International Experimental Toxicology Symposium on

PASSIVE SMOKING

Oct 23-25, 1986 · Essen, FRG

Thursday – Opening

1. Prof Steimle (Rector, Essen University)
   - referred to reports that 15% of all bronchial and airway disease is caused by PS – worldwide problem
2. Prof Goebell (Dean & Med School, Essen Univ)
   - undoubtedly greatest thing for prevention of cancer is elimination of tobacco
   - now is time to clarify the severity of effects of ETS on non-S
   - hopes the mtg will increase the # of non-S
3. Prof Norpoth (Director, Inst. of Hygiene and Occupational Medicine – organizer of the Symposium)
   - time to revisit the issue 2 years after Wynder's statement that legislation based on scientific data re: ETS would not be justified
   - asked for criticisms of papers to be on scientific grounds
   - also asked participants not to smoke

After the welcomes, Prof Schmidt of Mannheim interrupted the proceedings on behalf of the German Society Smoking and Health – he claimed the symposium was supported by the tobacco industry and that [illegible] society had protested for "flimsy reasons."

51212 3163

OPENING LECTURE

S.C. #6 author

A CRITICAL LOOK AT N-NITROSAMINES IN ENVIRONMENTAL TOBACCO SMOKE (ETS)

Dietrich Hoffmann, John D. Adams, and Klaus D. Brunnemann
American Health Foundation, Valhalla, NY 10595, U.S.A.

Cigarette smoke carcinogens are generally released into the environment as sidestream smoke (SS) constituents at twice their levels inhaled as mainstream smoke (MS) constituents. However, aromatic amines and nitrosamines in SS exceed MS levels up to 50 times. Regardless of the fact that SS is usually substantially diluted before being inhaled as an environmental pollutant, there are certain settings that provide hourly exposure to nitrosamines at levels equivalent to those inhaled with the MS of several cigarettes. The known carcinogenicity of several nitrosamines and especially of those derived from the tobacco alkaloids, raises concerns as to the health risk incurred from repeated prolonged exposure to ETS. In order to approach risk assessment, data on uptake and retention of environmental nitrosamines by passive smokers are needed. While measurements of nicotine and its major metabolite cotinine in physiological fluids provide biological markers for ETS exposure, assessment of the uptake of tobacco-specific nitrosamines (TSNA) would provide specific markers for carcinogen exposure. The carcinogenic TSNA derive exclusively from Nicotiana alkaloids, are metabolically activated to alkylating species and bind to globin. Data on the biological markers for ETS exposure and possibilities for assessment of TSNA as biological markers of carcinogen exposure will be discussed.

(acknowledged that breathing and inhaling are not the same)

These studies are supported by Grant NO. CA29580 from the U.S. National Cancer Institute.

IARC 1986 - concluded "passive smoking gives rise to some risk of cancer

cig smokers endogenously form nitrosamines - can't succeed in demonstrating endogenous nitrosamine formation in passive smokers, but he's trying very hard

nitrosamines (single dose of NNK) produce tumors in rats, mice, hamsters, but not rabbits

Hoffman now look at formation of globin adducts for NNK and NNN in order to assess uptake of TSNA by passive smokers — may take a year

WSE mtg: does not dispute that nitrosamines can come

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1st Session – Biological Markers of Passive Smoking

Estimation of Smoke Dosage and Mortality of Non-smokers from Environmental Tobacco Smoke.

M.A.H. Russell; Addiction Research Unit, Institute of Psychiatry, The Maudsley Hospital, London, England.

Russell did a passive smoking study in 73 and concluded no effects — he revisited his results in 1982 after hearing Hiragama

This paper shows how biochemical markers can be used to estimate smoke intake from passive smoking to complement epidemiological studies on the health risks and mortality to non-smokers. Using data from slow nicotine infusions given over one hour, we estimated that the nicotine intake from passive smoking averages about 0.014mg per hour among urban non-smokers leading their usual daily lives. This compares with 0.23mg/hr in a smokefilled public house, 0.36mg/hr during maximum exposure in an unventilated room, and 1.0 to 1.4mg nicotine per cigarette taken in by active smokers. Further studies indicate that exposure to gas phase components is some three times higher. Using these approaches we estimate that the mortality from passive smoking is about 1000 non-smokers per year in Britain and more than 4000 per year in the United States, assuming that the relation of dose risk can be extrapolated in linear fashion.

← 6 to six subject

Russell came to his extrapolations like this:

The average urban non-smoker has nicotine/cotinine levels ½ to ¾ of 1 percent of that found in smokers

Using Uk and US (Surgeon General's) figures for deaths related to active smoking, he multiplies the percentage of cotinine levels in non-smokers by those numbers to arrive at his estimates for deaths related to passive smoking

Russell is impressed that the number he gets by this method is similar to Repace's number for lung cancer related to ETS

This rather cavalier use of mathematics was pointedly attacked during the discussion period.

51212 3165

International Study on Exposure to other People' Smoke and Urinary Cotinine Levels in Nonsmokers.

Elio Riboli, (IARC)
Div. of Epidemiology & Biostatistics, International Agency for Research on Cancer, Lyon Cedex, France.

The study will explore the relationship between passive smoking history, as assessed by interview, and cotinine and thiocyanate levels in urine. This study aims to elucidate whether the conflicting results so far repoted on the association between lung cancer risk and passive smoking are due to differences in exposure ascertainment and/or to actual level of exposure.

Thirteen centres located in Canada, China, Federal Republic of Germany, Greece, Hong Kong, India, Italy, Japan, Poland and USA are participating in the study. Study subjects are non-smoking-women, half of whom are currently married to a smoker, the other half married to a non-smoker. Recent exposure at home, at work, in public places and in vehicles is investigated using an ad hoc questionnaire. Urine samples are taken on the day of the interview. Analyses of urine samples will be performed at the American Health Foundation Laboratories, New York. Both cotinine and creatinine will be measured in each urine sample, and their levels will be standardized according to the amount of creatinine present. Data collection will be completed by summer 1986, and preliminary results will be available by the end of 1986.

Prelim Results

1) at least 75% of subjects are exposed to ETS
2) # exposed by husband only is small
3) approx 50% of exposed subjects are exposed by husband + others
4) approx 50% of exposed subjects are exposed by others only

[Husband's Smoking Habit as indicator therefore appears open to question]

51212 3166

IARC APPROACHES TO MONITORING EXPOSURE OF PASSIVE SMOKING

I.K. O'NEILL & E. RIBOLI
International Agency for Research on Cancer, Lyon, France

Two related IARC projects seek to clarify actual exposure in passive smoking (PS), since no published or on-going epidemiological studies have involved physico-chemical measurements and yet severe inter-study discrepancies of volume and ventilation exist of indoor spaces and probably of substances in side-stream (SS) - mainstream (MS) smoke. An international group of researchers on physico-chemical measurements of PS, chose key methods for sampling, analysis and considered background information on SS/MS differences, ventilation, exposure assessment etc. This will appear in 1986, in the IARC manual series "Environmental Carcinogens: Selected Methods of Analysis". IARC is also coordinating an international study on the methods to assess exposure to passive smoking among non-smokers. The study will compare the information provided by a questionnaire and the levels of cotinine and thiocyanate measured in vivo. The presentation will discuss the need to clarify relative exposures, and give progress on these approaches.

IARC has concluded that passive smoking gives rise to some risk of cancer — but this risk may be undetectable unless extremely high exposures are involved

speaker acknowledged the limitations of cotinine because cotinine is not related to the carcinogens in smoke

51212 3167

Measuring problems in estimating the exposure to passive smoking using the excretion of cotinine in humans

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*** Forschungslabor Prof. Dr. Schievelbein, München, FRG

The evaluation of potential hazards of passive smoking is essentially based upon a sufficiently exact determination of actual exposure levels. During the last years, the measurement of nicotine and cotinine in plasma, saliva and urine has become a method of choice. A series of experiments in healthy volunteers who were exposed to graded side-stream smoke levels in a climatic chamber revealed a variety of methodological problems resulting in a variability of measured nicotine and cotinine levels which can - particularly at low exposure levels - exceed the influence of chosen trial factors like level and duration of exposure. Sources of this variation were quantified. Their impact on the validity of cotinine in urine as a marker for recent exposure to passive smoking will be discussed.

best sample to take is a 24-hr urine sample

RIA (radioimmunoassay) provided different results than GC (gas chromatography)

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Determination of nicotine and cotinine in human serum and urine. An interlaboratory study.

A. Biber*, G. Scherer**, I. Hoepfner**, F. Adlkofer**, J. E. Haddow***, G. J. Knight***;
*Forschungslabor Prof. Dr. Schievelbein, München, FRG
**Forschungsgesellschaft Rauchen und Gesundheit mbH, Hamburg, FRG
***Foundation for Blood Research, Scarborough, Maine, USA.

Nicotine and cotinine concentrations in body fluids are widely used as markers for tobacco smoke uptake by smoking and exposure to environmental tobacco smoke. Since analytical quality assurance of nicotine and cotinine measurements is still lacking, we initiated an interlaboratory study involving 10 laboratories in UK, Sweden, Finland, USA, Japan and W-Germany experienced in performing nicotine and cotinine determinations in biological fluids by radioimmuno assay (RIA) and/or gas chromatography (GC). Eighteen serum and 18 urine samples (derived from 8 smokers and 10 non-smokers; 2 non-smoker samples were spiked with known amounts of nicotine and cotinine) were analyzed. So far results are available from 5 laboratories. The GC derived values show fair agreement, but those determined by RIA demonstrate higher interlaboratory variability, especially in the urine samples. For both GC and RIA, relative variability is extremely high in the non-smoker samples.

Mathematical Modeling of Nicotine and Continine as Quantitative Biological Markers of Environmental Tabacco Smoke Exposure

(IAPAG)

S.L. Schwartz, R.T. Ball, P. Witorsch*,
Dept. of Pharmacology, Georgetown University,
School of Medicine, Washington, DC, and Dept. of
Medicine*, George Washington University, Washington,
DC, USA.

Computer software developed in our laboratory (CMATRIX) was used to design a physiological pharmacokinetic model of nicotine absorption, distribution, metabolism and excretion in man. The model accommodates inhalation of nicotine from various environmental settings and physiological conditions in man. It was also used to predict pharmacokinetic behavior of cotinine arising from nicotine metabolism. Model predicted variation in body-fluid nicotine levels confirm that nicotine is not an acceptable quantitative marker of ETS exposure. Though cotinine provides a more stable pattern, preticted interindividual variation suggests the need for specific strict sampling and monitoring guidelines for cotinine to be a reliable quantitative marker.

Based on morning session, Schwartz reworked this presentation.

His conclusion: attempts to use cotinine sampling as a quantitative analysis of ETS exposure is nonsense

51212 3170

DEPOSITION RATES OF SIDESTREAM TOBACCO SMOKE PARTICLES AND ASSOCIATED POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

T. Vu Duc, and C.K. Huynh
Institute of occupational medicine and industrial hygiene, Route de la Clochatte, CH-1052 Le Mont-sur-Lausanne, Switzerland.

Most of the people spend a great majority of their times, from 75 to 90% indoors and tobacco smokes can constitute the main source of pollution in the indoor environment. Exposure to environmental tobacco smokes have been associated with annoyance and irritations of the respiratory system as well as other health related problems.

In the context of energy conservation, a minimum ventilation rate was recommended, just enough to keep an acceptable comfort. To maintain a reduced level of pollutants, it is desirable to evacuate the most toxic substances. The fate and dissipation of cigarette smokes from the ambient atmosphere of a closed space have to be considered.

In our study, the side-stream smokes of cigarettes were generated in an experimental chamber. The depletion of total particulate matter, size distributed particles and their associated PAHs were investigated as a function of times. The smoking protocol according to the Coresta standards was followed. A Climet optical counter for the particle size distribution was used and PAHs were analyzed by gas chromatography-mass spectrometry. CO was used as a tracer of the stability of the atmosphere in the experimental chamber.

The results show that 70% of the particles below 0.3 μm remain in suspension after 8 hours, while the 0.5-1 μm particles dissipate in a relatively fast rate. The PAH levels follow a similar trend.

Based on chamber work (10 cigarettes smoked at once) researcher presented data on half-lives of different sized particles

[Dr. CR Green believes that such unrealistic conditions were used that Vu Duc's data were questionable due to coagulation]

Vu Duc also presented data on half-lives of smoke constituents

Tar = 9.6 hrs.
Nic = 8.7
PAH's = 3.0 to 8.8

51212 3171

Hydroxy-phenantrenes in urine of non-smokers and smokers.

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F. Adlkofer*;

* Forschungsgesellschaft Rauchen und Gesundheit mbH, Hamburg, FRG

** Biochemisches Institut für Umweltcarcinogene, Ahrensburg, FRG.

Two groups of smokers and non-smokers (each comprising 5 subjects) were put on a controlled diet low in polycyclic aromatic hydrocarbons (PAH). During the 1st day after admission (control day), smoking was not allowed, thus avoiding exposure of the non-smokers to environmental tobacco smoke (ETS). On the 2nd day, the non-smokers were exposed to ETS in an unventilated room for 8 hours. The concentration of ETS produced by the cigarette smokers was adjusted to 20-25 ppm CO. The 24h-urines of non-smokers (2 groups of 5 subjects each = NS) and smokers (2 groups of 5 subjects each = S) were collected during the control day (NS_c, S_c) and the exposure day (NS_e, S_e). Aliquots from individual subjects of each group were pooled and analysed for HO-phenantrenes (HO-PHE). The urinary HO-PHE excretion (µg/24h) is as follows:

	NS_c	NS_e	S_c	S_e
Group 1	4.39	4.84	4.48	5.24
Group 2	4.81	3.71	5.16	5.86

[increase in smokers not statistically significant)

In another experiment, 8 non-smokers were put on a diet low in PAH for 2 days and on a diet high in PAH for 2 further days. The urinary excretion of HO-PHE was nearly twice as high after the high-PAH diet as after the low-PAH diet. (but not associated with mutagenic activity in urine)

Our results indicate that HO-PHE in urine is a marker for dietary uptake of PAH. However, it does not clearly differentiate smokers and non-smokers and is certainly unsuitable for detecting PAH uptake from ETS exposure.

Ribol: (IARC) quarreled with her definition of statistical significance — he said the slight increase in HO-PHE's in smokers might be biologically significant

51212 3172

Degressive and regressive tumor antigen curves in active and passive smokers

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Investigation and analysis of more than 40,000 blood samples for tumor markers could fully confirm that smokers inhale an appreciable carcinogenic risk to the extent of their exposure: they show CEA values up to 7 ng/ml.
After total abstinence, the CEA titer may fall into the normal range in six to 18 months depending on the initial situation.
The overlapping specificity and sensitivity range as well as quality controls are referred to.
In 80% of otherwise healthy nonsmokers (age 20 to 40 years), the CEA titer was less than 2 ng/ml. A similar group (10) of passive smokers displayed values around 3 ng/ml.
Analyses of tumor markers can only reproduce a delayed retrospective view of a small subaspect of the problem of passive smoking.

Another marker which does not pan out to assess exposure

SECOND SESSION - TOXICITY OF SMOKE TO FETUS and INFANTS

FETAL GROWTH RETARDATION IN RATS CAUSED BY MATERNAL PASSIVE SMOKING DURING PREGNANCY

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Institute of Hygiene and Occupational Medicine, University Medical Center, Essen University, D-4300 Essen, FRG.

Although the relation between maternal active smoking and fetal development is well studied, less attempt has been made to evaluate the effects of maternal passive smoking on fetus. The few reports that exist, in this connection, are epidemiologic not experimental. We performed a preliminary experiment to determine the effects of involuntary smoking during pregnancy on the growth of fetus. As a model, pregnant Wistar rats were passively exposed to the sidestream smoke of a commercial brand of cigarettes during the first, second and the third week of pregnancy. The animals were individually exposed to the smoke in a 145.2 dm^3 glass chamber resembeling a room provided with normal air flow. The cigaretttes were smoked by a smoking machine under standard conditions. The uterine horns were removed a day before the natural delivery and the fetuses were examined macroscopically. Among the parameters studied, significant losses of body weights, body lengths and head circumferences were found for those whose mothers were passively exposed to the diluted sidestream smoke of 2 to 4 cigarettes. The most retardation of development was found to be for those whose mothers involuntarily smoked during the third week of pregnancy. However, no gross anomaly was observed. These studies are in process to examine the other parameters of growth affected by maternal passive smoking in rats.

M. would give no satisfactory answers on how controls were handled - it appeared that cage controls only were used

Dr Coggins said that photos of fetuses shown were in normal ranges

This study received very vigorous criticism in the

The effects of smoke exposure to infants

D.Schwartz-Bickenbach, S.Schulte-Hobein, S.Abt.,H.Nau,
Institut für Toxikologie und Embryonalpharmakologie, Garystr. 5,
1000 Berlin 33 (Free University of Berlin)

The consequences of smoke exposure via mother's milk and passive smoking on the physical and psychomotor development of infants during their first year of life were investigated in a group of children of smoking mothers and compared with a matched control group. The measurement of continine concentrations in mother's milk and urine of the infants represents an objective criterium for the actual nicotine exposure. So far our results show that

1. the intrauterine growth retardation often seen in the target group isn't caught up at twelve months;
2. smoking mothers nurse their babies for a shorter time than non smoking mothers do;
3. cotinine concentrations in mothers milk differ dependent on number of cigarettes smoked and nicotine contence of the cigarette sorts;
4. cotinine concentrations in the urine of the infants are 5 to 20 fold higher during the nursing period than after weaning;
5. the psychomotor development does not differ significantly between the infants of the target group and the control group.

this conclusion appeared to be contradicted

More smoking mothers nurse their babies than non-smoking mothers

Young woman making presentation appeared to get a little rattled when questioned, and the discussion lightened up on her.

51212 3175

Oct 24

Third Session: Uptake and Toxicity of Smoke

Uptake of Sidestream Smoke by Syrian Golden Hamsters

N.J. Haley, J.D. Adams, J. Alzofon, D. Hoffmann;
Naylor Dana Institute for Disease Prevention;
American Health Foundation; Valhalla, N.Y. 10595

An inhalation bioassay with syrian golden hamsters is being conducted to evaluate the toxic and carcinoenic potential of sidestream smoke (SS) relative to mainstream smoke (MS). A Hamburg II smoking machine was used to allow nose-only delivery of MS to hamsters and a modification allowed for the simultaneous collection of SS for whole body delivery to a different rack of animals. The tolerated dose of SS was determined by varying the air/smoke dilutions drawn through the animal restrainers. Preliminary data indicated that 20 % carboxyhemoglobin (COHb) could be obtained in SS exposed animals without fatality. Optimum exposure levels were determined. Monthly measurements of COHb, nicotine and cotinine indicate that the SS exposed animals are absorbing higher amounts of these smoke constituents than the MS counterparts. Tumor incidence and carcinogenicity data are being collected through complete necropsy and histology protocols and uptake data continue to be collected. These studies should help eludicate the carcinogenic potential of SS which has been suggested from its composition and from recent epidemiological data of cancer incidence in nonsmokers. This study was supported by Grant CA-29580 from the NCI.

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Dr. Coggins believes this study is methodologically very bad, and can cause some problems.

Study will continue until 80% of animals in longest living group die

using 2R1 Ky ref

started Dec 85

each SS chamber gets 37 mg TPM and 1.8 mg nicotine (more than MS exposure)

all groups gaining weight at similar rates

SS-exposed animals are taking up higher concentrations of constituents than MS

The Effect of Smoke Age and Dilution on the Cytotoxicity of Sidestream (Passive) Smoke.

Gerald Sonnenfeld and R.B. Griffith; Univ. of Lousville School of Medicine, Louisville, KY 40292 and College of Pharmacy, Univ. of Kentucky, Lexington, KY 40536-0081, USA.

Decreases in the mortality of monolayer cultures of L-929 cells exposed to sidestream (SS) smoke with increases in smoke age and dilution have been reported (Arch. Toxicol. 58:120, 1985) but smoke concentration and mathematical relationships had not been established. Additional studies (Griffith et al, pvt. communication) showed that the SS smoke generated in the interval between puffs was 1.68 ml/s, with a concentration of 5.36%. The SS concentration was directly correlated with % mortality (r=.987) to have 0% mortality at a concentration of 1.19% and a calculated mortality of 9562% at a concentration of 100%. The ln of % mortality was correlated with increases in smoke age (r=-.9999) and the regression equation was used to calculate 0 mortality at an age of 30 s and 393% mortality at the time of smoke generation. These data indicate that undiluted, unaged SS smoke is very toxic, but that cytotoxicity decreases rapidly with dilution and age. The importance of these results to passive smoking justifies additional studies.

SS more toxic than MS (in ZRI)

51212 3177

4TH SESSION - LUNG DEPOSITION OF SMOKE AND PULMONARY CHANGES

SG 86 author

Deposition of the Ultrafine Particulate Component of Sidestream Cigarette Smoke in the Human Respiratory Tract.

F.C. Hiller, P.J. Anderson, and J.D. Wilson. The University of Arkansas for Medical Sciences and the University of Arkansas Graduate Institute of Technology, Little Rock, Arkansas, U.S.A.

Sidestream cigarette smoke was generated into an inhalation chamber from which five normal male volunteers inhaled the smoke. Exhaled smoke was collected and analyzed using an electrical aerosol analyzer. Size distribution of the smoke aerosol after 15' aging and mixing was: count median diameter, 0.11 μm, mass median diameter 0.43 μm, and geometric standard deviation, 1.96. Deposition fraction measured as concentration difference for each size fraction between inhaled and exhaled aerosol (J. Appl. Physiol. 58:223, 1985) for each size interval was: 0.075 μm, 0.24 ± 0.04; 0.13 μm, 0.15 ± 0.04; 0.24 μm, 0.10 ± 0.04; and 0.42 μm, 0.07 ± 0.02. The declining deposition fraction as size approaches 0.5 μm is consistant with previous theoretical and experimental data. The deposition fractions are somewhat lower than that previously found for the same subjects inhaling a stable research aerosol using the same methods.

Deposition Fraction increases as particle size decreases

Study was of particles less than 1 micron

Size	Deposition Percentage
.42 micron	7 %
↓	
.072 micron	24 %

51212 3178

Physiological Changes in Pulmonary Development Related to Passive Smoking and its Interactions with Active Smoking.

M.D. Lebowitz, C.J. Holberg; Univ. Arizona, Div. Respiratory Sciences, Tucson, USA.

Effects on pulmonary development and changes in respiratory flows-small and large airways, were determined from age five to young adulthood. Pulmonary growth models were created using 1520 repeat physiological tests on 362 individuals studied in time for up to 12 years (average of 9 years). They had various initial respiratory characteristics. Mathematical calculations then determined independent effects of individual variability over time, passive and active smoking, and symptomotology. Interactions of passive and active smoking affected flows (small airways) adversely and size compensated flows were adversely affected independently. Studies of newborn physiology showed adverse effects of intrauterine exposure to smoking, especially decreased flows in females and increased volumes in males (associated with decreased size compensated flows), which appear to account for a majority of the effects of passive smoking. The remaining effects are additive or synergistic, in specific high risk groups. Exposure response characteristics are most critical, and will be discussed.

Extrapolation from Rat Studies with Environmental Tobacco Smoke (ETS) to Humans: Comparison of Particle Mass Deposition and of Clearance Behavior of ETS Compounds.

G. Oberdörster and F. Pott, Dept. RBB, Univ. of Rochester, Rochester, NY USA and Med. Inst. f. Umwelthygiene, Univ. of Düsseldorf, FRG.

Model calculations are performed and show, that inhalation via nose of monodisperse submicronic particles leads to lower deposition in rats than in humans for both the pulmonary and tracheobronchial region. Heterodispersity of ETS increases deposition in all regions. However, when the deposited surface area dose per generation is calculated a greater deposition occurs in rats than in humans in the transitional zone of the airways, i.e., the region of the terminal and respiratory bronchioles.

Dose to the airways is also determined by the clearance kinetics of the deposited substances - which in turn depends both on physical processes and the metabolism. As a consequence, the pulmonary retention of inhaled ETS components can be quite different in rats and humans. For example, we determined in separate studies that the pulmonary half time of Cd - an ETS compound - is about 80 days in rats and more than 600 days in primates. Thus, both lung deposition and lung clearance of ETS compounds have to be considered when extrapolating results from animal studies to humans.

Ultrastructural Changes in Respiratory Tract after Exposure to Cigarette's Main- and Sidestream Smoke.

M. Emara, M. Blank, and U. Lessner
Inst. of Anatomy, Univ.Med.Center, Essen University, Hufelandstr.55, D-4300 Essen, FRG.

SEM- and TEM-electron microscopic studies of cigarette smoke-treated respiratory epithelia of men and rats are described. Additional freeze-etching studies of the cilia have shown primary alterations after exposures to cigarette's smoke. Differences were found between ultrastructural changes following the exposure to main- and sidestream smoke. Cilia were the first to be affected by the smoke. The surface membranes were found to be wavy and rough, and the membrane proteins were minimized. There also were some changes in the architecture of microtubulis. These ultrastructural changes of cilia were seen to accompany cytoplasmic protrusions of the cellular surface. Bundles of cilia were enveloped with these latter cytoplasmic protrusions. An additional observation was the multiple alterations of cytoorganelles within the epithelium.

5TH SESSION - CHEMICAL ANALYSIS OF SMOKE

Personal Air Exposures and Breath Concentrations of Benzene and and Other Volatile Aromatic Compounds

L.A. Wallace, Harvard University School of Public Health, USA
E.D. Pellizzari, Research Triangle Institute, USA

Measurements of personal exposure and exhaled breath concentrations of 20 volatile organic compounds were made for 200 smokers and 300 nonsmokers in five U.S. cities (1980-84). Smokers showed significantly increased breath concentrations of six hydrocarbons: benzene, styrene, ethylbenzene, o-xylene, m+p-xylene, and octane. Homes with smokers had higher indoor air concentrations of the same compounds than homes without smokers during fall and winter. Passive smokers exposed at work had significantly higher levels of benzene and other aromatics in their breath than unexposed nonsmokers. Measuring exhaled breath and personal or indoor air for volatile aromatic compounds appears to be a feasible, although costly, way to determine exposure of passive smokers.

benzene associated with leukemia

Increased leukemia mortality rate for children of smokers

51212 3182

Gaschromatographic Determination of Polycyclic Aromatic Hydrocarbons, Azaarenes, Aromatic Amines in the Particle and Vapour Phase of Main- and Sidestream Smoke of Cigarettes.

C. Grimmer. K.-W. Naujack, G. Dettbarn
Biochemisches Institut für Umweltcarcinogene, D-2070 Großhansdorf

The particles and the semi volatiles of the main stream and side stream smoke from a mechanically smoked cigarette were collected on a filter system consisting of a cambridge filter as well as polystyrene beads coated with polydimethylsiloxane. The fractions of polycyclic aromatic hydrocarbons (PAH), azaarenes (N-PAC) and aromatic amines (NH_2-PAH) isolated by a specific enrichment procedure were separated by high resolution gas chromatography (fused silica columns) and characterized by a simultaneous detection of a FID, ECD and a nitrogen specific detector. The amount of the single compounds of PAH, N-PAC and NH_2-PAH in the particle and vapor phase of main- and side stream smoke, respectively was determined quantitatively.

Quantitative evaluation of cigarette sidestream smoke components under controlled experimental conditions.

C.J. Blake, J.J. Piade, W. Fink;
Philip Morris Europe, Research & Development, Neuchâtel, Switzerland.

The concentration of selected components of cigarette sidestream smoke was determined in a 18.2 m^3 stainless steel test chamber under controlled experimental conditions. The chamber was operated in the static mode. Concentrations were monitored as function of number of cigarettes smoked and of time. Among the components measured were CO, NO, NO2, HCN, NH3, formaldehyde, phenols and nicotine as well as aerosol particle mass.

Two different types of cigarettes were used for this study. Cigarettes were smoked under standard DIN smoking conditions (2 sec, 35-ml puff once per min) at 60% rel. humidity and 20°C. Sidestream smoke components were either continuously monitored by automatic computer controlled instrumentation or were trapped and analysed by GC2, HPLC or other techniques.

In all cases concentrations of investigated compounds are proportional to the number of cigarettes smoked in the range of 5 to 30 cigarettes. Changes in concentrations of smoke components as a function of time will be demonstrated. Maximum possible contribution of cigarette sidestream smoke to ETS as function of cigarette number is reported.

filters and increased air dilution do not drive SS values up

does not seem likely that any 1 compound can be used as a marker because of various decay rates

51212 3184

Analysis of environmental tobacco smoke (ETS) constituents in indoor air under controlled conditions.

H. Klus*, H. Begutter*, A. Nowak*, G. Pinterits**, I. Ultsch*, H. Wihlidal**;

* Austria Tabakwerke AG, Forschung und Entwicklung, Wien, Austria
** Österreichisches Forschungszentrum Seibersdorf GmbH, Seibersdorf, Austria.

The quality of indoor air was evaluated for some selected ETS constituents. Twenty experimental sessions with different subjects and ETS concentrations were carried out. Up to 4 subjects, smokers and nonsmokers, were present in an office room of 83,8 m^3 for 2h. During this period the window was closed. Opening of the door when subjects entered or left and the number of cigarettes, cigars and pipes, smoked ad libitum, were protocolled.

Carbon monoxide (CO) and nitrogen oxides (NO, NO_x) were monitored continuously. The concentrations were in the range of Δ3-14 ppm CO, Δ62-236 ppb NO, respectively. Maximal levels of NO_2 reached 22 ppb. Samples of air were analyzed for nicotine, formaldehyde, hydrogen cyanide, phenols, ammonia, acroleine, and N-nitrosamines. Furthermore, the relative amount of aerosole particulate matter was estimated.

In addition, the data obtained are compared with the background levels of all these substances measured in the same room without subjects prior to each single experiment.

Analysis of environmental tobacco smoke (ETS) constituents in indoor air under real life conditions.

M. Ball, M. Intorp, B. Schilling;
H.F. & Ph. F. Reemtsma, Rohtabak-Forschung-Entwicklung, Hamburg, FRG.

Methods have been developed for inconspicuous sampling of indoor air and analysis of selected air constituents with special consideration of ETS-related components.

Samples were taken from different localities like living room of a 4-person household, restaurant, pub, car and railway compartment, occupied by smokers and non-smokers respectively.

Air samples were analyzed for nicotine, formaldehyde, phenols, ammonia, hydrogen cyanide, carbon monoxide and N-nitrosamines.

The concentrations of carbon monoxide (CO) were in the range of 1 to more than 20 ppm. Maximal levels of CO were measured in air samples taken from cars, railway compartments and the living room of a 4-person household. They do not correlate to the responding values of ETS constituents e.g. nicotine.

Conclusion:
must measure more than 1 substance in order to get true picture of ETS exposure

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Estimation of Personal Exposure to Ambient Nicotine in Daily Environment

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To evaluate the actual Levels of passive exposure to tobacco smoke in various daily environments, exposure level to ambient nicotine was measured with a pocketable nicotine personal monitor attached to a nonsmoker. The personal monitor consists of a sampler tube containing an absorbent and a portable sampling pump. The collected nicotine on the tube was directly desorbed onto a GC column and analyzed.

Average exposure levels to ambient nicotine on both smoking seats and nonsmoking seats in airplane and in train were 13.3μg/m^3 and 5.3μg/m^3, and 16.7μg/m^3 and 2.0 μg/m^3, respectively. Amounts of nicotine passively inhaled in such smoky places as car, bar and coffee shop, though influenced mainly by the number of cigarette smoked and conditions of ventilation, were estimated to be less than 50μg/hr in this study.

A COMPARATIVE STUDY OF ENVIRONMENTAL TOBACCO SMOKE PARTICULATE MASS MEASUREMENTS IN AN ENVIRONMENTAL CHAMBER.

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ETS particulate mass is currently being measured by a number of research groups with several methods that have not been directly compared in controlled experiments. The purpose of this study is to provide a comparison among several commercially available instruments and a newly developed gravimetric/spectrophotometric method for assessing ETS particle mass concentrations. ETS was generated over a range of concentrations in an environmental chamber by introduction of sidestream only, by introduction of sidestream plus machine-smoked mainstream, and by human-smoking. Measurements were made with two TSI Model 5000 Aerosol Mass Monitors (piezoelectric), two GCA Minirams (nephelometry-based), one active and one passive, one PPM Handheld Aerosol Monitor (nephelometry-based), and a Fluoropore membrane filter collection procedure by which mass was determined both gravimetrically and by UV absorbance (325 nm). Measured mass concentrations were found to depend not only on the analytical procedure, but also on the method of ETS generation.

THE MEASUREMENT OF 'ENVIRONMENTAL TOBACCO SMOKE' PARTICULATES

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Mainstream tar yields from cigarettes, derived by traditional gravimetric methods, should not be compared directly with ambient smoke yields derived in the same way, because of a redistribution of chemical components between vapour and particulate phases, ageing effects and changes in particle size.

Light scattering methods for the routine measurement of atmospheric particulates depend upon a relevant calibration. When applied to atmospheric tobacco smoke calibration against mainstream smoke is inappropriate and even against undiluted sidestream smoke is open to question.

An alternative calibration for ambient smoke is proposed in which levels of particulate matter are measured directly using a cascade impactor with piezo-balance stages. Results demonstrate a five fold overestimate of concentration when light scattering devices are calibrated against undiluted sidestream smoke.

These problems need to be understood in all studies where extrapolation from the environment back to the cigarette is to be undertaken.

TPM is meaningless in terms of talking about ETS

A STUDY OF THE ATMOSPHERE IN LONDON UNDERGROUND TRAINS BEFORE AND AFTER THE PROHIBITION OF SMOKING

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In July of 1984, London Transport decided to enforce a ban on smoking from all of its Underground railway train compartments. This ruling presented an opportunity to study the effect of banning smoking on the ambient atmosphere of a popular public environment. In order to assess the contribution of tobacco smoke to any particular environment, it is necessary to measure a number of chemical constituents of smoke.

We shall describe methods of measuring carbon monoxide, airborne particulates and nicotine simultaneously using discrete, sensitive instrumentation contained in a portable package. In repeated investigations of smoking compartments before the ban, it was found that the levels of the selected compartments were far below industrial exposure limits, as recommended by the Occupational Safety and Health Administration (OSHA). Furthermore, it was illustrated that there was little difference in the concentration of carbon monoxide in train compartments whether smoking was permitted or not. The validity of the methods used to reach these conclusions will be discussed, along with the problem of placing the contribution of tobacco smoke to ambient atmospheres in true perspective.

EFFECT OF ENVIRONMENTAL TOBACCO SMOKE (ETS) ON AIR QUALITY WITHIN AIRCRAFT CABINS. G.B. Oldaker III and F.C. Conrad, Jr., R.J. Reynolds Tobacco Company, Research and Development, Bowman Gray Technical Center, Winston-Salem, NC 27102

Nicotine concentrations were determined in passenger cabins of B727 and B737 aircraft during domestic flights in order to assess the effectiveness of smoker segregation as a means of minimizing exposure of non-smokers to ETS. Sampling systems including constant flow pumps and sorbent tubes containing XAD-4 resin were used to collect integrated samples of nicotine. Sampling systems were concealed in briefcases to ensure that sampling operations were unobtrusive. Analysis involved desorption of nicotine into ethyl acetate and quantification with gas chromatography and nitrogen-phosphorus detection. Twenty-three samples were acquired in smoking sections and thirty-nine samples in no-smoking sections at the boundaries with smoking sections. In no-smoking, boundary sections, nicotine concentrations ranged from 0 to 40 $\mu g/m^3$ with an arithmetic mean of 11 $\mu g/m^3$. In smoking sections, nicotine concentrations ranged from 0 to 112 $\mu g/m^3$ with an arithmetic mean of 23 $\mu g/m^3$. Sampling times ranged from 13 to 179 minutes with an arithmetic mean of 57 minutes. Results indicate that no-smoking sections are essentially ETS-free for aircraft such as B727's and B737's, which have "once through" ventilation systems.

Also presented
particulate and CO data from later samples

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Sixth Session - Genetic Toxicology of Passive Smoking

Urinary mutagenicity after controlled exposures to environmental tobacco smoke (ETS).

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Twenty non-smokers were put on a defined diet low in polycyclic aromatic hydrocarbons (PAH). The first day following the night after admission was the control day during which exposure to tobacco smoke was avoided. On the second day the subjects were exposed to ETS in an unventilated room for 8 hours. Two separate experiments were carried out. In the first, the concentration of ETS produced by cigarette smokers was adjusted to 10 ppm CO, while the second it was set between 20-25 ppm. The urinary mutagenicity in the 24h urine samples as tested with the Salmonella (TA 98) microsome assay did not increase after exposure to 10 ppm CO, and increased only marginally under the extreme exposure conditions of 20-25 ppm CO. The increase was in the same order of magnitude as after a diet rich in leafy vegetables and charcoal broiled meat, and lower than in smoking controls.

We conclude that exposure of non-smokers to ETS does not increase their urinary mutagenicity, provided that exposure conditions are within a realistic range.

3 studies have reported increased urine mutagenicity after ETS exposure - this study sought controlled conditions

measuring mutagenicity is not a good indicator of the effect of ETS on human health

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URINARY EXCRETION OF MUTAGENS IN PASSIVE SMOKERS

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Six healthy young volunteers with no history of active smoking were asked to keep to their western diets avoiding the consumption of alcoholic beverages, excess coffee, any sort of medicament, and the known pro- and/or anti-mutagen-containing foods and drinks, 24 h before and during the experiments. They were exposed passively to cigarette smoke produced by four habitual smokers in an unventilated 48.6 m^3 room for 8 h. The carbon monoxide concentration was 18.85 ± 7.3 ppm during the 8-h exposure.

88 cigarettes were smoked

Frameshift mutagens were isolated from 10-h urine samples using chloroform and were tested for mutagenicity in the Salmonelle/mammalian microsome assay employing Salmonella typhimurium TA98. Although clearly enhanced, no significant mutagenic activity could be found with 25 ml equivalent urine/plate after passive exposure to cigarette smoke. The weak mutagenicities found were highly significant when 50 ml equivalent urine/plate was tested. No direct correlation was observed between urine-mutagenicity and the urinary cotinine concentration. The results obtained are discussed with reference to inconsistent reports in literature concerning the mutagenicity of urine after passive smoking.

Discussion generally shot down whole idea of mutagenicity studies

but Norpoth claimed the results are enough to call for the protection of non-smokers

CLASTOGENIC EFFECT OF PASSIVE SMOKING ON BONE MARROW POLYCHROMATIC ERYTHROCYTES OF NMRI-MICE

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Genotoxic effect of passive inhalation of cigarette's sidestream smoke on bone marrow polychromatic erythrocytes was studied using male NMRI mice. The animals were individually placed in a 145.2 dm^3 glass chamber resembling a room provided with normal air flow. They were exposed to the sidestream smoke of a commercial brand of cigarettes smoked by a smoking machine under standard conditions. Increased formation of micronuclei within polychromatic erythrocytes (PCEs) of femoral bone marrow 30 h after passive smoking was conducted to be due to the clastogenic effect of the smoke. Passive inhalation of the diluted sidestream smoke of a single cigarette resulted in a significant increase ($p < 0.01$) in the frequency of micronucleated PCEs. This clastogenic activity was found to be dose-dependent.

SEVENTH SESSION : SURVEYS IN EPIDEMIOLOGY OF PASSIVE SMOKING

Lung cancer and passive smoking : Association an artefact due to misclassification of smoking habits?

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1775 subjects were interviewed about their current smoking habits and use of nicotine-containing products and 1537 later provided a saliva sample for cotinine analysis. Of 808 non-smokers/users, 20 (2.5%) had cotinine values above 30 ng/ml, indicating their self-reports were false.

In another study, 540 subjects were interviewed in 1980 and in 1985. 7.2% of subjects who stated in 1985 they had never smoked had earlier reported current or ex-smoking. 12.8% of 1980 never smokers subsequently claimed to have smoked and started before 1980.

Coupled with evidence of strong concordance between spouse's smoking habits, these misclassification rates would produce an apparent increase in lung cancer risk in non-smokers married to smokers similar to that observed epidemiologically, even when no true passive smoking exists.

Conclusion

BIAS can completely explain reported apparent excess lung cancer in non-smokers married to smokers.

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Exposure Misclassification as an Interpretation of Some Case Control Studies of ETS and Lung Cancer in Nonsmoking Women.

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Several Case Control studies have reported a statistically significant relationship between environmental tabacco smoke ETS and lung cancer. In these studies exposure to ETS is retrospectively determined by interview of the subject or a close relative or friend. Apparent "dose-response" relationship have been taken as supporting the claim that ETS exposure, in fact, increases the risk of lung cancer, i.e. a causal interpretation. In such studies, several alternative explanations for a statistically significant result, including biased selection of subjects, confounding of ETS exposure with occupational, environmental or life style factors and misclassification must be considered. This paper presents an analysis of the potential contribution of differential misclassification of exposure in the case-control studies of Garfinkel, Correa and Trichopoulos. Differential misclassification rates in cases and controls is postulated to result from the tendency of respondents to inflate the amount of ETS exposure for the lung cancer cases and deflate the report of exposure for controls. Levels of overall misclassification which would account for the published results are calculated to be 3, 19 and 20 percent in the studies of Garfinkel, Correa and Trichopoulos, respectively. Several models of misclassification are shown to give virtually the same results, consistent with the discrete nature of the data. Finally, exposure misclassification, restricted to cases alone, is shown to mimic the dose response relationship. The existence of exposure misclassification is but one of the sources of potential error which must be evaluated before the case-control studies can be considered to support a causal relationship between ETS exposure and lung cancer in nonsmokers.

Mutagenic Determination of Passive Smoking.

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It has earlier been shown by means of the Ames Salmonella plate incorporation test that a major part of the particulate mutagenic activity in indoor air originates from tobacco smoking under moderate smoking conditions.

The mutagenic activity of environmental tobacco smoke (ETS) has been further characterized with potentially more sensitive microsuspension test systems and it is presently possible to determine and quantitate ETS with personal sampling and to discriminate ETS from other airborne mutagens by means of a simple fractionation with isolation of the basic, mutagenic components.

Mutagenicity of airborne particulate matter is thus a specific method for assessing exposure to ETS which can be used alone or in conjunction with vapor phase nicotine analysis.

he claims that ETS exposure can be quantified by taking mutagenicity samples of air

there was some agreement that this makes more sense than urine mutagenicity studies