E-6
Casino with no smoking allowed	1.11 E-6	4.6 E-6
Casino with smoking in smoking room	9.38 E -7	3.9 E-6

Passive Air Monitoring with Simultaneous Human ETS Exposure Trial in a Casino Lacking Smoking Restrictions

In this phase of the study, conducted in a casino where smoking was not restricted, the amount nicotine in air measured using passive monitors placed on the ceiling for a one week period was compared with the amount of nicotine in air measured using passive monitors attached in the breathing zone of two volunteers who spent 5 hours in the same casino during the hours of operation. Additionally, the urine and saliva of the two volunteers was collected at specific time intervals to allow for the measurement of nicotine and cotinine levels so that the extent to which the exposure to ETS in air translated into an absorbed dose could be quantified with nicotine and its main metabolite cotinine serving as markers for ETS. The results from the analysis of the passive monitors used this phase of the study are illustrated in table 3. Figure 9 illustrates the comparison of the results of the concentration of the nicotine in air for in the casino with no smoking restrictions from the first phase of the study with the results from passive monitors placed in approximately the same location in the second phase of the study.

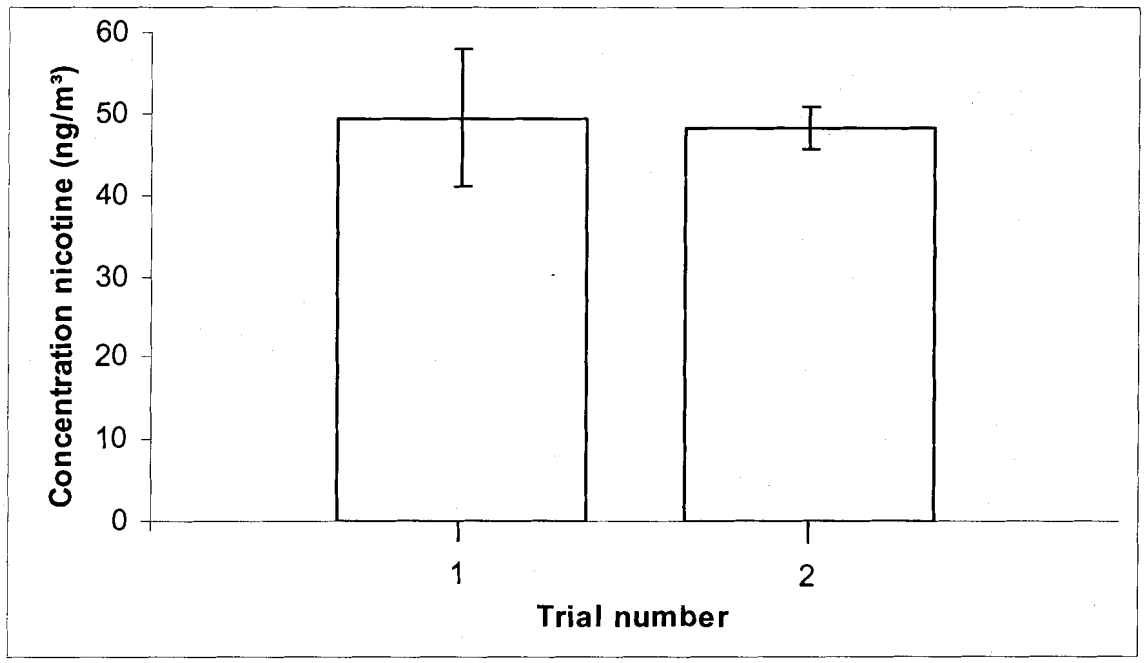
The results indicate that the ETS nicotine in air level measured using the monitors affixed to the casino ceiling did not differ significantly between the two trials. The results also provided evidence consistent with the suspicion that the level of ETS nicotine in air is apparently underestimated when measured using passive monitors affixed to the ceiling. The level of ETS nicotine in air measured using the passive monitors worn attached in the breathing zone of the volunteers was an order of magnitude greater than that measured using monitors affixed to the ceiling although still approximately two orders of magnitude less than what would be expected in an environment where smoking was permitted without any effective restrictions based on

the levels previously published for this type of workplace (Jarvis et al., 1992; Trout et al., 1998).

Table 3: Nicotine in air concentrations calculated from passive monitors on ceiling of casino and from passive monitors in breathing zone of two volunteers

Location of Passive Monitors	Average concentration of nicotine in air (ng/m^3)	Standard error
Monitors from casino ceiling (no smoking restrictions)	48.3	1.3
Breathing zone monitors (no smoking restrictions in casino)	875.1	71.4

Figure 9: Comparison of two separate measurements of the concentration of nicotine in the air in a casino where smoking was not restricted.



As was done in the initial phase of the study, the nicotine levels observed were then used with equations 1, 2 and 3 to calculate daily average dose of nicotine, mean plasma cotinine concentrations and steady state saliva cotinine concentrations. The

results of these calculations are listed in table 4. Again it can be seen that the steady state saliva cotinine levels based on both the monitors affixed to the ceiling and the monitors worn by the volunteers exceed the saliva cotinine concentration *de minimis* risk level for lung cancer and heart disease, but are still less than the *de manifestis* level reported by Repace and Lowrey (1993).

Table 4: Predicted steady state saliva cotinine levels based on average concentration of nicotine in air from passive monitors

Location of passive monitors	Daily average dose of ETS nicotine (µg/day)	Mean plasma cotinine concentration (µg/ml)	Steady state saliva cotinine concentration (µg/ml)
Ceiling of casino smoking permitted	0.21	1.91 E-6	2.21 E-6
Breathing zone of volunteers	3.91	3.48 E-5	4.03 E-5

To calculate the theoretical risk levels, the air nicotine levels were substituted into equations 4 and 5 from Repace and Lowrey (1993), giving a risk of lung cancer of 6.4E-6 based on the monitors placed on the ceiling and 1.17E-4 based on the monitors worn by the volunteers. As mentioned previously, risk of death from heart disease can be assumed to be ten times the risk of lung cancer death. This would indicate that while neither the ETS air nicotine levels measured using monitors placed on the ceiling nor those measured using monitors worn by the volunteers exceed the *de manifestis* risk level for lung cancer, the air level measured using monitors worn by the volunteers did exceed the *de manifestis* level for risk of death from cardiovascular disease.

The results of the analysis of the saliva collected from the volunteers for nicotine and cotinine during this phase are illustrated in figures 10 and 11. As can be seen in the figures, both the measurements of nicotine and cotinine in saliva are inconsistent with what would be expected. In neither case was the initial cotinine or nicotine level near zero nor did it return to near zero towards the end of the sample collection period. Given that the initial measurements of nicotine and cotinine are not consistent with the expected levels and the problems with the analysis, it is difficult to draw any substantive inferences from the results.

Figure 10: Average nicotine in saliva over time for two volunteers exposed to ETS in a casino lacking smoking restrictions

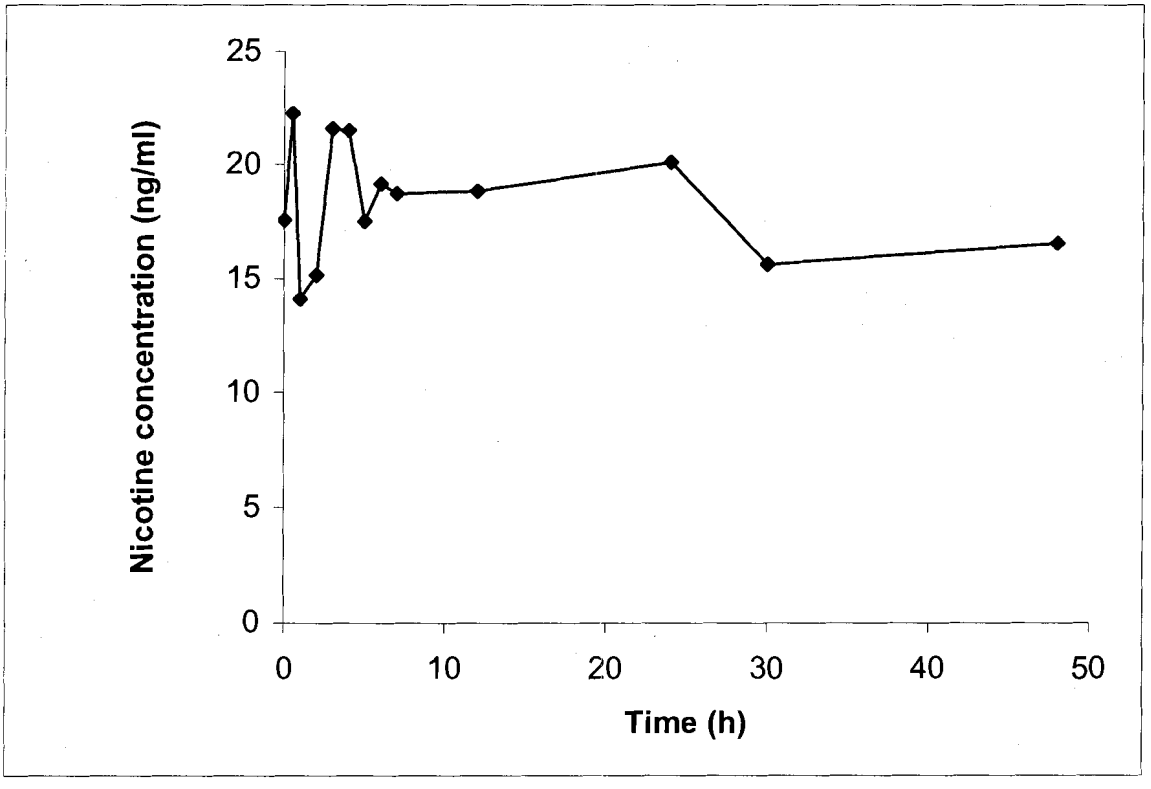


Figure 11 Average cotinine in saliva over time for two volunteers exposed to ETS in a casino lacking smoking restrictions

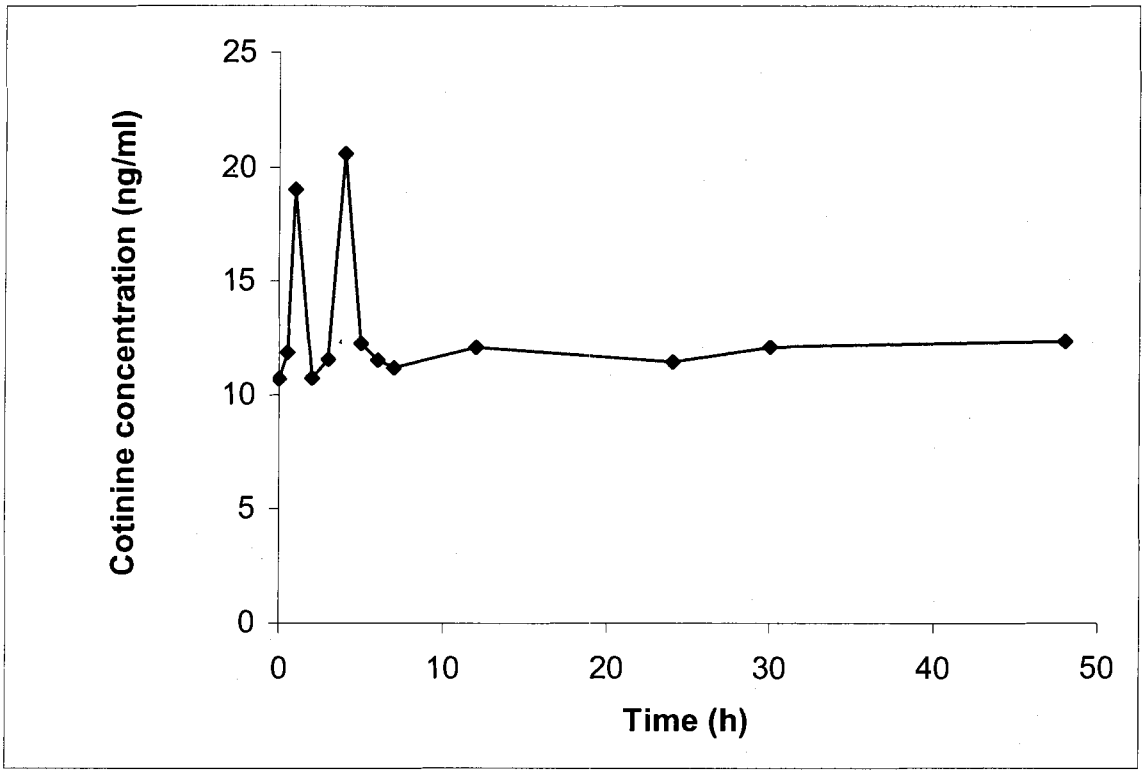
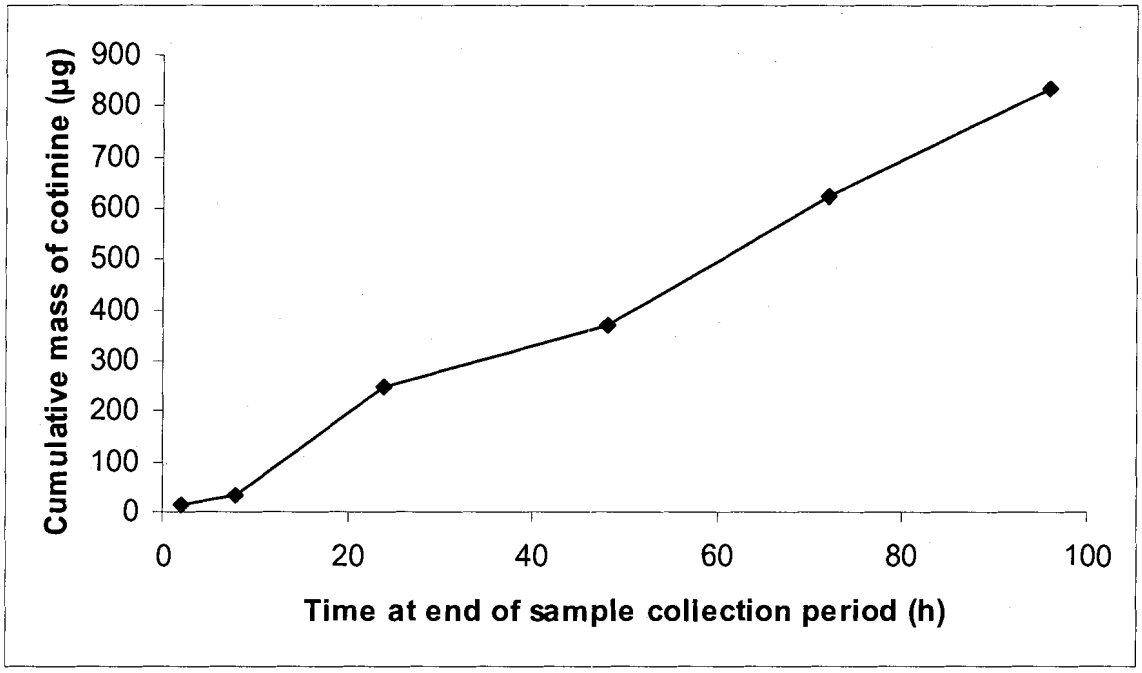


Figure 12 illustrates the average cumulative cotinine in urine over time, although as for the analysis of cotinine and nicotine in saliva, there were also problems with the analysis for cotinine in urine using the GC/MS. In several cases no peaks were obtained in the sample analysis so cotinine in those samples could not be quantified. While the extraction used for the analysis of the filters from the passive monitors was modified for use in the analysis of the urine and saliva samples for cotinine and nicotine, it did not yield suitable results. The problem appeared to stem, at least in part, from a lack of sensitivity of the GC/MS at the time of analysis.

Figure 12: Average cumulative cotinine in urine over time from two volunteers exposed to ETS in a casino lacking smoking restrictions



Human ETS Exposure Trial in a Bingo Smoking Room

Saliva and urine were analysed using radioimmunoassay for concentration of nicotine and cotinine. The results of the analysis of nicotine in the saliva of the 6 volunteers are presented in figures 13 to 18. The results of the analysis for cotinine in saliva over time are presented in figures 19 to 24.

Figure 13: Volunteer a concentration nicotine in saliva over time

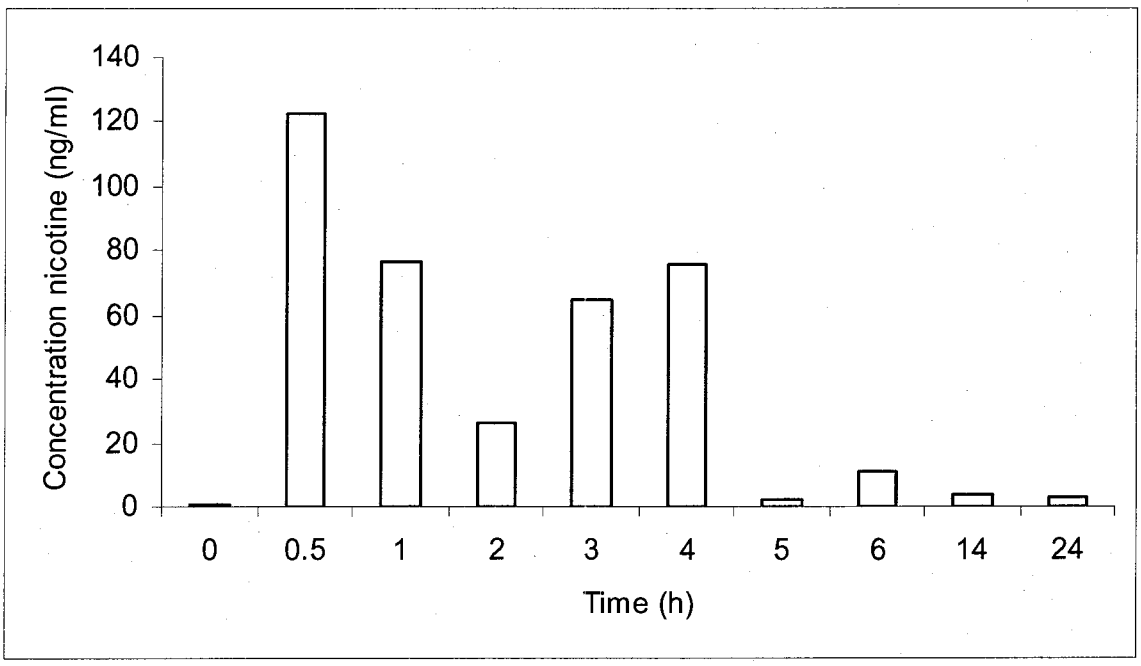


Figure 14: Volunteer b concentration nicotine in saliva over time

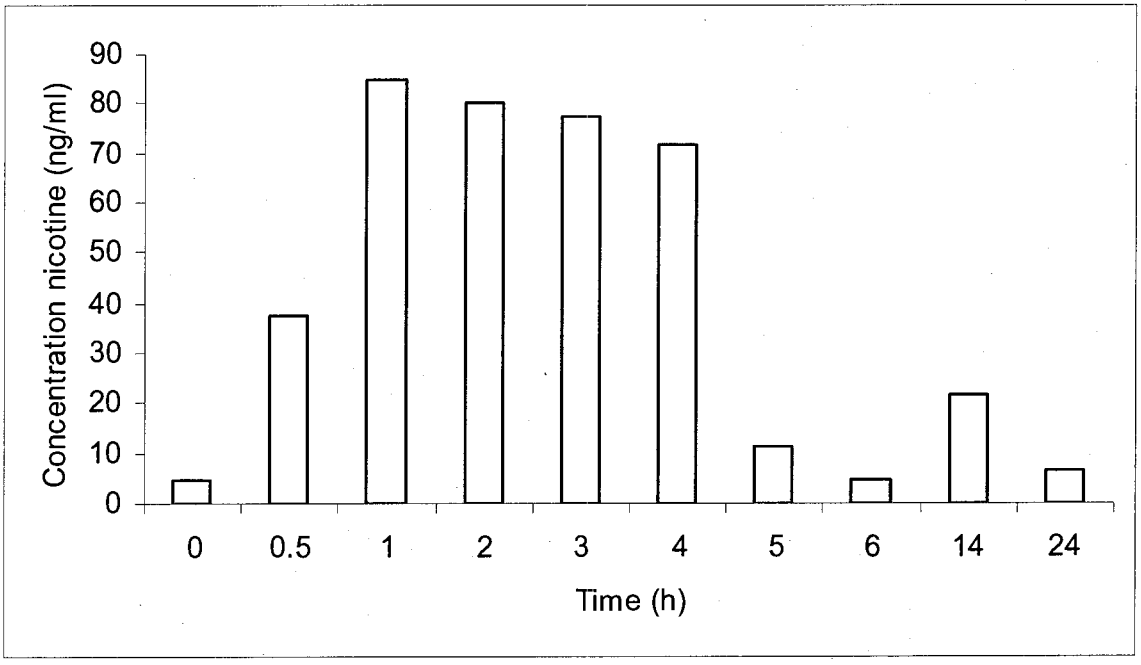


Figure 15: Volunteer c concentration nicotine in saliva over time

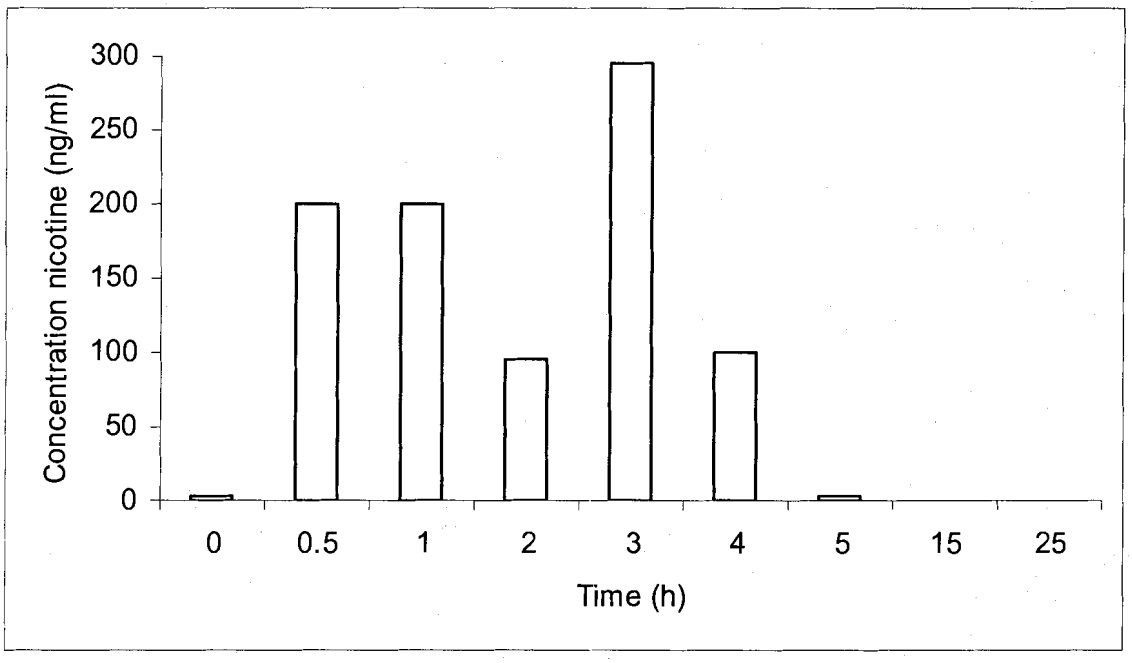


Figure 16: Volunteer d concentration nicotine in saliva over time

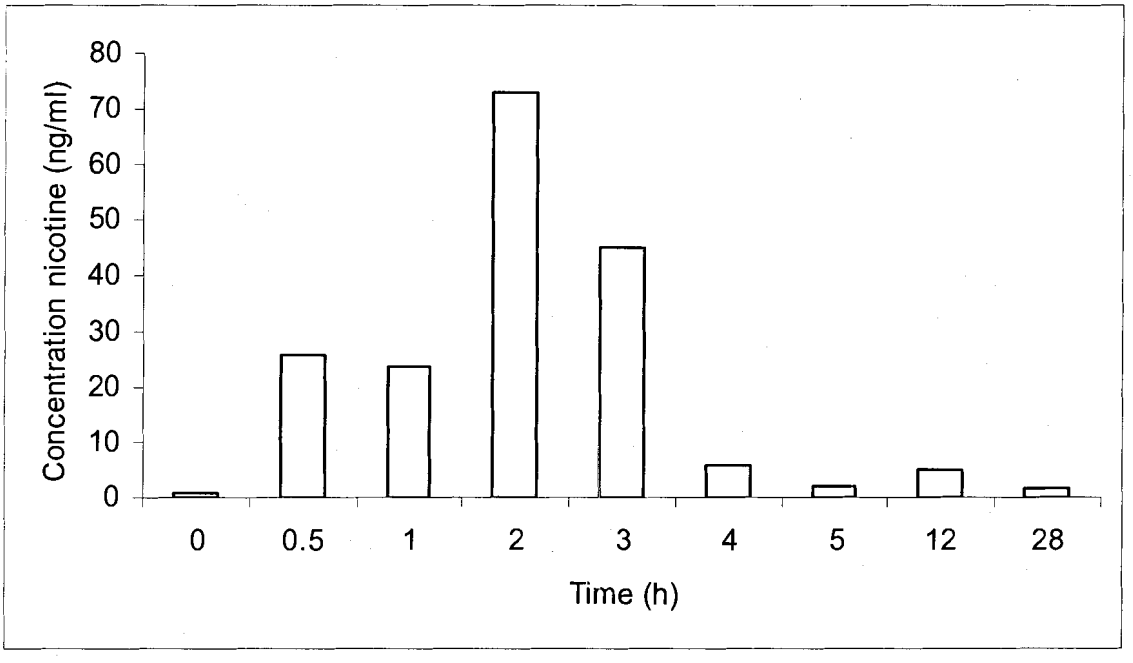


Figure 17: Volunteer e concentration nicotine in saliva over time

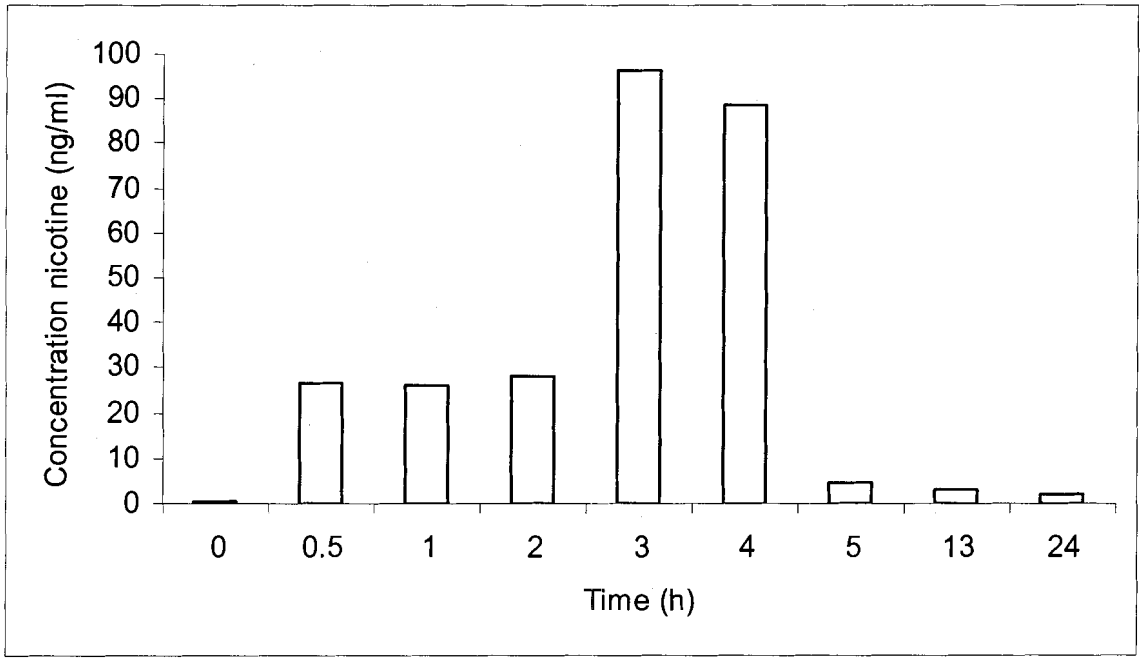


Figure 18: Volunteer f concentration nicotine in saliva over time

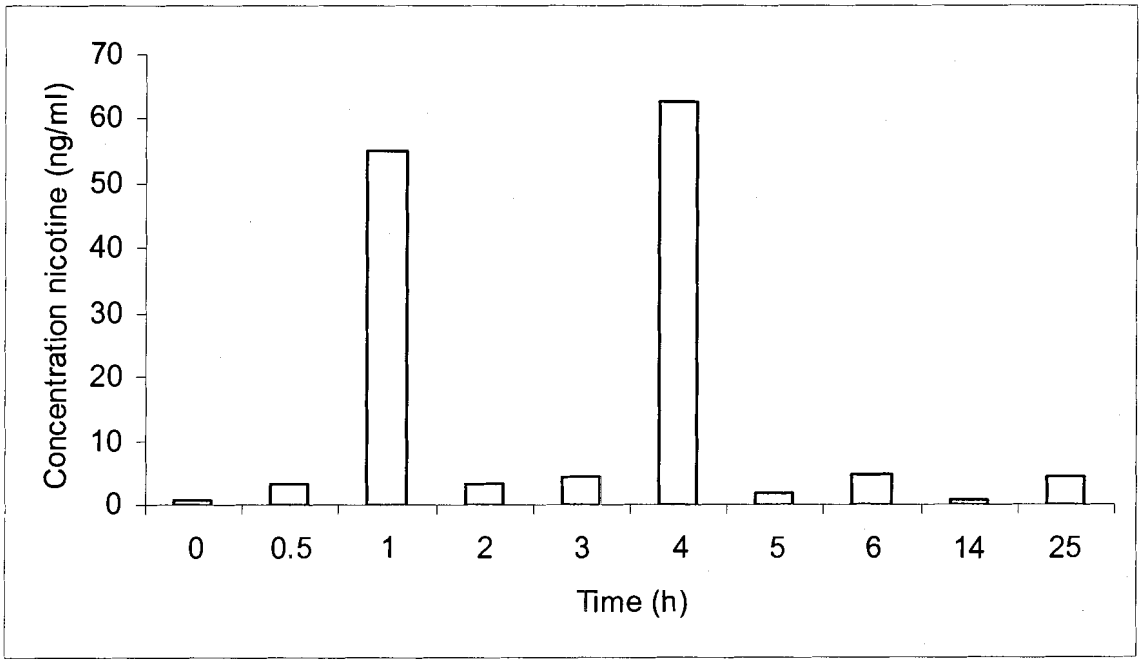


Figure 19: Volunteer a concentration cotinine in saliva over time

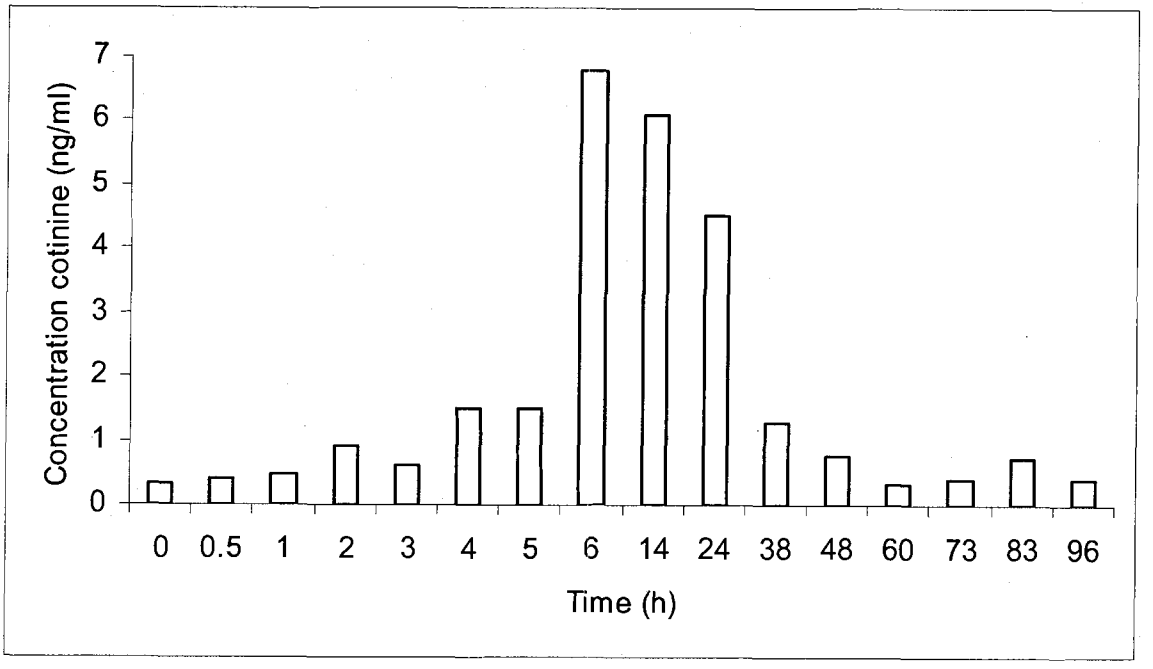


Figure 20: Volunteer b concentration cotinine in saliva over time

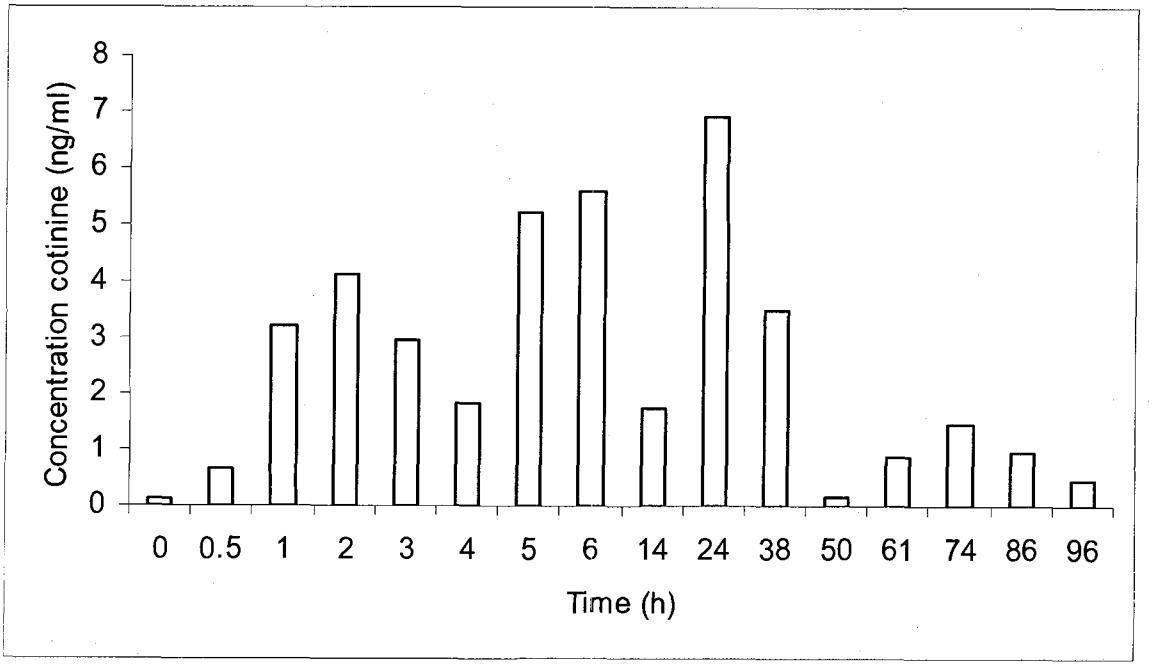


Figure 21: Volunteer c concentration cotinine in saliva over time

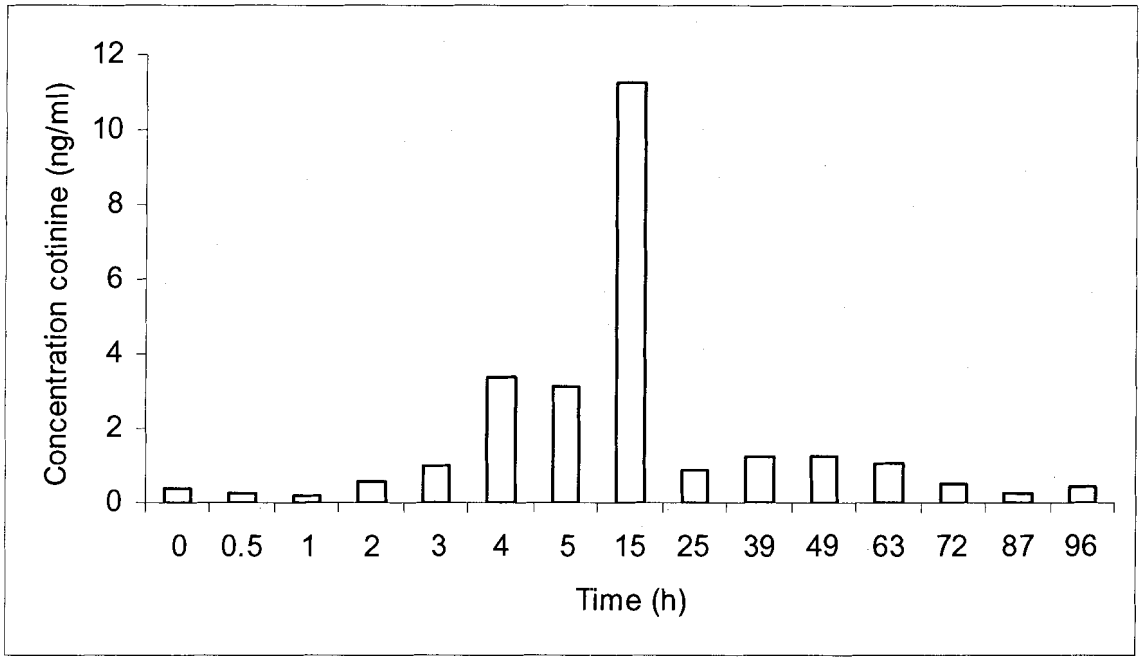


Figure 22: Volunteer d concentration cotinine in saliva over time

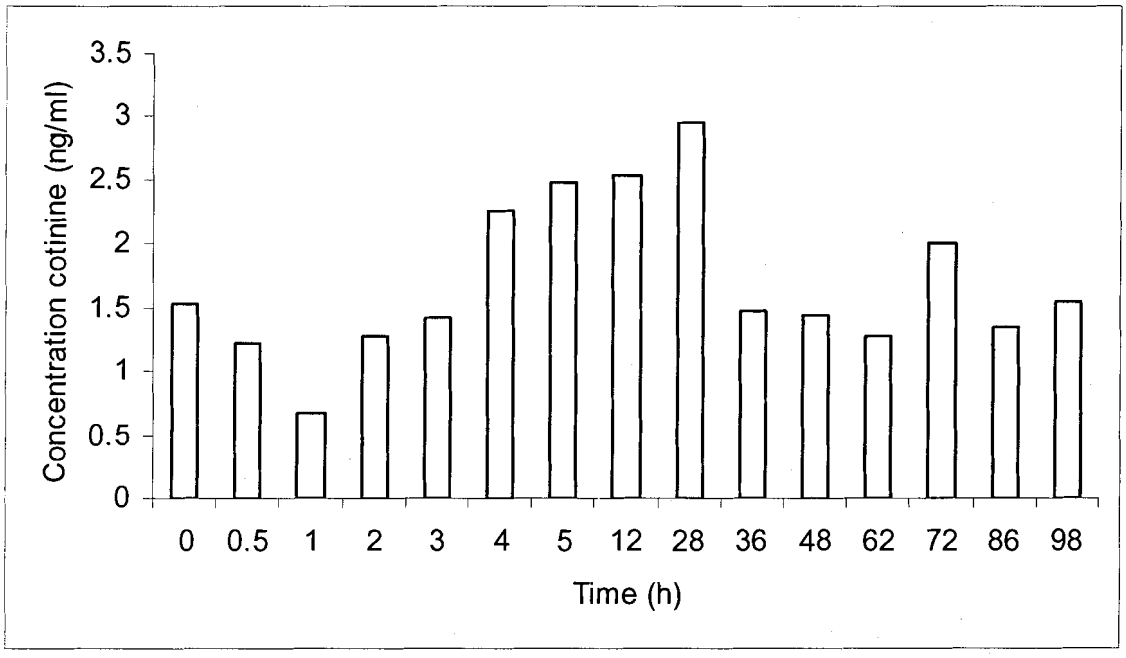


Figure 23: Volunteer e concentration cotinine in saliva over time

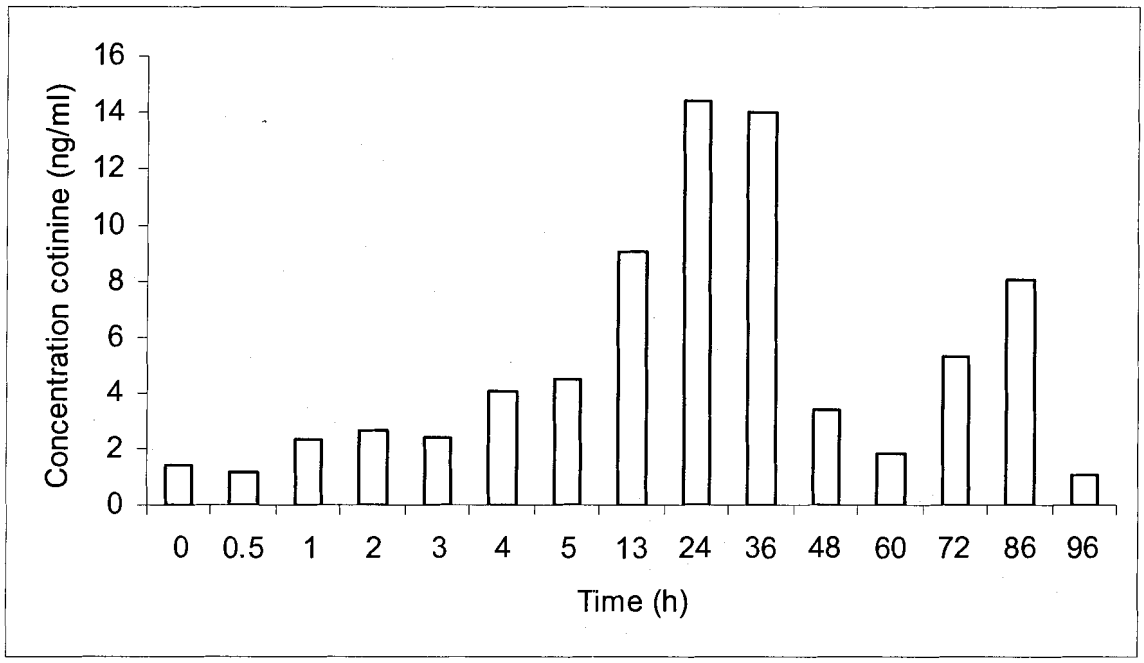
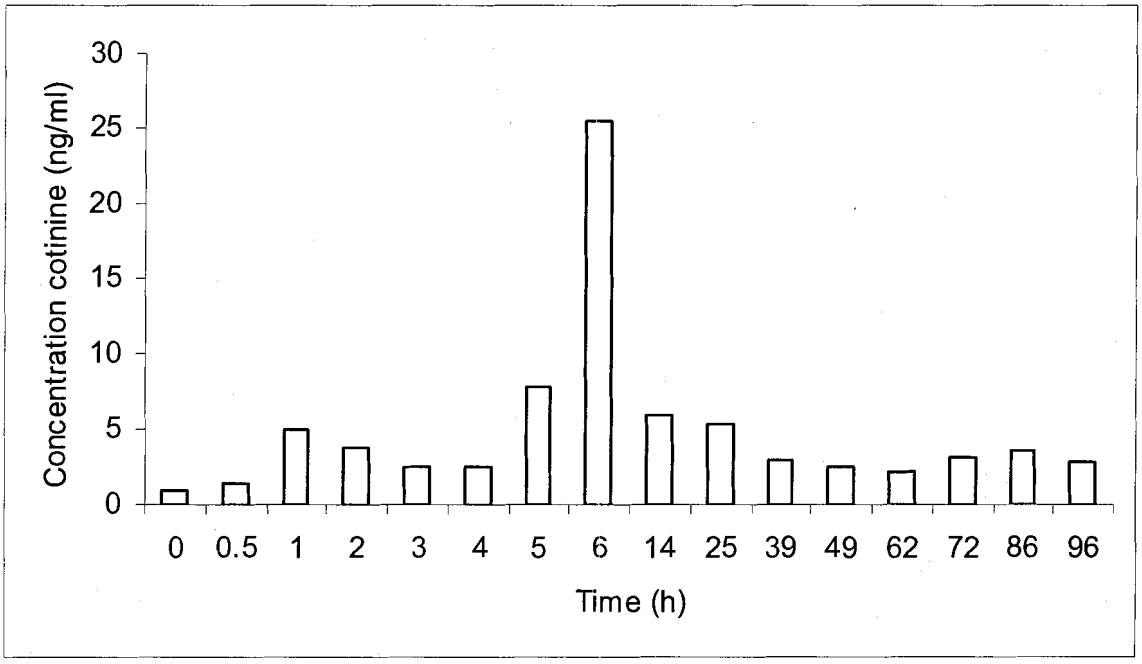


Figure 24: Volunteer f concentration cotinine in saliva over time



In all cases, the nicotine and cotinine levels start at or near zero. This confirms both the non-smoking status of the volunteers as well as the lack of recent ETS exposure prior to the commencement of the planned exposure period as a part of this phase of the study. As can be seen in the figures, there is a large degree of individual variability in the results for both nicotine and cotinine in saliva over time. This is consistent with the findings of Jenkins and Counts (1999). While there is individual variability, when the salivary concentration of cotinine and nicotine over time amongst the volunteers is averaged, the plots are in keeping with the cotinine and nicotine levels over time that would be expected based on what has been observed in previous studies where nicotine was either injected or ingested (Curvall et al., 1990; De Schepper, Van Hecken, Daenens, and Van Rossum, 1987; Robinson, Balter, and Schwartz, 1992; Benowitz, Kuyt, Jacob, Jones, and Osman, 1983; Benowitz and Jacob, 1993). As seen in figure 25, the average concentration of nicotine in saliva for the 6 volunteers has an initial rapid increase corresponding with the start of exposure to second hand smoke and a subsequent decline in nicotine level over a relatively short period of time. This is consistent with what would be expected given the short half-life of nicotine in the body of approximately two hours (Benowitz and Jacob, 1993). The average concentration of nicotine over time appears to have two peaks with a slight decline in between. This may be explained by the brief intermission that occurred in the bingo play while the volunteers were in the smoking room. During this intermission, many of the smokers in the smoking room left to visit the washroom or concession. During a previous visit to the bingo hall, it was noted that some smokers also went outside during the intermission to get some fresh air.

With respect to cotinine concentrations, figure 26 illustrates the change in average cotinine concentration in saliva of the six volunteers over time subsequent to

initiation of exposure to second hand smoke. In this case there is a slightly delayed increase in cotinine level after the initial exposure to second hand smoke and a much slower decline in cotinine levels subsequent to termination of exposure. This is consistent with the time for metabolism of nicotine to produce the cotinine resulting in a delay in the onset of the increase of cotinine. The slower decline in cotinine level is consistent with the longer half-life of cotinine of approximately 18 -20 hours (Benowitz and Jacob, 1993; Benowitz et al., 1983). As was the case with the plot of average nicotine concentration in saliva, there are two peaks in the average cotinine concentration in saliva, with the timing of these peaks appearing to coincide with the intermission in bingo play. The apparent presence of two peaks with only a slight decline in cotinine levels is also consistent with the longer half-life of cotinine in that there would be less time for cotinine levels to decline compared to what was observed with the nicotine levels given the relatively short duration of the break.

Figure 25: Average concentration nicotine in saliva over time

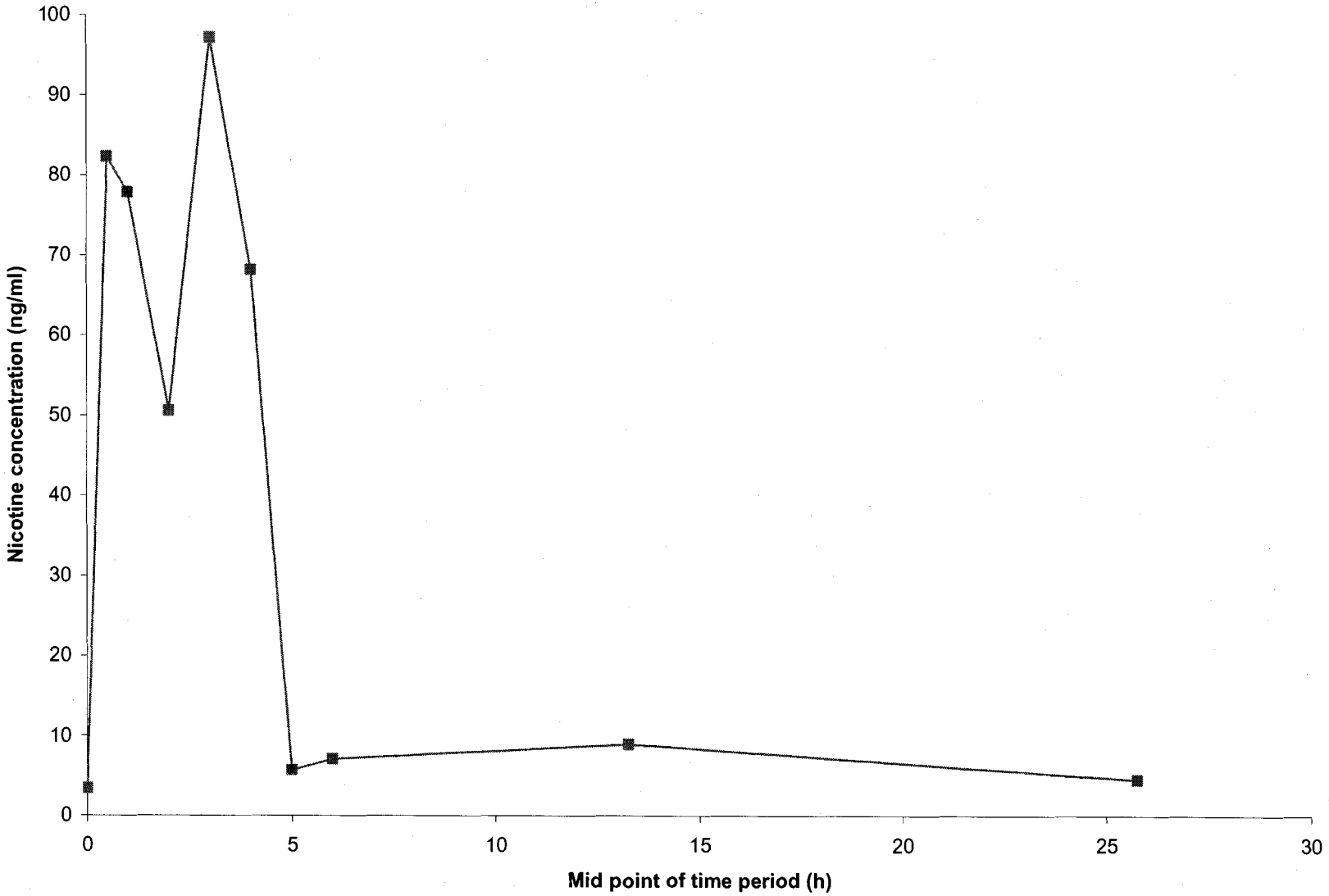
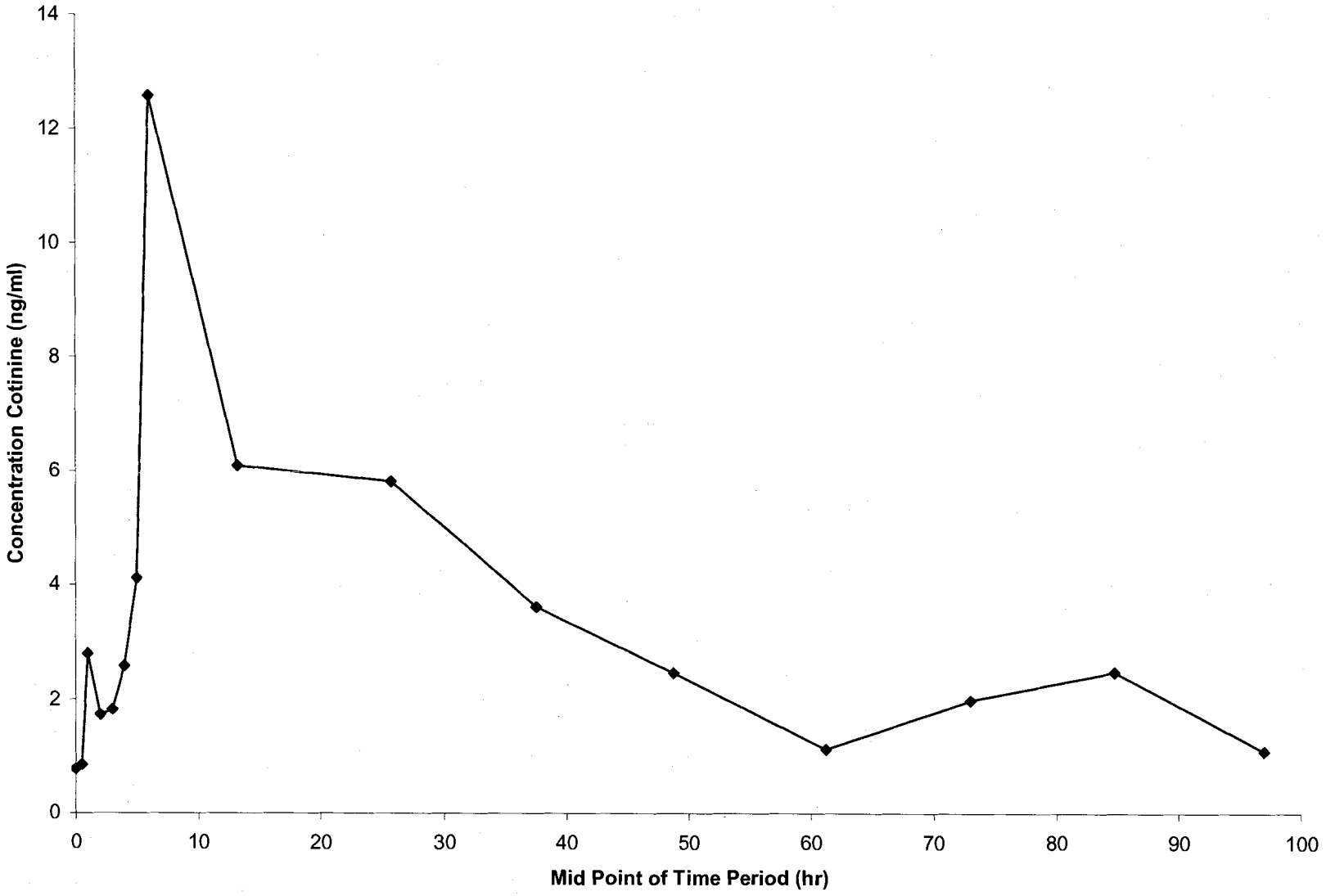

Nicotine concentration (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Mid point of time period (h)

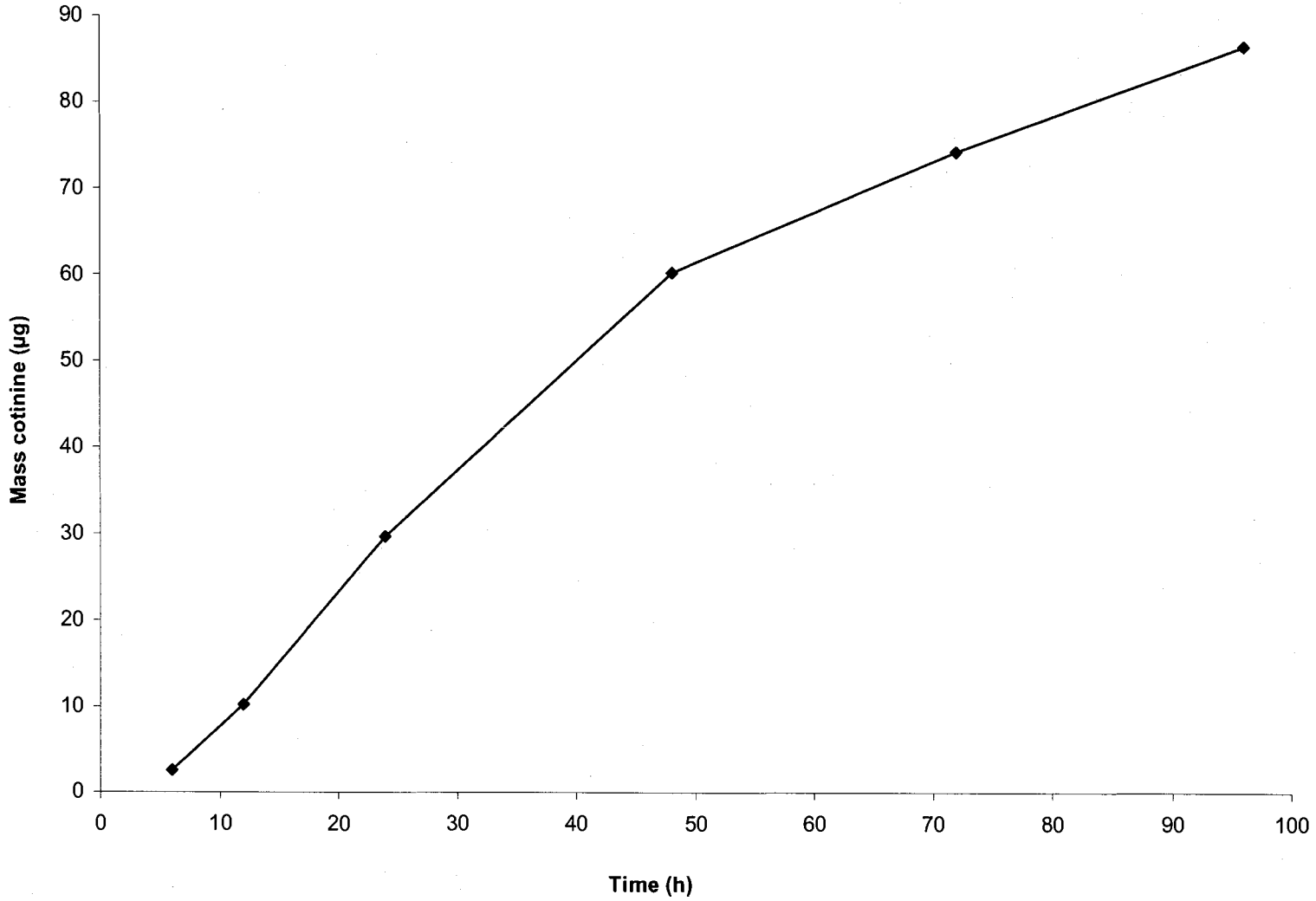
Figure 26: Average concentration cotinine in saliva over time


Concentration Cotinine (ng/ml)
0
2
4
6
8
10
12
14
0
10
20
30
40
50
60
70
80
90
100
Mid Point of Time Period (hr)

As can be seen in figure 26, the time of sample collection for the determination of cotinine concentration is quite important when risk is to be calculated. If the level of cotinine in the blood were to not reach steady state as a result of the normal pattern of exposure for the person being tested, there could be a fairly large difference in the risk that will be calculated depending on the timing of the sample collection relative to exposure to ETS. Of course this point becomes moot if the cotinine level were to reach steady state as may be the case with repeated exposures of relatively longer duration. While exposure may be sufficient in both length and frequency in some provinces and states across North America, in British Columbia employers are required to ensure their employees' length of exposure in the smoking room is no more than 20% of their shift which further complicates the situation (WCB, 2003).

Figure 27 illustrates the average cumulative cotinine in urine from the start of the exposure to 4 days (96 hours) after the initiation of exposure. As can be seen in the figure, the rate of increase in cumulative level of cotinine in urine commences with a rather gradual incline. This is consistent with what would be expected as the amount of nicotine is just beginning to build up. Once the amount of nicotine absorbed into the body has increased given the relatively short half-life of nicotine, and much longer half-life of cotinine, it would be expected that the amount of cotinine would begin to build up faster. As expected, figure 27 shows the rate at which the cumulative cotinine is found to increase begins to be more rapid as both the amount of nicotine absorbed begins to increase thus allowing for its metabolism to cotinine, and the longer half-life of cotinine dictates that the cotinine is not further metabolised or eliminated as quickly. As time progresses, and exposure ends, the rate of increase in cumulative cotinine then begins to slow. As the amount of nicotine remaining to be metabolised decreases, the rate of

Figure 27 Average cumulative cotinine in urine over time


Mass cotinine (µg)
0
10
20
30
40
50
60
70
80
90
0
10
20
30
40
50
60
70
80
90
100
Time (h)

increase in cumulative cotinine is not as great. The rate of increase is still increasing as the longer half-life of cotinine as compared to nicotine, dictates a net build up of cotinine.

In the last phase of the study, there was also an attempt to measure the concentration of nicotine in air so that exposure as measured by the air concentration of nicotine could be compared with the nicotine in blood as measured using the nicotine in saliva concentrations. The nicotine in air was collected using the passive monitors as had been used in the two previous phases of the study. Results of the analysis of the filters from the passive monitors worn by the volunteers in the bingo smoking room are not reported. All attempts to analyse the filters were unsuccessful and the author was unable to get any of the methods attempted to produce a suitable standard curve which would allow the work to proceed to the analysis of the filters. Initial attempts were conducted using the radioimmunoassay for the analysis of nicotine on the filters. This method had not been reported previously as having been used for this type of analysis. Contact with the author further confirmed that to her knowledge filters from air monitoring had not been analysed using radioimmunoassay. While she did suggest that the method used by Klein and Koren (1999) to analyse hair for nicotine might be able to be modified for use with filters from air sampling, she did however also indicate that there could be problems with interference with the buffer or other parts of the immunoassay that would prevent the analysis from being completed successfully. The analysis was attempted using the radioimmunoassay method that had been used for this phase to quantify the nicotine in saliva. The aqueous solution from the first part of the extraction of the filters was analysed for nicotine using the same protocol for radioimmunoassay that was used to analyse saliva for nicotine. The initial attempts proved fruitless. Attempts to modify this protocol similar to what had been done to analyse for nicotine in hair and as well as to try to prevent potential interference with the buffer by the sodium

bisulfate on the treated filter papers also proved fruitless as did another trial using the full extraction of the nicotine into the organic solvent and then extracting back into aqueous solution prior to analysis. In all cases, there was an inability to produce a consistent recovery of nicotine.

Trials to analyse the filters from the passive monitors using the original method for filter analysis from the first two phases of the study also proved unsuccessful in obtaining a consistent recovery of nicotine from the filter papers. In this case the difficulty may have been from the use of nicotine-d_3 salicylate salt instead of the pure methyl-d_3 nicotine as was used in phase one and two may have been the route of the problem. Another potential cause of a lack of acceptable results in the analysis of the filters in this phase of the project could have been insufficient trials with minor changes to the method to get adequate results.

While the monitors weren't analysed and thus did not allow for a direct analysis of the amount nicotine in the air in the Bingo smoking room and thus of the volunteers exposure to nicotine compared with the amount of nicotine that was absorbed by the volunteers based on the nicotine and cotinine levels in their saliva, it is still possible to compare the average cotinine level found in this portion of the study with the levels set as the *de minimis* and *de manifestis* risk levels for lung cancer and heart disease. It is also possible to calculate the amount of nicotine in the air based on the saliva levels of either cotinine or nicotine. From this information, it is also possible to calculate the risk of lung cancer and heart disease that that would correlate with that level of exposure on an ongoing basis.

De minimis and *de manifestis* risk levels for lung cancer based on Repace et al. (1998) are 0.4 pg/ml and 0.14 ng/ml respectively and heart disease mortality risk levels are about one tenth as much 0.04 pg/ml and 0.014 ng/ml. The peak average level of

cotinine (at 6 hours) measured in this portion of the study was 12.6 ng/ml. This clearly exceeds the *de manifestis* risk level for lung cancer and heart disease. In addition, this level exceeds the 0.4 and 0.04 ng/ml levels for lung cancer and heart disease respectively that would correspond with the significant risk or regulatory action level of 1 death in 1000 workers that is frequently used by OSHA. This does however assume that the peak average level used for this comparison, is representative of the cotinine level that would end up as the steady state level given regular daily exposure for the full work shift. Using equations 1 through 3 from the initial phase of the study, and again assuming the peak average cotinine level corresponds with the steady state cotinine level, the level of nicotine in air can be calculated to be 273.42 $\mu g/m^3$. Consequently, substituting the level of nicotine in air into equations 4 and 5 to calculate intake rate and risk of lung cancer, a relative risk value of 0.109 is obtained. The concentration of nicotine in air and consequently the calculated risk of lung cancer are 3 orders of magnitude greater than the concentration of nicotine in air that was observed in the casino during the human exposure trial, as well as the subsequent level of risk calculated for that level of nicotine. Since all persons in the smoking room of the bingo (other than the volunteers for this study) were smoking it is likely that the concentration of nicotine in air that the volunteers were exposed to in the bingo was greater than that which was present in the casino. It is however unlikely that it was actually 1000 times greater. Part of this discrepancy may be explained by the fact that the level of nicotine found in the air in the casino, as mentioned previously, is believed to be on the low side. That the level of nicotine in the air measured in the casino was on the low side is confirmed, at least in part, by the fact that the average cotinine in saliva level that was used to calculate the theoretical nicotine in air concentration in the bingo smoking room is within the range seen previously for non-smokers exposed to high levels of ETS, and the concentration of nicotine in air calculated from the salivary cotinine level is consistent

with the levels seen previously (Jarvis et al., 1992; Repace and Lowery, 1993; Maskarinec, Jenkins, Counts, and Dindal, 2000). The average salivary cotinine level was also less than the level that has been used previously to discriminate between nonsmokers and occasional smokers, 15 ng/ml (Etzel, 1990).

This leaves the calculation of risk as the source of a potential problem. Given the concentration of nicotine in air that was calculated, a risk of 1 lung cancer death per 10 persons exposed to ETS at the level calculated for the Bingo smoking room seems high. Repace and Lowrey (1993) had previously estimated that for the "most exposed" non-smokers (having a cotinine in plasma concentration of 10 ng/ml) risk was in the order of 2 lung cancer deaths per 100 persons exposed at that level. This points to the possibility that the rate of increase in risk of lung cancer and heart disease decreases with increasing ETS exposure. This would be consistent with the contention of Kiyohara et al. (2003) who suggest that as the amount of exposure to the chemicals in tobacco smoke increases, and one goes beyond low-dose exposure, the effect of phenotype or genotype becomes less evident. This may thus be a similar non-linear dose-response relationship to that contented by Law et al. (1997) with respect to the risk of ischaemic heart disease caused by exposure to second hand smoke being almost half that of smoking 20 cigarettes per day while the level of exposure is only about 1% of smoking. At lower doses those that are genetically most susceptible illustrate the negative effect under consideration, as the dose increases, those that are more susceptible are now included as a part of the over all response and what was initially a dramatic rate of increase in the negative effect slows.

CONCLUSIONS

The level of air nicotine that was observed through the use of the passive monitors mounted on the ceiling of the casinos, particularly in the casino where smoking was permitted, was almost three orders of magnitude less than expected based on previous studies where the concentration of nicotine in air in this type of environment had been measured with personal breathing zone monitors. The large discrepancy was most likely due to the use of locations on the ceiling and high up on walls as the sites to mount the passive monitors to avoid tampering vs. the use of locations in the facilities that were more representative of the breathing zone. This theory was supported with the results of the trial involving ETS exposure of volunteers wearing passive monitors conducted simultaneous with passive air monitoring of nicotine with the monitors placed on or near the ceiling. While the nicotine level that was measured using the passive monitors affixed to the ceiling was very similar between the two phases, the level measured using the passive monitors attached to the volunteers in their breathing zone was much greater, and more consistent with the nicotine level that could be expected based on the levels found in other studies. The importance of ensuring that passive monitors are placed in the breathing zone was clearly illustrated by these results.

While the level of nicotine in the air measured using passive monitors affixed to the ceiling or the wall at ceiling height was less than would be expected, a trend was apparent. The level of nicotine in the air was observed to be significantly less in the casinos where smoking was restricted to a structurally separate, separately ventilated smoking room or not permitted within the establishment at all than the level that was observed in the casino where smoking was not restricted. Although a trend was

observed, firm conclusions can not be drawn from these results due to the aforementioned issue with placement of the monitors on the ceiling and near the ceiling on the walls of the facilities being studied– rather than within the breathing zone. Future comparisons of different levels of restrictions on indoor smoking in order to determine the benefits with respect to risk of negative outcomes, and based on measurement of nicotine levels, should be done with all airborne nicotine measurements performed in the breathing zone. An alternative to this would be to conduct further studies to see if a consistent relationship could be observed between nicotine levels when measured near the ceiling of a room and the corresponding level measured in the breathing zone.

In the trial with urine and saliva samples collected from volunteers exposed to ETS in the bingo smoking room, there was a fair degree of variation among the various volunteers. This was not unexpected and is consistent with the findings of Jenkins and Counts (1999). As outlined by Benowitz and Jacob (1987), the rate of nicotine excretion is dependent on the acidity of urine with the rate being higher in acidic urine due to re-absorption. Differing rates of nicotine excretion would affect both nicotine and cotinine levels. This illustrates the importance of having samples from a group of exposed individuals and drawing conclusions based on average levels of such a group rather than making any conclusions based on individual nicotine or cotinine levels.

In the present study, cotinine concentrations were examined after a single 4 hour exposure to ETS. While this length of exposure was sufficient to observe a noticeable change in cotinine levels beyond baseline levels, more work is needed to look at cotinine concentrations after repeated regular exposure to ETS in order to determine if the time of sample collection is significant with respect to determining the risk of various negative outcomes based on concentration of cotinine in saliva. In addition, given the effects that were under consideration (lung cancer and cardiovascular disease) are as a result of

chronic exposure to ETS, it would be important to examine the trend in cotinine levels in those people with repeated exposures to ETS and in particular to determine if cotinine levels reached steady state and if so what was the steady state level of cotinine in order to have a clearer picture of the risk of long term consequences from their exposure.

The present study also illustrated the need for further work on the calculation of risk based on the cotinine levels of those exposed. Using the formulae developed by Repace et al. (1998) a level of risk of lung cancer of 1 in 10 was calculated based on the peak average salivary cotinine level. This is in excess of the level of risk cited by Repace and Lowery (1993) of 2 lung cancer deaths per 100 persons for the most heavily exposed non-smokers. It appears likely that there is a non-linear dose response relationship with respect to exposure to ETS and negative health outcomes, or at least with respect to the outcome of death from lung cancer or heart disease. Future studies should examine more closely the relationship between level of exposure to ETS and risk of lung cancer or heart disease death as exposure approaches the maximum that is to be expected for those passively exposed.

APPENDICES

Appendix A – Volunteer Consent Form for Human Exposure to ETS in a Casino Lacking Smoking Restrictions

SIMON FRASER UNIVERSITY

INFORMED CONSENT BY SUBJECTS TO PARTICIPATE IN A RESEARCH PROJECT OR EXPERIMENT

The University and those conducting this project subscribe to the ethical conduct of research and to the protection at all times of the interests, comfort, and safety of subjects. This form and the information it contains are given to you for your own protection and full understanding of the procedures. Your signature on this form will signify that you have received a document which describes the procedures, possible risks, and benefits of this research project, that you have received an adequate opportunity to consider the information in the document, and that you voluntarily agree to participate in the project.

Any information that is obtained during this study will be kept confidential to the full extent permitted by law. Knowledge of your identity is not required. You will not be required to write your name or any other identifying information on the research materials. Materials will be held in a secure location and will be destroyed after the completion of the study. However, it is possible that, as a result of legal action, the researcher may be required to divulge information obtained in the course of this research to a court or other legal body.

Having been asked by Dr. Francis Law of the Dept of Biological Sciences of Simon Fraser University to participate in a research project experiment, I have read the procedures specified in the document.

I understand the procedures to be used in this experiment and the personal **risks are minimal** to me in taking part.

I understand that I may withdraw my participation in this experiment at any time.

I also understand that I may register any complaint I might have about the experiment with the researcher named above or with the Chairman of the Dept. of Biological Sciences of Simon Fraser University

I may obtain copies of the results of this study, upon its completion, by contacting:
Dr. Francis Law

I have been informed that the research material will be held confidential by the Principal Investigator.

I understand that my supervisor or employer may require me to obtain his or her permission prior to my participation in a study such as this.

I agree to participate by:

wearing a passive nicotine monitor on my collar and remaining in a casino where public cigarette smoking is allowed for 5 hours. I will provide saliva and urine samples to the researcher as described in the document referred to above,during the 5-hr exposure period·and the following 3 days.

NAME (please type or print legibly): _____

ADDRESS: _____

_____

SIGNATURE: _____ **WITNESS:** _____

DATE: _____

ONCE SIGNED, A COPY OF THIS CONSENT FORM AND A SUBJECT FEEDBACK FORM SHOULD BE PROVIDED TO THE SUBJECT.

Appendix B – Volunteer Information Sheet for Human Exposure to ETS in a Casino Lacking Smoking Restrictions

SIMON FRASER UNIVERSITY

INFORMATION SHEET FOR SUBJECTS

This form describes the proposed tests involving physical, psychological, or any other invasive testing.

Title of Project: **Assessing Exposure of Non-Smokers to Environmental Tobacco in Public Places of Vancouver**

Description of the procedures to be followed and a statement of the risks to the subjects and benefits of the research.

Six healthy male non-smokers between 20-35 years old will be recruited for the exposure study. Non-smokers are defined as those who reported no smoking of cigarettes, pipes or cigars and do not live with someone who smokes cigarettes, pipes or cigars. One sample each of saliva and urine will be collected from the volunteers immediately before the study.

The volunteer will be sent to a bingo hall or casino where public cigarette smoking is allowed and remained there for 3-5 hr. A passive monitor for nicotine will be clipped to the collar near the breathing zone of each volunteer. At different time points during and after environmental tobacco smoke exposure (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 30, 48, 54, 72, 80, and 96 hr) a saliva sample (2 ml) will be collected by asking the volunteer to spit into labelled sample vials. Urine will be collected for 72 hr over the following time intervals: 0-2, 2-8, 8-24, 24-48, and 48-72. The volume of the urine samples will be measured and aliquots (20 ml) will be taken and stored at –20 ^{o}C.

The proposed study will be conducted concurrently to an environmental tobacco smoke monitoring study supported by the Vancouver/Richmond Health Board. The study will help to decrease the uncertainties involved in assessing the exposure doses of non-smokers to tobacco smoke, thus providing a more accurate estimation of lung cancer risks from chronic exposure. The risks to the experimental subjects are minimal.

Appendix C – Subject Feedback Form for Human Exposure to ETS in a Casino Lacking Smoking Restrictions

SIMON FRASER UNIVERSITY
UNIVERSITY RESEARCH ETHICS REVIEW COMMITTEE

SUBJECT FEEDBACK FORM

Completion of this form is **OPTIONAL**, and is not a requirement of participation in the project. However, if you have served as a subject in a project and would care to comment on the procedures involved, you may complete the following form and send it to the Chair, University Research Ethics Review Committee. All information received will be treated in a strictly confidential manner.

Name of Principal Investigator: Dr. Francis Law

Title of Project: Assessing Exposure of Non-Smokers to Environmental Tobacco Smoke in Public Places in Vancouver

Dept./School/Faculty: Biological Sciences

Did you sign an Informed Consent Form before participating in the project? ____

Were there significant deviations from the originally stated procedures? _____

I wish to comment on my involvement in the above project which took place:

_____
(Date) (Place) (Time)

Comments: _____

_____

_____

_____

_____

_____

Completion of this section is optional

Your name: _____

Address: _____

Telephone: (w)_____ (h)_____

This form should be sent to the Chair, University Research Ethics Review Committee, c/o Office of the Vice-President, Research, Simon Fraser University, Burnaby, BC, V5A 1S6.

Appendix D – Guide for Suggested Saliva and Urine Sampling Times

Saliva sampling times

Sample time (t + x hrs)	Projected sample time	Actual sample time
Baseline sample (t - x hrs)	Prior to start of experiment	
0.5	7:30 p.m. Sept. 14	
1	8	
2	9	
3	10	
4	11	
5	12 a.m. Sept. 15	
6	1	
7	2	
8	3	
12	7	
24	7 p.m.	
30	1 a.m. Sept. 16	
48	7 p.m.	
54	1 a.m. Sept. 17	
72	7 p.m.	
80	1 a.m. Sept. 18	
96	7 p.m.	

t = start time of 7:00 p.m. September 14, 2001

Urine Sample Times

Sample time (t + x hrs)	Projected sample time	Actual sample time
Baseline Sample (t – x hrs)	Prior to start of experiment	
0-2	7-9 p.m. Sept. 14	
2-8	9 p.m. - 3 a.m. Sept. 15	
8-24	3 a.m. - 7 p.m. Sept. 15	
24-48	7 p.m. Sept. 15 - 7 p.m. Sept. 16	
48-72	7 p.m. Sept. 16 - 7 p.m. Sept. 17	

t = start time of 7:00 p.m. September 14, 2001

Appendix E – Volunteer Consent Form for Human ETS Exposure in the Smoking Room of a Bingo

SIMON FRASER UNIVERSITY

INFORMED CONSENT BY SUBJECTS TO PARTICIPATE IN A RESEARCH PROJECT OR EXPERIMENT

The University and those conducting this project subscribe to the ethical conduct of research and to the protection at all times of the interests, comfort, and safety of subjects. This form and the information it contains are given to you for your own protection and full understanding of the procedures. Your signature on this form will signify that you have received a document which describes the procedures, possible risks, and benefits of this research project, that you have received an adequate opportunity to consider the information in the document, and that you voluntarily agree to participate in the project.

Any information that is obtained during this study will be kept confidential to the full extent permitted by law. Knowledge of your identity is not required. You will not be required to write your name or any other identifying information on the research materials. Materials will be held in a secure location and will be destroyed after the completion of the study. However, it is possible that, as a result of legal action, the researcher may be required to divulge information obtained in the course of this research to a court or other legal body.

Having been asked by Dr. Francis Law of the Dept of Biological Sciences of Simon Fraser University to participate in a research project experiment, I have read the procedures specified in the document.

I understand the procedures to be used in this experiment and the personal **risks are minimal** to me in taking part.

I understand that I may withdraw my participation in this experiment at any time.

I also understand that I may register any complaint I might have about the experiment with the researcher named above or with the Chairman of the Dept. of Biological Sciences of Simon Fraser University

I may obtain copies of the results of this study, upon its completion, by contacting:
Dr.Francis Law

I have been informed that the research material will be held confidential by the Principal Investigator

I understand that my supervisor or employer may require me to obtain his or her permission prior to my participation in a study such as this.

I agree to participate by:

wearing a passive nicotine monitor on my collar and remaining in a smoking room in a bingo hall where public cigarette smoking is allowed for up to 5 hours. I will provide saliva and urine samples to the researcher as described in the document referred to above,during the exposure period·and the following 4 days.

NAME (please type or print legibly): _____

ADDRESS: _____

_____

SIGNATURE: _____ **WITNESS:** _____

DATE: _____

ONCE SIGNED, A COPY OF THIS CONSENT FORM AND A SUBJECT FEEDBACK FORM SHOULD BE PROVIDED TO THE SUBJECT.

Appendix F – Volunteer Information Sheet for Human ETS Exposure in the Smoking Room of a Bingo

SIMON FRASER UNIVERSITY

INFORMATION SHEET FOR SUBJECTS

This form describes the proposed tests involving physical, psychological, or any other invasive testing.

Title of Project: **Assessing Exposure of Non-Smokers to Environmental Tobacco in Public Places of Vancouver**

Description of the procedures to be followed and a statement of the risks to the subjects and benefits of the research.

Six healthy non-smokers between 20-45 years old will be recruited for the exposure study. Non-smokers are defined as those who reported no smoking of cigarettes, pipes or cigars and do not live with someone who smokes cigarettes, pipes or cigars. One sample each of saliva and urine will be collected from the volunteers immediately before the study.

The volunteers will be sent to the smoking room of a bingo hall or casino where public cigarette smoking is allowed and remain there for 3-5 hr. A passive monitor for nicotine will be clipped to the collar near the breathing zone of each volunteer. At different time points during and after environmental tobacco smoke exposure (0.5, 1, 2, 3, 4, 5, 6, 12, 24, 30, 48, 60, 72, 84, and 96 hr) a saliva sample (<2 ml) will be collected by asking the volunteer to place a cotton salivette in their mouth for 2-3 minutes and then deposit the salivette in a plastic tube. Urine will be collected for 96 hr over the following time intervals: 0-6, 6-12, 12-24, 24-48, 48-72 and 72-96. The volume of the urine for the time periods will be measured and aliquots (20-50 ml) for each time period will be stored at –20 °C.

The proposed study will be conducted subsequent to an environmental tobacco smoke monitoring study supported by the Vancouver/Richmond Health Board. The study will help to decrease the uncertainties involved in assessing the exposure doses of non-smokers to tobacco smoke, thus providing a more accurate estimation of lung cancer and cardiovascular disease risks from chronic exposure. The risks to the experimental subjects are minimal.

Appendix G – Protocol for Volunteers for Human ETS Exposure in the Smoking Room of a Bingo

Protocol for Volunteers in ETS Study

Study Information

- Study will be conducted on January 31, 2003 at the Abbotsford Bingo (time in the smoking room will be from approximately 6:00pm-10pm) with collection of saliva and urine lasting for 4 days post-exposure
- Participants must be non-tobacco users (i.e. have not smoked or used any smokeless tobacco products or nicotine replacement therapy for at least 1 year)
- Participants should not have any regular ETS exposure in their home or work/school environment
- Exposure to ETS should be minimized as much as possible for the 4 days prior to and 4 days after study exposure
- Alcohol should not be consumed for the 24 hour period prior to, as well as the duration of the study
- Each participant must complete a consent form
- Participants will be reimbursed $150 for their participation which will be provided as soon as possible after participation in the study (a form must be completed and a check will be mailed by the university)
- Bingo is required to be played (requirement of the bingo) and cost is to be covered by the participant (estimated at $20-30 depending on number of cards played and whether playing electronic or manual dab)
- Participants will need to be present at the bingo hall at 5:30 pm for a brief orientation by bingo staff, to have a personal monitor affixed to their collar and to go over last minute details.
- A "kit" with sample collection tubes and bottles etc. can be picked up on the afternoon of January 30 or morning of January 31 at Dr. Law's lab (b9223). Email (rash@sfu.ca) or phone me 604-291-3680 to arrange to get the kit.

Pre-exposure Samples

- A saliva sample and a urine sample are required to be collected in the afternoon prior to attending the bingo hall.
- The saliva sample can be collected as outlined below.
- The urine sample should be collected as outlined below although does not have to be collected in the large collection bottle as long as total volume is obtained (i.e. small sample bottle can be filled and emptied and refilled provided you know the total volume).

Saliva Sampling

- Saliva samples should be collected at, or as close as possible to, the recommended sampling times (see table provided)
- Saliva samples should be taken prior to, or three hours after brushing teeth and 24 hours after any dental work
- Prior to each sample, the participant's mouth should be rinsed with water, and the water swallowed
- Wait for approximately ten minutes
- Without touching the cotton, insert a cotton salivette from top compartment of supplied plastic tube into mouth
- Chew softly on salivette for 2 – 3 minutes and move around in mouth allowing it to soak up as much saliva as possible
- Again without touching the salivette, deposit it back in the plastic tube (top portion) and recap.
- Label tube with time of sample (use supplied table for time (hour) since study start time
- Place saliva sample in freezer as soon as possible and store frozen until delivery to or pick up by Randy

Urine Sampling

- Urine should be collected for the recommended sampling time periods (see table provided) but it is more important to accurately reflect the time period for which the urine is collected than to stick to the recommended sampling time periods
- It is important to collect urine at least once per sample time
- Collect all urine for the designated time periods in the supplied large collection bottle
- At end of time period, confirm total volume of urine and record on urine sample bottle along with time period of urine collection
- Pour a sample of collected urine into labeled sample bottle and discard remaining urine (down toilet)
- Store urine in sample bottle upright in freezer until pick up by or delivery to Randy
- Rinse collection bottle with tap water and empty in preparation for next time period sample collection

Appendix H – Saliva and Urine Sampling Schedule for Human ETS Exposure in the Smoking Room of a Bingo

Saliva sampling times

Sample time (t + x hrs)	Projected sample time	Actual sample time
Baseline sample (t - x hrs)	Prior to start of experiment	
0.5	6:00 p.m. Jan. 31	
1	6:30	
2	7	
3	8	
4	9	
5	10	
6	11	
12	6 a.m. Feb. 1	
24	6 p.m.	
36	6 a.m. Feb. 2	
48	6 p.m.	
60	6 a.m. Feb. 3	
72	6 p.m.	
84	6 a.m. Feb. 4	
96	6 p.m.	

t = start time of 6:00 p.m. January 31, 2003

Urine Sample Times

Sample time (t + x hrs)	Projected sample time	Actual sample time
Baseline Sample (t – x hrs)	Prior to start of experiment	
0-6	6 p.m. Jan. 31 – 12 a.m. Feb. 1	
6-12	12 a.m. – 6 a.m. Feb. 1	
12-24	6 a.m. – 6 p.m. Feb. 1	
24-48	6 p.m. Feb. 1 – 6 p.m. Feb. 2	
48-72	6 p.m. Feb. 2 – 6 p.m. Feb. 3	
72-96	6 p.m. Feb. 3 – 6 p.m. Feb. 4	

t = start time of 6:00 p.m. January 31, 2003

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