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March 20, 1998

VIA FEDERAL EXPRESS

Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
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VIA EXPRESS MAIL

Dr. C.W. Jameson
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Re: Comments on NTP's proposal for listing environmental tobacco smoke (ETS) in the Report on Carcinogens, Ninth Edition

Dear Dr. Jameson:

Philip Morris U.S.A. welcomes this opportunity to comment on the National Toxicology Program's (NTP's) intent to review environmental tobacco smoke (ETS) for possible listing in the *Report on Carcinogens, Ninth Edition*, as noticed in 63 *Fed. Reg.* 5565, February 3, 1998. We understand that these comments, postmarked March 20, 1998, 45 days after the date of publication of the notice, will be accepted as timely and provided to NTP's reviewers. We are sending one bound copy by Federal Express and one unbound copy by Express Mail for your convenience in photocopying.

Philip Morris believes that, at this time, a scientific classification of ETS as a "known human carcinogen" would be premature because existing scientific support with respect to ETS is neither persuasive nor conclusive, and because a number of important critical scientific questions remain unanswered.

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We therefore submit, for your reference, a listing of currently available studies and reviews from the scientific literature that do not support the classification of ETS as a known human carcinogen. [Attached to this correspondence, Appendix 1]

Philip Morris USA also is committed to addressing remaining questions regarding ETS through support of scientific research. We have, for example, initiated an on-going research program which is evaluating the biological activity of an ETS surrogate (room-aged sidestream smoke (RASS)). Our submission contains a publication on the methodology for analyzing RASS, as well as a publication of a subchronic study of inhalation exposure of rats to RASS. [See Appendix 2] The biological effects observed to date are indicative of mild irritation that is both reversible and non-progressive. We anticipate completion of the study within two years; as additional data are published, they will be forwarded to you for your review.

We have also supported research and analysis of the smoke components, tobacco specific nitrosamines (TSNAs), that have been purported to be causally associated with lung cancer. A comparative analysis of TSNA metabolism in lung and liver from the A/J mouse, Fischer 344 rat and human strongly indicates that activation predominates in the mouse and rat, but that in human tissues, a detoxification process predominates. This suggests that though TSNAs may be animal carcinogens (consistent with the metabolic profile reported here), in humans, the metabolic profile of TSNAs is **not** consistent with carcinogenic activity. [See Appendix 3]

In addition to the foregoing, Philip Morris U.S.A. submits the following materials for consideration in NTP's review of ETS for listing in the *Ninth Report on Carcinogens*. Included are the following items:

- A discussion of the recent report from the International Agency for Research on Cancer (IARC) on its multi-center epidemiologic study of ETS exposure and lung cancer risk in Europe (IARC Biennial Report 1996/1997). The IARC study, the largest, and according to IARC, the "most comprehensive" epidemiological study specifically designed to assess the potential lung cancer risk of spousal, workplace and childhood ETS exposure, reported no statistically significant risk estimates for spousal, workplace, spousal plus workplace or childhood exposures. [See Appendix 4]

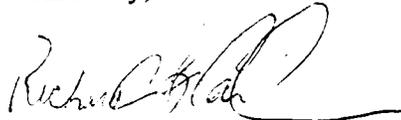
- An additional analysis of the United States Environmental Protection Agency's (U.S. EPA) 1992 Risk Assessment on ETS, which addresses, using Monte Carlo simulations, the uncertainty associated with two critical factors that influence the attributable deaths estimated by EPA.¹ Our analysis indicates that proper consideration of just these two factors reduces the EPA's attributable risk number by two-thirds. Further consideration of factors such as diet, socio-economic status, family history of lung disease, etc., as well as data from epidemiologic and exposure studies in the workplace, social settings, etc., would be expected to further reduce this estimate. [See Appendix 5]
- Our analysis of methodologies utilizing cotinine as a measure of either smoking status or ETS exposure. The technological measurement short-comings and the metabolic complexities of these issues lead us to describe what we believe to be a more systematic and rigorous approach to this question. Given the current situation, we believe that the use of single point measurements using cotinine alone cannot be used as a quantitative measure of ETS exposure. [See Appendix 6]
- An analysis of the smoke component, benzo(a)pyrene (B(a)P), that has been purported to be causally associated with lung cancer. This analysis suggests the following: (i) that the levels of B(a)P that would be expected in environments where ETS is present are within the background levels found in urban air and (ii) that sources other than ETS account for more than 90 percent (>90%) of the total body burden of B(a)P. [See Appendix 7]
- Philip Morris' 1997 submission to the California Environmental Protection Agency (Cal/EPA) addressing the alleged association between reported exposure to ETS and human cancer. [See Appendix 8]

1. For the purpose of demonstrating the fragility of the U.S. EPA's attributable death calculations in its Risk Assessment on ETS (1992), we utilized EPA's relative risk point estimate. It is important to note that we do not believe that this point estimate is scientifically justified. In fact, the data indicate that one cannot distinguish the association between reported exposure to ETS and lung cancer described by the EPA from no association.

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We believe that the underlying science, including ETS exposure as determined by personal monitoring, distributional analysis of exposure information, human epidemiological and biomonitoring data and animal studies, support the scientific position that environmental tobacco smoke cannot be classified as a known human carcinogen.

Sincerely,

A handwritten signature in black ink, appearing to read "Richard A. Carchman", with a long horizontal flourish extending to the right.

Richard A. Carchman, Ph.D.

RAC/jk

Enclosure(s)

Submission by Philip Morris U.S.A.

to

The National Toxicology Program

Volume I

Appendices 1-5

March 20, 1998

Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 1 -

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Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 2 -

**Long-Term Inhalation Study on
Room-Aged Sidestream Smoke
and Diesel Engine Exhaust**

March 20, 1998

Introduction

Philip Morris is funding a long-term inhalation study on room-aged sidestream smoke (RASS) and diesel engine exhaust (DEE), currently underway at INBIFO (Institut für biologische Forschung, Köln, Germany), with the objective of comparing classic and mechanistic endpoints considered to be relevant in experimental carcinogenesis.

Appended to this chapter are the following manuscripts prepared and submitted or accepted for publication based on several completed pilot studies:

- Haussmann, H.-J., Anskeit, E., Becker, D., Kuhl, P., Stinn, W., Teredesai, A., Voncken, P., Walk, R.-A., Comparison of fresh and room-aged cigarette sidestream smoke in a subchronic inhalation study on rats, *Toxicol. Sci.* 41: 100-116 (1998). [Tab A]
- Haussmann, H.-J., Anskeit, E., Gerstenberg, B., Göcke, W., Kuhl, P., Schepers, G., Stabbert, R., Stinn, W., Teredesai, A., Terpstra, P., Tewes, F., Twelve-month inhalation study on room-aged sidestream smoke in rats (Submitted). [Tab B]
- Voncken, P., Stinn, W., Haussmann, H.-J., Anskeit, E., Influence of aging and surface contact on the composition of cigarette sidestream smoke -- Models for environmental tobacco smoke, In: Dungworth, D.L., Mauderly, J.L., Oberdörster, G. (Eds.): *Toxic and carcinogenic effects of solid particles in the respiratory tract*, Washington: ILSI Press, ILSI Monographs, pp. 637-641 (1994). [Tab C]

Concept of the Long-Term Inhalation Study

The study will correlate mechanistic data gained from intermediate biomarker assays with tumor development, as well as compare these data for the two test materials. The study is designed to investigate the test materials at toxicologically relevant concentrations that bear a more realistic relationship to human exposure.

This study will also contribute to the discussion on the biological plausibility of the tumorigenic risk purportedly attributable to ETS and DEE exposure. Mechanistic investigations on the interaction between the test model and the test materials will be an integral part of the study in order to provide a comprehensive interpretation of effects. The integration of mechanistic endpoints in long-term bioassays and the use of these data in risk assessment has been recommended by the NTP Board of Scientific Counselors (1992) and the International Agency for Research on Cancer (IARC) Working Group (1992), as well as in the Guidelines for Carcinogen Risk Assessment recently proposed by the U.S. Environmental Protection Agency (EPA) (1996).

The study has been designed to generally comply with regulatory requirements and recommendations (OECD guideline 451, 1986; NTP, 1991). There are two major exceptions from these guidelines in the present study design. The use of only two instead of three dose levels is motivated by the comparative nature of the study design; it is not the intention to perform a classical carcinogenesis study with the determination of a no-effect level. Also, doses have not been selected

based primarily on the concept of maximum tolerated dose (MTD), but rather are linked to certain multiples of realistic human environmental concentrations.

In the following, critical parameters of the study concept will be discussed.

Test Atmosphere Definitions

Environmental tobacco smoke (ETS) is a complex mixture mainly composed of aged sidestream smoke (SS) as well as of small amounts of exhaled mainstream smoke (MS) (First, 1985; Baker and Proctor, 1990). Since "real" ETS cannot be generated for long-term laboratory research, RASS will be used in the long-term study as a surrogate for ETS. It will be obtained by diluting and aging SS generated from the standard reference cigarette 1R4F in a controlled, noninert environment. International standards applying to the generation of MS will be adapted to produce SS. Generally, the same chemical compounds are found in SS, RASS, and ETS, but there are quantitative differences in the concentrations and phase distribution (Guerin et al., 1992; Voncken et al., 1994).

DEE is a complex combustion aerosol like SS. The composition of DEE depends on the fuel and lubricants used as well as on the engine and its mode of use. In the long-term study, standard fuel and lubricant will be used, and international standards for diesel engine operation and the dilution of DEE will be applied (EPA, 1990).

Both ETS and DEE are reported in the environment at similar concentrations. The most significant difference in the chemical composition of the two combustion aerosols is the insoluble carbon core of DEE particles, whereas ETS particles are almost completely soluble (Zaebst et al., 1991).

Test Atmosphere Generation

RASS will be generated from the SS of the standard reference cigarette 1R4F, a lower yield filter cigarette supplied by the University of Kentucky (Tobacco and Health Research Institute, 1990). The 1R4F is in line with current consumer preference.

Dilution and aging of SS involve physicochemical changes (e.g., particle/gas phase distribution and particle size distribution), particle losses due to deposition and adsorption on surfaces, and chemical reactions (Benner et al., 1989; Eatough et al., 1989 and 1990; Baker and Proctor, 1990). In comparison to other SS constituents, nicotine has been shown to interact most readily with surfaces (Neurath et al., 1991; Voncken et al., 1994). To quantify ETS concentrations in field studies, particle and nicotine concentrations have most frequently been determined. Due to different kinetics, the ratio of particle/nicotine mass concentrations changes with time and has thus been considered as a determinant to characterize ETS and fresh SS (Eatough et al., 1990; Nelson et al., 1992; Guerin et al., 1992; Sterling et al., 1996). Average concentration ratios of 4 up to 100 were

determined in field studies and 2 to 4 in fresh SS.¹ This ratio may be distorted at lower ETS concentrations by unaccountable portions of nonsmoke-related particles (dust).

In order to approach “real” ETS conditions as far as possible, RASS with a mean age of at least 30 min will be used in the long-term study. A mean air residence time of 30 min (corresponding to 2 air changes per hour) can be found in less ventilated rooms.

The RASS concentrations (3 and 10 $\mu\text{g}/\text{l}$) required for the long-term study may be obtained either by aging SS at the high TPM concentration and subsequent dilution to the lower concentrations or by aging SS separately at each of the RASS concentrations required. As the intention of the long-term study is to investigate a single test atmosphere at two different concentrations, the former approach is preferred.

In our previous investigations, the chemical composition of RASS was found to change with the amount and type of surface materials in the aging room (e.g., with paper or wool) (Voncken et al., 1994). In general, particles and particle-associated components decreased, while most vapor phase components remained unchanged. In order to provide reasonable aging of the SS and to optimize the reproducibility of the setup, a noninert empty room with defined characteristics will be used in the long-term study.

1. The ratio indicative of fresh SS was also found in previous subchronic and chronic inhalation studies on rats (Adlkofer et al., 1988; von Meyerinck et al., 1989; Coggins et al., 1992; Coggins et al., 1993; Rajini and Witschi, 1994; Witschi et al., 1995).

In most field studies as well as in all published experimental inhalation studies, the particle concentration has been used as the key analyte to determine the concentration of ETS and ETS surrogates. In order to be able to compare our analytical and biological results with published data, TPM concentration will be used as the key analyte in the long-term study. In addition, the particle concentration has been the key analyte in all DEE inhalation studies as well as the basis for DEE risk assessment.

DEE will be generated using standard fuel and lubricant and a passenger car engine frequently in use in Europe. The engine will be driven using a standardized protocol to simulate city driving behavior.

Fresh DEE will be used as the test atmosphere to keep comparability with previously published DEE long-term inhalation studies (Karagianes et al., 1979; Iwai et al., 1986; Ishinishi et al., 1986; Vallyathan et al., 1986; Mauderly et al., 1987; Brightwell et al., 1989; Heinrich et al., 1986, 1992, and 1995; Nikula et al., 1995). Fresh DEE may be less environmentally relevant but its use in long-term inhalation studies is consistent with published literature.

A comparison of DEE composition in published inhalation studies revealed some differences. The most important difference may be the proportion of elemental carbon in the particulate phase. In the most recent published long-term DEE inhalation studies, elemental carbon was reported to be 60 % (Heinrich et al., 1995) and 92 % (Nikula et al., 1995) of TPM. It is well known that the proportion of elemental carbon in DEE particulates fluctuates (Hering et al., 1990;

Watts, 1995). Major factors contributing to this fluctuation include, apart from emission control devices, the engine type, duty cycle, fuel, and lubricant consumption. Elemental carbon generally accounts for about 40 to 60 % of DEE particulate matter mass (Klingenberg et al., 1991; Zaebst et al., 1991).

As for SS, DEE concentrations in experimental studies are commonly based on particulate matter mass as collected on glass fiber filters. Sometimes, soot seems to be synonymously used.

Dose Levels

According to the principle of maximum tolerated dose (MTD), the highest test substance dose in a carcinogenicity study should be “sufficiently high to elicit signs of minimal toxicity without substantially altering the normal life span due to effects other than tumors. Signs of toxicity are those that may be indicated by alterations in certain serum enzyme levels or slight depression of body weight gain (less than 10 %)” (OECD Guideline 451, 1986). Setting the upper limit of doses to the MTD level should prevent toxicity from substantially interfering with tumorigenicity. On the other hand, dosing as high as this limit should prevent false negative carcinogenicity studies.

Historically, the MTD has been frequently determined in pilot subchronic/90-day studies, and histopathological lesions other than those that may be related to carcinogenesis were

also considered (Morrow et al., 1996). However, in only approximately 20 % of the NTP inhalation studies, reduced body weight was used as the rationale for selecting the highest dose. In one study, a multiple of the human therapeutic dose was applied. In the long-term studies completed by the NTP, only 60 % had reduced body weight at the highest dose, and the magnitude of these body weight effects did not correlate with the carcinogenic response of the test substances. This is in line with recent trends within the NTP in the interpretation of the MTD concept, i.e., from the “maximum tolerated dose” to the “minimally toxic dose,” which would also consider slight body weight effects in the long-term study as minimally toxic. A special workshop dealt with establishing a rationale for aerosol exposure concentrations in long-term inhalation studies (Lewis et al., 1989). Emphasis was given to substances which are relatively insoluble and of low systemic and respiratory tract toxicity. This workshop concluded that the highest dose should only minimally affect pulmonary clearance. This is in line with the concept of a functionally defined MTD (MFTD) for highest exposure concentrations in long-term bioassays (Muhle et al., 1990a), which should permit the extrapolation of observed biological effects to realistic concentrations to which humans are exposed. The MFTD concept considers biokinetic and mechanistic aspects (NTP Board of Scientific Counselors, 1992). However, more specific recommendations with regard to the type or degree of respiratory tract responses that constitute evidence of an appropriate minimally toxic response or an MFTD were not made (Lewis et al., 1989; Morrow et al., 1996). Especially, the relevance of particle overload in the lungs and the resulting inflammatory response and cell proliferation with regard to risk assessment remains open (Oberdörster, 1995).

Altered serum enzyme levels or hematological changes as signs of toxicity were not found in a subchronic SS inhalation study at a TPM concentration of 4 $\mu\text{g}/\text{l}$ (Adlkofer et al., 1988). At concentrations of up to 10 $\mu\text{g}/\text{l}$, no clinical signs of toxicity were found using “aged and diluted SS” (Coggins et al., 1993).

The rationale for the selection of the SS concentration of 4 $\mu\text{g TPM}/\text{l}$ in one fully published long-term SS inhalation study was not made quite clear (Witschi et al., 1995). At this concentration, there was no significant effect on body weight. In a previous (pilot?) study performed by the same laboratory, cell proliferation was observed in bronchi and bronchioli at 1 $\mu\text{g TPM}/\text{l}$ (Rajini and Witschi, 1994). The same effects as well as cell proliferation in the nasal epithelia were seen during the initial part of the chronic study (4 $\mu\text{g TPM}/\text{l}$). Obviously, the dose for the chronic study was selected based on the response in the pilot study of biomarkers considered to be related to carcinogenesis. Taking this approach for the dose selection, i.e., subchronic responses in biomarkers related to carcinogenesis, a RASS dose of 10 $\mu\text{g TPM}/\text{l}$ for the present study would seem to be justified based on the biomarker responses noted above. In a second study, Witschi et al. (1997) used a TPM concentration of roughly 90 $\mu\text{g}/\text{l}$.

In the long-term study on female rats performed by Heinrich et al. (1995), the body weight of the high dose DEE group started to deviate significantly from the control on study day 200 (7 $\mu\text{g TPM}/\text{l}$, 18 hours/day, 5 days/week, weekly particle dose: 630 hours $\times \mu\text{g}/\text{l}$). The final body weight difference was 17 %. Nikula et al. (1995) found a body weight gain reduction of approximately 10 % for both sexes following about 180 days of inhalation (6.5 $\mu\text{g TPM}/\text{l}$, 16

hours/day, 5 days/week weekly particle dose: 520 hours x $\mu\text{g/l}$). This body weight gain reduction did not further increase for the male rats but increased to approximately 20 % for the female rats following approximately 500 days of inhalation. Thus, these long-term DEE inhalation studies which were both positive for lung tumors do not meet the historical MTD definition (10 % body weight gain reduction within 90 days), but are in line with the minimally toxic dose concept (body weight differences at chronic time points).

The survival of DEE-exposed rats in the study by Heinrich et al. (1995) was not affected, as was the case for female rats in the study by Nikula et al. (1995). However, the male rats in the latter study showed reduced survival in the high dose group.

Slight changes in serum clinical chemical and hematological data were reported following DEE inhalation (Ishinishi et al., 1986; Lewis et al., 1986). The relevance of these changes is unclear. These parameters have not been regularly investigated in long-term DEE inhalation studies.

DEE inhalation at the high doses mentioned above reproducibly resulted in an impairment of the macrophage-associated particle clearance from the lungs, which is connected to a particle overload phenomenon (Proceedings of Symposium on Particle Lung Interactions: Overload Related Phenomena, 1990; HEI, 1995). According to a current hypothesis, the overloaded macrophages attract PMNL to the alveolar lumen as an inflammatory response. This persisting inflammation may ultimately result in hyperplastic and metaplastic changes leading to lung fibrosis

and carcinogenesis. The definition of an MTD or MFTD as well as the relevance of tumors arising under particle or other overload conditions for human risk assessment is under discussion. Excessive particle overload as found in high dose long-term DEE inhalation studies is certainly no condition which can be readily extrapolated to the human situation. On the other hand, prolonged particle clearance as well as associated inflammation, cell proliferation, and fibrotic changes can also be found in humans, such as coal workers, occupationally exposed to particles Oberdörster, 1995).

Deposition of soot particles in the lungs, carbon-loaded alveolar macrophages, and influx of PMNL and lymphocytes were also observed in our subchronic DEE pilot inhalation study. The magnitude of these biomarker responses suggests that the dosing regimen selected would be sufficient to elicit lung tumors in a long-term study.

The tumorigenic response to DEE in long-term inhalation studies on rats varies from 4 (spontaneous tumor prevalence) to 54 % lung tumor prevalence at approximately $7 \mu\text{g}$ particles/l (Karagianes et al., 1979; Brightwell et al., 1986; Mauderly et al., 1986; Heinrich et al., 1992). This large variance has been mainly attributed to differences in the exposure regimen (Heinrich et al. 1986), but major differences in test atmosphere generation are also probable. For weekly particle doses, a threshold of approximately 120 hours $\times \mu\text{g}/\text{l}$ has been suggested for lung tumorigenicity (Nikula et al., 1995). The lung soot burden is generally considered to be the major determining factor for particle-associated tumor responses. No relevant difference in the lung particle burden or associated effects were found when changing the daily exposure pattern or rate of delivery (Henderson et al., 1992). Thus, the relatively short daily exposure duration applicable to head-only

exposure can be compensated by increasing the DEE particle concentrations over those usually used in whole-body exposure studies for longer daily durations.

Taking together the foregoing discussion on the applicability of the MTD concept to the present test atmospheres, the dose levels chosen seem to be compatible with the minimally toxic dose concept. Another aspect for setting dose levels is the intention to correlate the responses of selected biomarkers to the lung tumor response. This requires the use of doses which have been shown to be positive in previously published long-term inhalation studies. This rationale fully applies to DEE. It cannot be applied to RASS, since only few data are available on the long-term responsiveness of mice (Witschi et al., 1995; Witschi and Pinkerton, 1996; Witschi et al., 1997) but none on rats.

Maximum mean ETS concentrations in terms of smoke-related, respirable suspended particles (RSP) are reported to be approximately $0.1 \mu\text{g}/\text{l}$ in residences, offices, transportation vehicles, or other places where smoking occurs (EPA, 1992; Guerin et al., 1992). $600\text{-}1000 \mu\text{g}/\text{m}^3$ seems to be the upper limit for the most extreme ETS concentrations reported for all types of occupied spaces. In the present study, the highest RASS particle concentration of $10 \mu\text{g}/\text{l}$ will exceed extreme human exposure concentrations by a factor of 10 and typical concentrations by a factor higher than 100. For the low dose group of this study, a TPM concentration of $3 \mu\text{g}/\text{l}$ will be used. As for the planned high RASS concentration, the high DEE concentration in the present study will exceed extreme and normal mean environmental DEE concentrations by factors of 10 and 100,

respectively (Woskie et al., 1988; Lehmann, 1991; Pott, 1991; Elbers and Muratyan, 1991; Kühn and Bireft, 1992; projected ambient concentrations by McClellan, 1986; Brightwell et al., 1989).

Due to the comparative nature of the present study design as well as the relationship of the doses to reported environmental concentrations, the use of the same particle concentrations for both test atmospheres is considered straightforward.

Animal Model

Rats, mice, and hamsters have been preferred in carcinogenicity studies “because of their relatively short life span, . . . their widespread use in pharmacological and toxicological studies, their susceptibility to tumor induction, and the availability of sufficiently characterized strains” (OECD Guideline 451, 1986). The same recommendation was made by Lewis et al. (1989), particularly for aerosol inhalation studies.

For head-only exposure, rats are technically more suitable than mice and hamsters. This fact and our long experience with the rat in inhalation studies recommend its use in this long-term inhalation study. The mouse could be considered as a second species for possible future work.

In order to facilitate the detection of a low prevalence of induced tumors in the test groups, the spontaneous tumor prevalence in target organs should be as low as possible. The spontaneous prevalence of nasal cavity tumors seems to be negligible (i.e., <1 %) in the three rat

strains considered. However, approximately 4-fold spontaneous lung tumor prevalences were reported for the Fischer 344 rat (Goodman et al., 1978; Solleveld et al., 1984; Haseman et al., 1985) compared to the Sprague Dawley (MacKenzie and Garner, 1973; Prejean et al., 1973) and Wistar rat (Vandenberghe, 1990; Kroes et al., 1988; Deerberg et al., 1980, 1982; Rehm et al., 1984; Ueberberg and Luetzen, 1979; Takizawa and Miyamoto, 1976; Boorman and Hollander, 1973; Bomhard et al., 1986). Fischer 344 rats have been used in carcinogenicity studies performed by the NTP, a preference which was based on the availability of historical data rather than scientific reasons (Gregory, 1992).

Test animals should be exposed to the test material for a major portion of their life span (OECD Guideline 451, 1986). Survival in all groups should be not less than 50 % at 24 months for rats in order for a negative test result to be accepted. In past years, the longevity of laboratory rat strains has decreased substantially, possibly due to breeding targets for rapid growth (White, 1992). The Sprague Dawley rat, which has been used in our previous MS and SS inhalation studies, is no longer considered to fulfill the above requirement (British Society of Toxicological Pathologists, 1992; White, 1992; Mariani et al., 1992). In addition, the increasingly high body weight and associated large size of adult Sprague Dawley rats do not recommend their use in a long-term head-only inhalation study.

Based on the reported low spontaneous lung tumor prevalence, the sufficient longevity and the body weight development suitable for head-only exposure, the Wistar rat seems to be particularly appropriate for the proposed long-term inhalation study. The suitability of the

Wistar rat was corroborated in a recent historical control study. In addition, besides the Fischer 344 rat, the female Wistar rat was used in previous DEE inhalation studies (Heinrich et al., 1986, 1995).

Exposure Regimen

The head-only or nose-only exposure modes are most appropriate for rat inhalation studies with aerosols (Pauluhn, 1984; Phalen et al., 1984; Hahn, 1993). These modes diminish test substance deposition on the fur of the animals and subsequent dermal or oral uptake by grooming, which was observed following whole-body exposure (Wolff et al., 1982; Iwasaki et al., 1988; Mauderly et al. 1989; Chen et al., 1995). In a mainstream cigarette smoke inhalation study, the two exposure modes were compared: plasma and urinary nicotine concentrations were 5- to 6-fold higher in whole-body compared to nose-only exposed rats when normalized to the nicotine concentrations in the test atmospheres (Mauderly et al., 1989). In whole-body exposure, the filtering of the test atmosphere by the fur may impair reproducible uptake by inhalation. In addition, the test atmosphere is in contact with the animal excretion products. In previous studies we found that nicotine and reactive test atmosphere components such as formaldehyde are efficiently trapped by urine and feces.

Thus, to enhance the controllability of the test atmosphere administration to the rat as well as the route of the test substance uptake by the rats, the head-only exposure mode will be used in the present study.

In inhalation studies, "long-term exposures are usually patterned on projected industrial experience, giving the animals a daily exposure of 6 hours . . . for 5 days a week (intermittent exposure)" (OECD Guideline 451, 1986). In all published DEE inhalation studies with rats, whole-body exposure was used. The daily exposure duration lasted up to 19 hours/day (Heinrich et al., 1986). Head-only exposure should not last longer than 7 hours/day due to the restraint of the rats and other technical reasons. Recently published inhalation studies on man-made vitreous fibers used head-only exposure for 6 hours/day, 5 days/week, 24 months (Smith et al., 1987; Hesterberg et al., 1993). In order to maximize our weekly doses, the present study will be conducted for 6 hours/day and 7 days/week.

Depending on the longevity of the rat strain used, it is recommended to terminate carcinogenicity studies after 24 or 30 months of exposure (OECD Guideline 451, 1986). Studies with DEE-exposed rats demonstrated that nearly 80 % of the exposure-related tumors were observed later than 24 months of inhalation (Mauderly et al., 1987). However, for the expression of tumors in this late stage of the rats' lifespan, a continuation of the inhalation period over 24 months does not seem to be necessary, since the particle load will not be substantially cleared under these conditions due to a complete loss of clearance. No information is available for SS-induced pulmonary tumorigenicity in rats, but Witschi et al. (1997) followed a similar exposure regimen for their A/J mice, i.e., inhalation followed by a postinhalation observation period. For the present study, an inhalation period of 24 months followed by a postinhalation period of 6 months is planned.

Size of Experimental Groups

Fifty animals per sex and exposure group are considered to be sufficient to adequately evaluate a long-term bioassay (OECD Guideline 451, 1986). Assuming a spontaneous tumor prevalence of 2 %, the 51 rats per group and sex planned for the present study would be sufficient to detect an increased tumor probability of 16 % (1st order error $\alpha = 0.05$, 2nd order error $\beta = 0.10$). This is in the range of the expected lung tumor prevalence in the high dose DEE group based on the comparison of our planned weekly high DEE particle dose with published results (Karagianes et al., 1979; Iwai et al., 1986; Ishinishi et al., 1986; Vallyathan et al., 1986; Mauderly et al., 1987; Brightwell et al., 1989; Heinrich et al., 1986, 1992, and 1995; Nikula et al., 1995). Combining the results from both sexes would even allow detection of an increased tumor probability of 10 %.

Mechanistic Endpoints

In evaluating the carcinogenic risk of test materials to humans or establishing relative potencies of toxicity and carcinogenicity between species or test materials, increasing emphasis is being placed on mechanistic investigations (NTP Board of Scientific Counselors, 1992; IARC Working Group, 1992; EPA, 1996). Of special interest are preneoplastic changes that might be present before tumor manifestation or at concentrations lower than those which would be required for tumor development. These mechanistic investigations may comprise studies on pharmacokinetics, including target organ dose monitoring; genotoxicity including mutations and repair mechanisms; cell proliferation; cell differentiation; immunosuppression; and inflammatory

or fibrotic processes. Especially for particle inhalation studies, deposition and/or clearance studies are recommended (Lewis et al., 1989).

In the present study, in addition to classical pathology, several endpoints are planned to be investigated which are thought to be mechanistically related to chronic disease and chemical carcinogenesis in the respiratory tract. The reasons for choosing these endpoints is the current mechanistic understanding of the processes under investigation as well as in-house scientific expertise. The extent of these investigations is limited by the available number of rats in a long-term head-only inhalation study.

Biomonitoring for both RASS and DEE will include the determination of the carboxyhemoglobin proportion in the blood and of the steady-state content of aminobiphenyl adducts to hemoglobin. For RASS, nicotine metabolites in urine will serve as a specific monitor. If possible, TSNA-derived hemoglobin adducts will also be investigated. In addition, the green autofluorescence of alveolar macrophages is considered for use as an estimate for the steady-state lung particle dose. The feasibility of this parameter has not yet been fully validated. .

The determination of the lung burden of inhaled particles or the pulmonary clearance efficacy are mandatory in chronic inhalation studies using particle-containing aerosols (Lewis et al., 1989). For DEE lung burden, nonlinear time-response relationships but linear dose-response relationships were found (McClellan, 1986; Heinrich et al., 1992). The nonlinearity has been

attributed to particle overload which impairs pulmonary clearance. For SS-exposed rats, particle overload has not been published.

The oxidative modification of deoxyguanosine by forming 8-OHdG is a rather frequent event due to the permanent cellular oxidative stress (Fraga et al., 1990). Therefore, efficient repair mechanisms are set in operation which, in conjunction with the impaired catabolism of the modified base, result in the urinary excretion of 8-OH-dG. Guanidine oxidation reportedly leads to base mispairing, resulting in G:C to T:A transversions (Cheng et al., 1992). In some experimental systems, a correlation between the presence of 8-OH-dG in DNA and tumor development was observed (Floyd, 1990). This endpoint has never been investigated in long-term DEE inhalation studies to date. In the present study, both the excretion of 8-OH-dG in urine during inhalation as well as the respiratory tract tissue level of this base modification will be investigated.

Bulky DNA adducts were observed in a subchronic SS inhalation study at 10 μ g TPM/l in lungs, heart, and larynx tissue using the 32 P-postlabeling technique (Lee et al., 1993; Brown et al., 1995). This effect was not observed at lower concentrations. This type of adduct could not be found in ETS-exposed nonsmokers (Scherer et al., 1993) but has been described for smokers (Phillips et al., 1990). DNA adducts were also detected in rat lungs following subchronic DEE inhalation (Bond et al., 1990). Although the 32 P-postlabeling technique seems to be the most sensitive method to detect DNA adducts, it lacks specificity. In the present study, DNA adducts will be evaluated. For this purpose, we prefer to use specific mass spectrometric methods which still

have to be established in our laboratory. If the latter is not possible, samples will be extramurally analyzed by the ³²P-postlabeling technique.

Classical histopathology remains a basic endpoint for use in the present study. Apart from the microscopic confirmation and classification of tumors, persistent hyperplastic, metaplastic, and dysplastic tissue changes are considered as essential indicators of preneoplastic and neoplastic lesion induction. In subchronic inhalation studies on SS, hyperplasia and metaplasia of nasal and laryngeal epithelia were found in the rat (von Meyerinck et al., 1989; Coggins et al., 1993; Teredesai and Prühs, 1994). Due to the general reversibility of these findings after cessation of the SS inhalation, these changes have been considered an adaptive response to the irritating activity of the test atmosphere (Burger et al., 1989).

Mutations are a prerequisite for initiating carcinogenesis, and most probably also play a role in epigenetically induced carcinogenesis such as by particle overload (Driscoll et al., 1996). Apart from the latter study, there is limited expertise in the detection of early mutations in rat long-term studies. The single-strand DNA conformation assay can be used to detect unknown mutations. Recently, we have been able to increase the sensitivity of this assay by several orders of magnitude. The attempt to further increase the assay sensitivity was limited by the fidelity of the DNA polymerase used in the polymerase chain reaction to amplify DNA probes. The present assay sensitivity is not considered to be sufficient enough to detect early mutations to fulfill the function of an intermediate biomarker. However, the assay will contribute to tumor differentiation in the final part of this study. Emphasis will be given to mutations in the *p53* tumor suppressor gene. Mutations

of this gene can be found in about half of all human cancer cases. The location and characteristics of these mutations may reveal clues about their etiology. The predominant base changes in *p53* in human lung cancers (G:C to T:A transversions) were suggested to be indicative of causal lesions on the nontranscribed DNA strand by polycyclic aromatic hydrocarbons (Harris, 1993). Possible correlations of *p53* mutations with cigarette smoking have recently been discussed (Suzuki et al., 1992; Spruck et al., 1993; Habuchi et al., 1993; Brennan et al., 1995). The mutational activation of the protooncogene *ras*, another endpoint frequently associated with lung tumor development (Carbone and Minna, 1992), was not considered for the present study since the rat seems to be less sensitive to *ras* mutations by agents positive in the mouse and hamster such as N-nitrosamines (Belinsky et al., 1990).

Activation of cell proliferation, in conjunction with changes at the DNA level, is considered to be essential for initiation and tumor development. Cell division is necessary for conversion of adducts or DNA strand breaks to mutations or gaps, and also allows for mitotic recombination. However, the direct correlation between increased cell proliferation and development of neoplasia in target organs or morphological sites has been questioned (Yoshida et al., 1993). Cell proliferation, which does not give rise to formation of neoplasia, may simply be induced by the cytotoxicity of the test material in the absence of initiation. On the other hand, persistent cell proliferation may increase the probability of converting spontaneous DNA lesions to neoplastic changes. In subchronic inhalation studies on aged and diluted SS, an increased incorporation of BrdU into the rat nasal respiratory epithelium was found at a concentration of 10 μg TPM/l (Brown et al., 1995). Following a shorter period (5 days) of inhalation, the effect was

already seen at 1 $\mu\text{g/l}$. A similar pattern of response was found for the A/J mouse at concentrations of 1 and 4 $\mu\text{g TPM/l}$ (Rajini and Witschi, 1994; Witschi et al., 1995), whereas the C57BI/6 mouse did not respond. Following DEE inhalation, a transient initial increase in rat lungs was described by Wright (1986). Following chronic DEE inhalation, increased cell proliferation measured by ^3H -thymidine incorporation was detected in the bronchi and bronchioli as well as at particular sites in rat lungs (McClellan et al., 1986). Cell proliferation will be investigated in the present study using BrdU incorporation.

Cytokeratins have been used for the differential characterization of preneoplastic and neoplastic changes in epithelial tissues (Broers et al., 1988; Moll et al., 1988; Lindberg and Rheinwald, 1989; Schaafsma et al., 1990; Smedts et al., 1990). In contrast to the well-defined human cytokeratin expression patterns, those of the rat have been less extensively characterized. Alterations in cytokeratin expression were found to precede the histological expression of squamous metaplasia in several epithelial tissues in vitamin A-deficient rats (Gijbels et al., 1992). Recently, rat lung tumor types could be differentiated using monoclonal antibodies to human cytokeratins (Kal et al., 1993).

Inflammatory and fibrotic processes have often been associated with particle overload and carcinogenesis (Heinrich et al., 1986; Henderson et al., 1988; Muhle et al., 1990b; Morrow, 1992; Oberdörster, 1995). The PMNL proportion of lavagable bronchoalveolar cells as a sign of persistent acute inflammation was found to increase time- and dose-dependently following chronic exposure to DEE or toner.

Lactate dehydrogenase (LDH) activity is a cellular enzyme which is commonly determined extracellularly, e.g., in the supernatant of lung lavage, as a measure for cell lysis or cytotoxicity. Increased LDH activity was found in the lavage of DEE-exposed rats (Henderson et al., 1988) and may either be ascribed to alveolar macrophage lysis or to damage to the alveolar epithelium subsequent to particle overload. The determination of lung lavage LDH activity will enhance the interpretation of data in the present study.

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Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 3 -

**4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a
tobacco-specific *N*-nitrosamine, and funded research
on the metabolism of NNK**

March 20, 1998

Background

Based on experimental data available prior to 1984, the International Agency for Research on Cancer (IARC) concluded that “There is *sufficient evidence* for the carcinogenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to experimental animals,” but “No data on humans were available” (IARC, 1985). In a footnote it was stated: “In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals as if they presented a carcinogenic risk to humans.” As far as we are aware, no regulatory agency has further evaluated the carcinogenicity of this compound to humans.

Following internal company review of all scientific literature published after the IARC assessment on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), it was concluded that:

- NNK is a carcinogen in the A/J mouse, F344 rat, and Syrian golden hamster, inducing mainly tumors of the lung, and to a lesser extent, tumors of the liver.
- Extensive evidence exists to demonstrate that human metabolism of NNK differs significantly from that observed in laboratory animals. However, relevant data have not been obtained under identical experimental conditions to allow a valid comparison of the metabolism of NNK in laboratory animal and human tissues.

- Current data do not support the assumption that NNK, present in tobacco products and cigarette smoke, induces lung and liver tumors in man.

This section of our submission briefly reviews published experimental data which formed the basis for our conclusions. To obtain additional scientific data on the metabolism of NNK in different animal species which would be relevant for toxicological assessment of the potential biological activity of this compound to man, Philip Morris is currently funding a research project at the Walther-Straub Institute for Pharmacology and Toxicology, University of Munich, Germany. This research will provide comparative experimental data on the *in vitro* metabolism of NNK in lung and liver of the A/J mouse, F344 rat, Syrian golden hamster, and man.

Introduction

N-Nitroso compounds represent a large diverse group of chemicals of which some, but not all, have been shown to induce a wide range of tumors in experimental animal models (Preussmann and Steward, 1984). According to recent analytical data, total human exposure to exogenous *N*-nitrosamines is estimated to be 1.10 $\mu\text{mol/day}$; the major sources are the diet (0.79 $\mu\text{mol/day}$, 80-120 $\mu\text{g/day}$; 72%), occupational exposure (0.15-0.30 $\mu\text{mol/day}$; 25%), cigarette smoking (0.02 $\mu\text{mol/day}$, 3.4 $\mu\text{g/day}$; 2%), and miscellaneous minor sources, including pharmaceutical products, cosmetics, indoor and outdoor air (0.001 $\mu\text{mol/day}$, 0.1 $\mu\text{g/day}$; 1%) (Tricker, 1997). Cigarette smoking accounts for only 2% of the estimated total exogenous exposure to *N*-nitroso compounds; however, tobacco-specific *N*-nitrosamines (TSNA), as their name implies,

are considered to occur only in tobacco and tobacco smoke (Hecht and Hoffmann, 1989). To date seven different TSNA derived from the nitrosation of nicotine and other minor tobacco alkaloids have been identified (Amin et al., 1995). [Figure 1] (The following abbreviations will be used in addition to NNK: NNN, *N*-nitrosornicotine; NAB, *N*-nitrosoanabasine; NAT, *N*-nitrosoanatabine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; iso-NNAL, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; and iso-NNAC, 4-(methylnitrosamino)-1-(3-pyridyl)butyric acid.)

TSNA Present in Mainstream Cigarette Smoke and Indoor Air

The U.S. National Academy of Sciences has previously estimated that a smoker of domestic filter cigarettes has a total *N*-nitrosamine exposure of 16.2 $\mu\text{g}/\text{day}$ (6.1 μg NNN and 2.9 μg NNK), based on the assumption that the average smoker consumes 20 cigarettes/day (Assembly of Life Sciences, 1981). This exposure estimate was based on unpublished analytical data provided by Hecht and Hoffmann of the American Health Foundation, Valhalla, NY. More recent data for filter cigarettes [Table 1] yields a lower exposure estimate of 3.4 $\mu\text{g}/\text{day}$ (1.5 μg NNN and 1.0 μg NNK) (Tricker, 1997).

Since TSNA are also transferred to sidestream cigarette smoke (Adams et al., 1987), and presumably exhaled by smokers, trace levels occur in environmental tobacco smoke (ETS) present in indoor air (Brunnemann et al., 1992; Klus et al., 1992; Tricker et al., 1994). Extensive smoking under poor ventilation conditions results in mean ETS concentrations of 2.8 ± 1.6 (range n.d.-6.0) ng/m^3 NNN and 4.9 ± 9.6 (range n.d.-13.5) ng/m^3 NNK (Klus et al., 1992). Similar levels

Figure 1. Proposed formation of tobacco-specific *N*-nitrosamines.

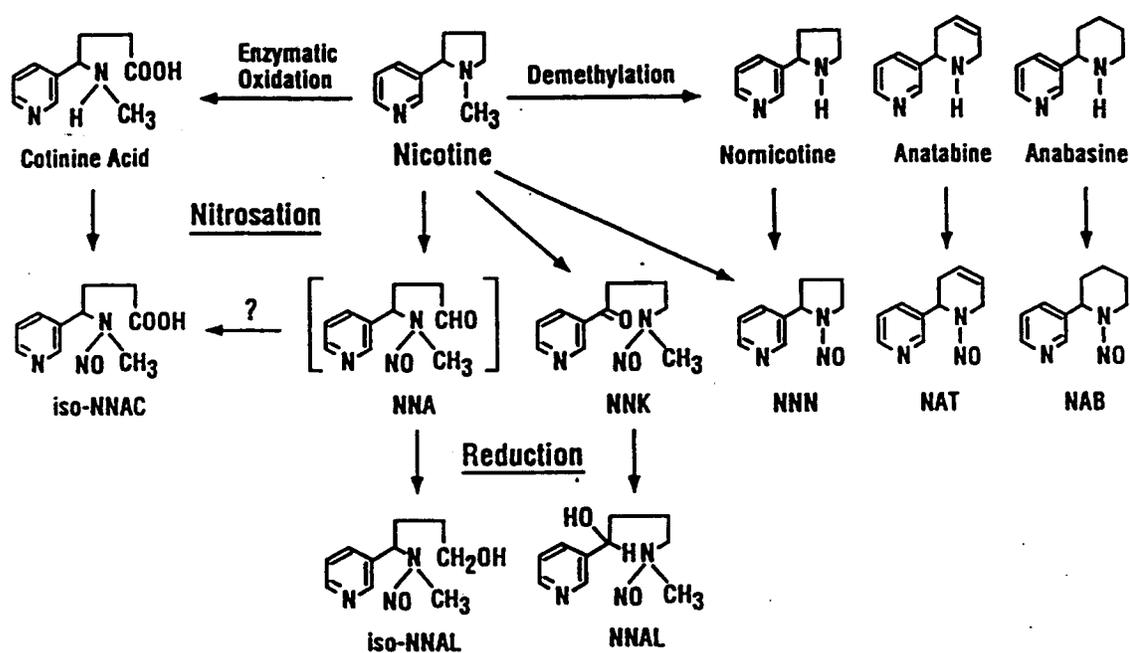


Table 1. Estimates of exposure to TSNA in smokers.

TSNA	Exposure estimate ($\mu\text{g}/\text{day}$) For a smoker of 20 filter cigarettes		
	1981 ¹	1991 ²	1994 ³
NNK	3.0	1.0	1.6
NNN	6.2	1.5	1.0
NAB	--	--	0.2
NAT	7.4	--	1.8
NAB/NAT	--	1.5	--

-
1. Academy of Life Sciences (1981).
 2. Tricker et al. (1991).
 3. Hoffmann et al. (1994).

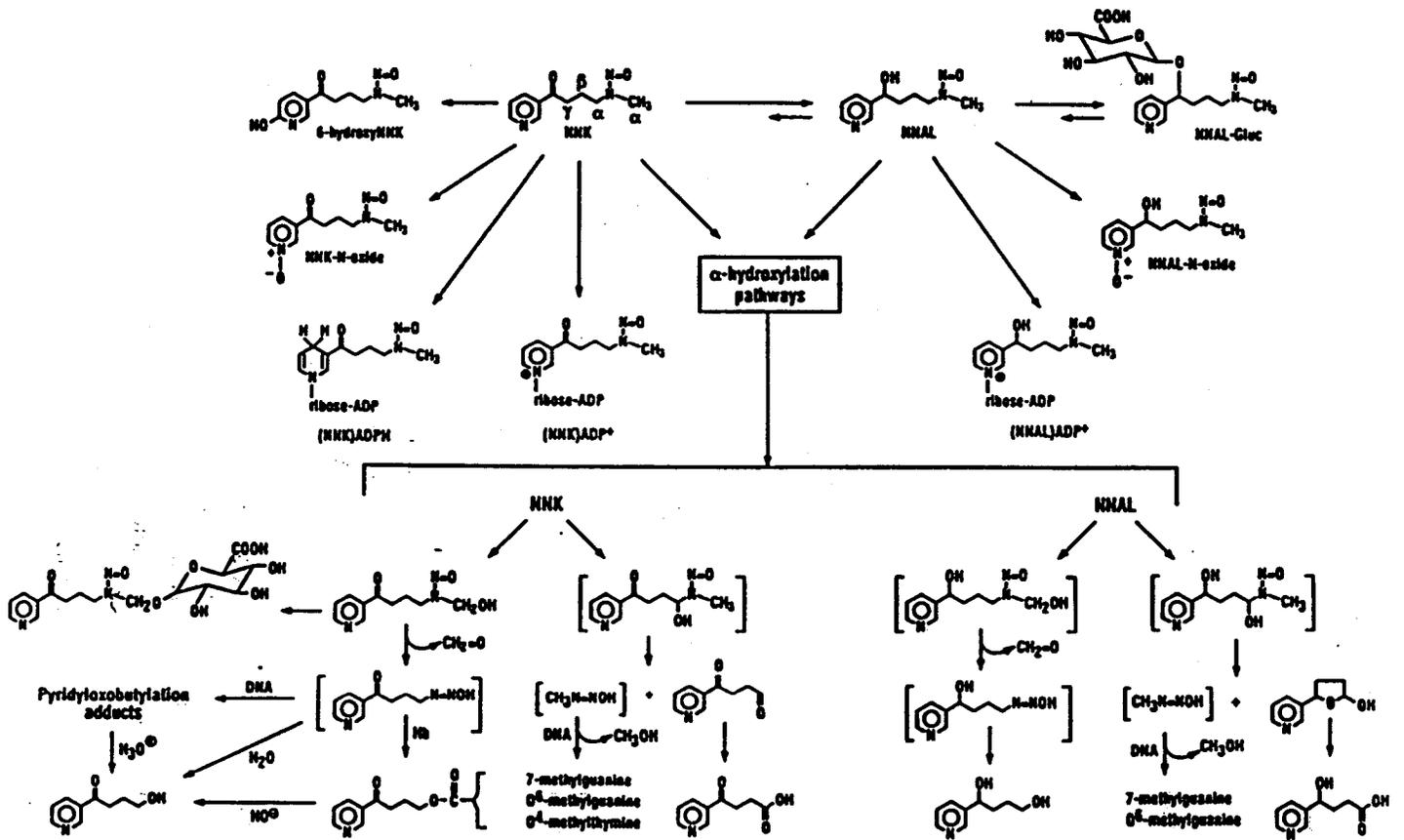
occur in the home; 0.8 ± 1.2 (range n.d.-3.3) ng/m^3 NNN and 4.0 ± 4.6 (range n.d.-14.3) ng/m^3 NNK (Tricker et al., 1994), and other venues (Brunnemann et al., 1992).

Putative Metabolism of NNK in Laboratory Animals

The major reported pathways of NNK metabolism in experimental animals involve carbonyl reduction, α -hydroxylation of the methylene and methyl groups adjacent to the *N*-nitroso group, and pyridine-*N*-oxidation [Figure 2].

Carbonyl reduction of NNK to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is probably a detoxification pathway of NNK metabolism since it provides the functional hydroxy moiety necessary for glucuronidation to [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]- β -*O*-D-glucosiduronic acid (NNAL-Gluc) (Maser et al., 1996; Kim and Wells, 1996). α -Hydroxylation of the NNK methyl carbon results in the formation of an unstable intermediate which spontaneously decomposes to yield formaldehyde and 4-(3-pyridyl)-4-oxobutanediazohydroxide, a potential pyridyloxobutylating agent, which can react with water to yield 4-hydroxy-1-(3-pyridyl)-1-butanone (keto alcohol). α -Hydroxylation of the methylene group in NNK produces 4-hydroxy-4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone which spontaneously decomposes to methane diazohydroxide, a potential methylating species, and stable 4-oxo-1-(3-pyridyl)-1-butanone (keto aldehyde), which is further oxidized to 4-oxo-4-(3-pyridyl)butyric acid (keto acid). Glucuronidation of 4-((hydroxymethyl)nitrosamino)-1-(3-pyridyl)-1-butanone, the initial

Figure 2. Putative metabolism of NNK in laboratory animals (Hecht, 1996).



intermediate formed in the “keto alcohol” pathway, may be a detoxification route (Murphy et al., 1995).

α -Hydroxylation of the methylene and methyl groups adjacent to the *N*-nitroso group in NNAL produces unstable metabolic intermediates before ultimately forming stable 4-hydroxy-4-(3-pyridyl)butric acid (**hydroxy acid**) and 4-hydroxy-1-(3-pyridyl)-1-butanol (**diol**). α -Hydroxylation of the methyl group and ultimate formation of the diol is a putative detoxification pathway since this pathway has not been reported to result in adduct formation, while α -hydroxylation of the methylene group (“hydroxy acid” pathway) can potentially result in methylation of cellular macromolecules. NNAL is a poor substrate for α -hydroxylation compared to NNK (Hecht and Trushin, 1988; Belinsky et al. 1989; Staretz et al. 1997a), and consequently exhibits less biological activity than NNK (Liu et al., 1990; Castonguay et al., 1983a; Hoffmann et al., 1993).

Pyridyl-*N*-oxidation of both NNK and NNAL to yield 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone (**NNK-*N*-oxide**) and 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanol (**NNAL-*N*-oxide**), respectively, are considered to be detoxification pathways of NNK metabolism (Liu et al., 1990; Castonguay et al., 1983a; Hecht, 1994; Staretz et al., 1997a).

The determination of hydroxy acid, keto acid and keto alcohol as stable end products of NNK metabolism by α -hydroxylation pathways represents the metabolic activation of the host to produce reactive intermediates with the potential to form adducts with DNA or other cellular macromolecules.

Urinary NNK Metabolite Excretion in Laboratory Animals and Man

Urinary excretion profiles provide evidence of species-dependent differences in metabolic activation of NNK by α -hydroxylation and detoxification [Table 2].

Urinary NNK metabolite profiles in the A/J mouse and F344 rat (Morse et al., 1990) are highly dose dependent [Table 3]. Similar studies have not been performed in other animal species. The data in Table 3 indicate that α -hydroxylation pathways account for about 50% of NNK metabolism in the A/J mouse and F344 rat, regardless of the administered dose. At the lowest administered dose of NNK (1 μ g/kg body weight), excretion of NNAL and NNAL-Gluc does not occur in either species, although NNAL is apparently formed and further metabolized by pyridyl-N-oxidation. NNK-N-oxide excretion increases with decreasing administration of NNK.

Human biomonitoring studies report the presence of NNAL, NNAL-Gluc and NNAL-N-oxide in 24-h urine of smokers maintaining constant smoking habits (Hecht et al. 1995; Carmella et al., 1997). NNK-N-oxide is not a urinary metabolite of NNK in smokers (Carmella et al., 1997). Other metabolites of NNK detected in experimental animal excretion studies, such as stable end products of α -hydroxylation, are not specific to NNK metabolism since they are also formed during metabolism of NNN and nicotine. Combined urinary excretion of NNAL, NNAL-Gluc and NNAL-N-oxide in urine of smokers (Hecht et al., 1995; Carmella et al., 1997), when calculated in molar equivalents of NNK, balances well with predicted total NNK exposure [Table 4]. These data

Table 2. Urinary excretion of NNK metabolites in different species.

% urinary excretion of NNK metabolites

Metabolite	A/J mice ¹ 0.1 mg/kg i.p. 48-h excretion	F344 rat ² 0.1 mg/kg i.p. 24-h excretion	F344 rat ¹ 0.1 mg/kg i.p. 48-h excretion	Patas monkey ³ 0.1 mg/kg i.v. 24-h excretion
Hydroxy acid*	34	16.0	28	41.9-42.9
Keto acid*	19	37.8	21	24.6-26.6
Keto alcohol	--	<0.06	--	--
NNAL	1	3.3	7	n.d.-2.0
NNAL-Gluc	3	0.6	2	19.1-19.9
NNK-N-oxide	8	11.7	7	13.6-15.7
NNAL-N-oxide	14	11.4	16	7.7-15.7
NNK	--	0.8	--	n.d.-0.1
6-hydroxy-NNK	--	1.0	--	--
Total α -hydroxylation*	53	54	49	58.1

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1. Morse et al. (1990).
 2. Murphy et al. (1995).
 3. Hecht et al. (1993a).

Table 3. Dose response for excretion of NNK metabolites.¹

Metabolite	% 24-h excretion of NNK metabolites at different dose levels (mg/kg i.p.)					
	103.5	10.35	1.035	0.103	0.010	0.001
A/J mouse:						
Hydroxy acid*	18	32	37	34	27	35
Keto acid*	11	16	26	19	27	23
NNAL	29	11	2	1	--	--
NNAL-Gluc	22	8	4	3	--	--
NNK-N-oxide	--	--	5	8	10	7
NNAL-N-oxide	11	13	8	14	7	6
Total α -hydroxylation*	29	48	63	53	54	58
F344 rat:						
Hydroxy acid*	26	24	20	28	16	14
Keto acid*	24	39	45	21	38	40
NNAL	25	12	6	7	4	--
NNAL-Gluc	8	3	2	2	2	--
NNK-N-oxide	3	3	6	7	14	14
NNAL-N-oxide	6	6	8	16	13	12
Total α -hydroxylation*	50	63	66	49	54	54

1. Morse et al. (1990).

Table 4. Biomonitoring of NNK metabolites in human urine.

Smoker	Cigarettes/day	Total excretion of NNK Metabolites (nmol/day) ¹	Theoretical NNK exposure and excretion	
			Exposure ($\mu\text{g/day}$) ²	Excretion ($\mu\text{g/day}$)
1	18.2±2.2	7.32	0.91-1.46	1.52
2	16.7±1.1	6.24	0.85-1.34	1.29
3	16.8±1.1	4.63	0.84-1.34	0.96
4	15.0±1.1	4.68	0.75-1.20	0.97
5	15.8±0.4	4.75	0.79-1.26	0.98
6	9.5±1.2	Data incomplete	--	--
7	14.2±1.1	2.41	0.71-1.14	0.50
8	13.6±0.7	Data incomplete	--	--
9	19.1±1.7	4.30	0.95-1.53	0.89
10	8.0±1.4	3.74	0.40-0.64	0.77
11	15.9±1.0	Data incomplete	--	--
Mean			0.78-1.24	0.99
S.D.				0.31

-
1. Total excretion of NNAL, NNAL-Gluc and NNAL-N-oxide. To convert to μg NNK multiply by 207.
 2. Estimated exposure range based on mainstream cigarette smoke NNK concentrations (Tricker et al., 1991; Hoffman et al., 1994).

provide evidence that NNK metabolism and excretion in man differs significantly from that observed in laboratory animals [Table 2].

Total NNAL plus NNAL-Gluc excretion in 5 nonsmokers measured on 2 occasions is reported to be 8.4 ± 11.2 ng/day, and increases after experimental exposure to sidestream smoke used as a surrogate for ETS (Hecht et al., 1993b). Another study reported 17 of 29 nonsmokers to have a mean excretion of 8.8 ± 9.4 (range 0.8-31) ng/day total NNAL plus NNAL-Gluc compared to 0.68 ± 0.41 (range 0.08-1.68) $\mu\text{g/day}$ in smokers (Meger et al., 1998). Twelve of 29 nonsmokers had no detectable levels of NNAL plus NNAL-Gluc in urine.

Metabolism of NNK by Laboratory Animal and Human Microsomes

Microsomes from lung and liver have been extensively used to investigate metabolism of NNK under various experimental conditions [Appendix 1]. Despite differences in the individual study protocols (NNK substrate concentration and time of incubation), rodent lung and liver microsomes metabolize NNK to yield significant levels of α -hydroxylation products. Contrary to this, lung and liver microsomes of human origin primarily metabolize NNK by keto reduction to NNAL in the absence of significant α -hydroxylation. Only limited kinetic data are available to document interspecies differences in NNK metabolism by lung and liver microsomes.

The available kinetic parameters for NNK metabolism by lung microsomes [Table 5] provide further indication that laboratory animals (A/J mouse and patas monkey) primarily metabolize NNK by α -hydroxylation with no significant formation of NNAL. Human lung microsomes primarily metabolize NNK to NNAL, with no significant formation of α -hydroxylation products. The biological relevance of data for metabolism of NNK by human lung microsomes is partially comprised by the high experimental substrate range compared to actual human exposure to NNK (19-135 ng/filter cigarette; 70-650 pmol [Tricker et al., 1991]). No data are available for microsomes isolated from rat lung.

Metabolism of NNK by hepatic microsomes is less well documented [Table 6]. Liver microsomes from the patas monkey primarily metabolize NNK via α -hydroxylation with no significant formation of NNAL at low NNK substrate concentrations, while at high substrate concentrations metabolism to NNAL would be predicted to occur. Human liver microsomes primarily metabolize NNK to NNAL, and to a lesser extent NNAL-N-oxide, at low substrate concentrations, with no significant formation of α -hydroxylation products. The formation of NNAL-N-oxide at low substrate concentrations supports the presence of this metabolite in human urine (Carmella et al., 1997). Only kinetic parameters for NNK α -hydroxylation pathways have been reported for A/J mouse and F344 rat liver microsomes.

In conclusion, kinetic parameters of NNK metabolism in lung and liver microsomes provide strong evidence that significant differences occur in metabolism between laboratory animals and man. At low levels of exposure to NNK, human metabolism is characterized by reduction of

Table 5. Metabolism of NNK by lung microsomes.Kinetic parameters: K_m (μM), V_{max} (pmol/min/mg protein)

Metabolite	Mouse ¹		Rat		Patas monkey ²		Human ³	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Keto alcohol	5.6	56	No published data		4.9	19.1	--	--
Keto aldehyde	--	--			10.3	5.3	653	4.6
Keto acid	9.2	4.2			--	--	--	--
Hydroxy acid	--	--			--	--	526	2.9
NNAL	2541	1322			902	479	573	335
NNAL-N-oxide	4.7	54			--	--	--	--
NNK-N-oxide	--	--			5.4	19.1	531	7.7
Substrate range	1-100 μM NNK				1-20 μM NNK		7-200 μM NNK	

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1. Smith et al. (1990).
 2. Smith et al. (1997).
 3. Smith et al. (1992).

Table 6. Metabolism of NNK by liver microsomes.

Kinetic parameters: K_m (μM), V_{max} (pmol/min/mg protein)

Metabolite	Mouse ¹		Rat ²		Patas monkey ³		Human ⁴	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Keto alcohol	73.8	239	211	156	474	37.7	1200	500
Keto aldehyde	19.1	173	234	153	8.2	37.4	367	60
NNAL	--	--	--	--	474	3470	56	282
NNAL-N-oxide	--	--	--	--	--	--	1600	3300
							53	19
NNK-N-oxide	--	--	--	--	--	--	4500	560
							--	--
Substrate range	1-100 μM NNK		1-200 μM NNK		1-50 μM NNK		5-2000 μM NNK	

1. Peterson et al. (1991).

2. Guo et al. (1992).

3. Smith et al. (1997).

4. Patten et al. (1996).

NNK to NNAL, in the absence of significant α -hydroxylation, while laboratory animals metabolize NNK to yield significant levels of α -hydroxylation products.

Metabolism of NNK by Laboratory Animal and Human Tissues

Similar profiles are observed for metabolism of NNK by microsomes and tissue samples [Appendix 1]. The data confirm that significant differences occur between laboratory animal and human metabolism of NNK. The major pathways of NNK metabolism in rodent tissues yield significant levels of α -hydroxylation products while human tissues primarily reduce NNK to NNAL.

DNA and Hemoglobin Adduct Formation by NNK

Metabolism of NNK by α -hydroxylation is assumed to be a critical event resulting in DNA-reactive intermediates; however, the exact role of methylating and pyridyloxobutylating species in different animal species and organs remains unclear. Metabolism of NNK to the keto acid via a methylating intermediate is thought to be a critical determinant of NNK-induced tumorigenesis in the A/J mouse lung (Peterson and Hecht, 1991; Belinsky et al., 1992) and hamster liver (Liu et al., 1992). Although NNK-induced methylation to yield O⁶-methylguanine in Clara cells appears to be a suitable indicator of the carcinogenic potency of NNK in the rat lung (Belinsky et al., 1990), pyridyloxobutylation is thought to be the critical event in tumor induction in rat lung (Staretz et al., 1997b) and liver (Liu et al., 1992). Both NNK and NNN can be metabolized to a

pyridyloxobutylating species in the rat lung (Hecht et al., 1988) to yield a N²-(pyridyloxobutyl)deoxyguanosine (HPB) adduct with DNA (Spratt et al., 1989). Linear dose-response relationships are not observed for either NNK-induced methylation (Belinsky et al., 1990; Murphy et al., 1990a) or NNK-induced pyridyloxobutylation in the rat lung (Murphy et al., 1990a). An HPB-releasing adduct with hemoglobin has been proposed as a surrogate marker for DNA adduct formation by both NNK and NNN (Carmella and Hecht, 1987; Peterson et al., 1990). However, the exact mechanism of adduct formation still remains to be determined (Murphy and Coletta, 1993).

Only two studies have investigated the presence of HPB-releasing DNA adducts in human lung (Foiles et al., 1991; Blömeke et al., 1996). Mean levels of 11±16 and 9.0±2.3 fmol HPB/mg DNA have been reported in smokers and nonsmokers, respectively (Foiles et al., 1991). Since the response for the analytical reagent blank was equivalent to 38±16 fmol HPB and 1-2 mg DNA were used for analysis, the reported levels are well below the blank response and could easily be due to an analytical artifact. No HPB-releasing DNA adducts were detected in 16 lung tissue samples from current smokers and 16 from nonsmokers (Blömeke et al., 1996). The levels of 7-methylguanine in 80 lung tissue samples could not be explained by differences in tobacco exposure (measured by serum cotinine), gender, age, or ethnicity (Blömeke et al., 1996). These data suggest that exposure to NNK via smoking does not result in HPB adduction to lung DNA or increase the background level of lung DNA methylation.

Determination of hemoglobin adducts as a possible surrogate for tissue DNA adducts shows less than a 3-fold difference in the mean level of HPB-releasing hemoglobin adducts in

smokers (163 fmol HPB/g hemoglobin) compared to nonsmokers (68 fmol HPB/g hemoglobin) (Hecht, 1994). A smaller difference was observed in another study (54.7±8.9 vs. 26.7±4.1 fmol/g hemoglobin) and self-reported exposure of nonsmokers to environmental tobacco smoke did not increase the background level of HPB-releasing hemoglobin adducts (Richter et al., 1995). Both studies indicated elevated hemoglobin adduct levels were only apparent in about 10% of smokers compared to all subjects.

Concluding Remarks

The above studies provide intensive evidence that human metabolism of NNK differs significantly from that observed in laboratory animals. These differences are evident from *in vitro* studies of NNK metabolism in microsomes and tissue samples, excretion profiles of NNK metabolites, and absence of a clear differentiation in NNK/NNN-derived DNA and hemoglobin adducts in smokers and nonsmokers. These data provide little support for the assumption that NNK in tobacco products and cigarette smoke induces similar biological effects in the lung and liver as reported in laboratory animals.

Research Funded by Philip Morris on the Metabolism of NNK

A research project has been funded at the Walther-Straub Institute for Pharmacology and Toxicology, University of Munich, Germany. The research has two major objectives:

1. To define the major routes of *in vitro* NNK and NNAL metabolism under identical experimental conditions in lung and liver of the A/J mouse, F344 rat, Syrian golden hamster, and man. The three animal species chosen represent those most often used for chronic bioassays of NNK.
2. To determine pharmacokinetic constants (K_m and V_{max}) for each pathway of NNK and NNAL metabolism in both organs of all four species.

Experimental Design

Lung and liver are removed from laboratory animals killed by decapitation. Human peripheral lung and liver tissue are collected from excess material removed at surgery from patients undergoing routine surgical procedures. Human tissues are rejected from subjects having current treatment with immuno-suppressants or other drugs known to induce or suppress metabolism, or a history of alcohol abuse. Undamaged macroscopically normal tissue is received in the laboratory stored in ice-cold Hanks medium within 30 min of removal.

Metabolic studies are performed using precision cut liver slices and lung tissue in dynamic culture (Vickers, 1994). Compared to using microsomes or isolated cells (extensively used in previous studies as summarized in **Appendix I**), tissue samples maintain structural heterogeneity with intact phase I and phase II metabolism, and cell interaction and communication are preserved to a certain extent, thus resembling the situation in the intact organ.

Radiolabeled [5-³H]NNK or [5-³H]NNAL (sp. act. 25-30 Ci/mmol) are incubated over a substrate concentration range of 0.01-100.0 μ M with precision cut liver slices and lung tissue for 6 h in Krebs-Henseleit buffer (pH 7.4) under standard laboratory conditions for dynamic culture (Vickers, 1994). The substrate concentration range was selected to include the lowest possible concentration to approach the physiologically relevant concentration in man, and substrate concentrations predicted to occur in animal bioassays. Each incubation is performed in triplicate using lung and liver samples from at least 5 different laboratory animals. Metabolite profiles are determined by reversed-phase HPLC with radioflow detection (Richter and Tricker, 1994). Pharmacokinetic parameters (*K_m* and *V_{max}*) are calculated from Lineweaver-Burk plots with reaction velocities showing linear response to time. Pharmacokinetic parameters will be determined for lung and liver from different human donors.

Current Results

Preliminary results from this research have already been presented at two scientific meetings:

1. AACR Special Conference in Cancer Research "DNA methylation, imprinting, and the epigenetics of cancer," Las Croabas, Puerto Rico, December 12-16, 1997.
2. 37th Annual Meeting of the Society of Toxicology, Seattle, March 1-5, 1998.

Further results will be presented at the 89th Annual Meeting of the American Association for Cancer Research, New Orleans, March 28-April 1, 1998.

The data currently available from this research project [Table 7, Table 8] demonstrate that NNK metabolism in lung of the A/J mouse and F344 rat results in significant levels of α -hydroxylation products at high tissue substrate concentrations predicted to occur in animal bioassay protocols. The experimental conditions and data support the reported biological activity of NNK in the A/J mouse and F344 rat lung; metabolism of NNK at high substrate concentrations yields the keto acid via a methylation pathway in the A/J mouse lung, while formation of the keto alcohol via the pyridyloxobutylation pathway occurs in the F344 rat lung. These two pathways are considered critical for lung tumorigenesis in A/J mouse lung (Peterson and Hecht, 1991; Belinsky et al., 1992) and F344 rat lung (Staretz et al., 1997b), respectively. Metabolism of NNK by human lung results primarily in the formation of NNAL in the absence of formation of significant levels of α -hydroxylation products [Figure 3]. The kinetic data [Table 7] provide support for pyridyl-N-oxidation of NNAL, but not NNK, and are consistent with the reported occurrence of NNAL-N-oxide but not NNK-N-oxide in human urine (Carmella et al., 1997). The kinetic data do not support significant formation of keto alcohol via the pyridyloxobutylation pathway at low NNK substrate

Table 7. Metabolism of NNK in lung tissue.

Kinetic parameters: K_m (μM), V_{max} (pmol/min/mg protein)

Metabolite	A/J Mouse		SG Hamster		F344 Rat		Human	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
0.01-1.0 μM NNK								
NNAL	1.7	168	No	No	3.6	309	1.0	311
Keto acid	0.3	45	current	current	5.2	182	0.4	11
Keto alcohol	0.6	141	data	data	2.7	177	1.2	26
Hydroxy alcohol	1.0	56			n.d.	--	0.7	25
NNK-N-oxide	0.7	184			6.8	498	n.d.	n.d.
NNAL-N-oxide	n.d.	n.d.			n.d.	--	0.6	19
0.01-100 μM NNK								
NNAL	39.0	4309			--	--	239	65640
Keto acid	10.1	317			317.0	15620	--	--
Keto alcohol	7.5	556			90.0	8398	--	--
Hydroxy alcohol	160.0	3405			2.4	107	--	--
NNK-N-oxide	25.1	25.1			68.0	7995	41240	413500
NNAL-N-oxide	n.d.	n.d.			0.9	21	--	--

Substrate range: 0.01-100.0 μM NNK

n.d., no detectable formation; --, Michaelis-Menton kinetics could not be fitted.

Table 8. Metabolism of NNK in liver tissue.

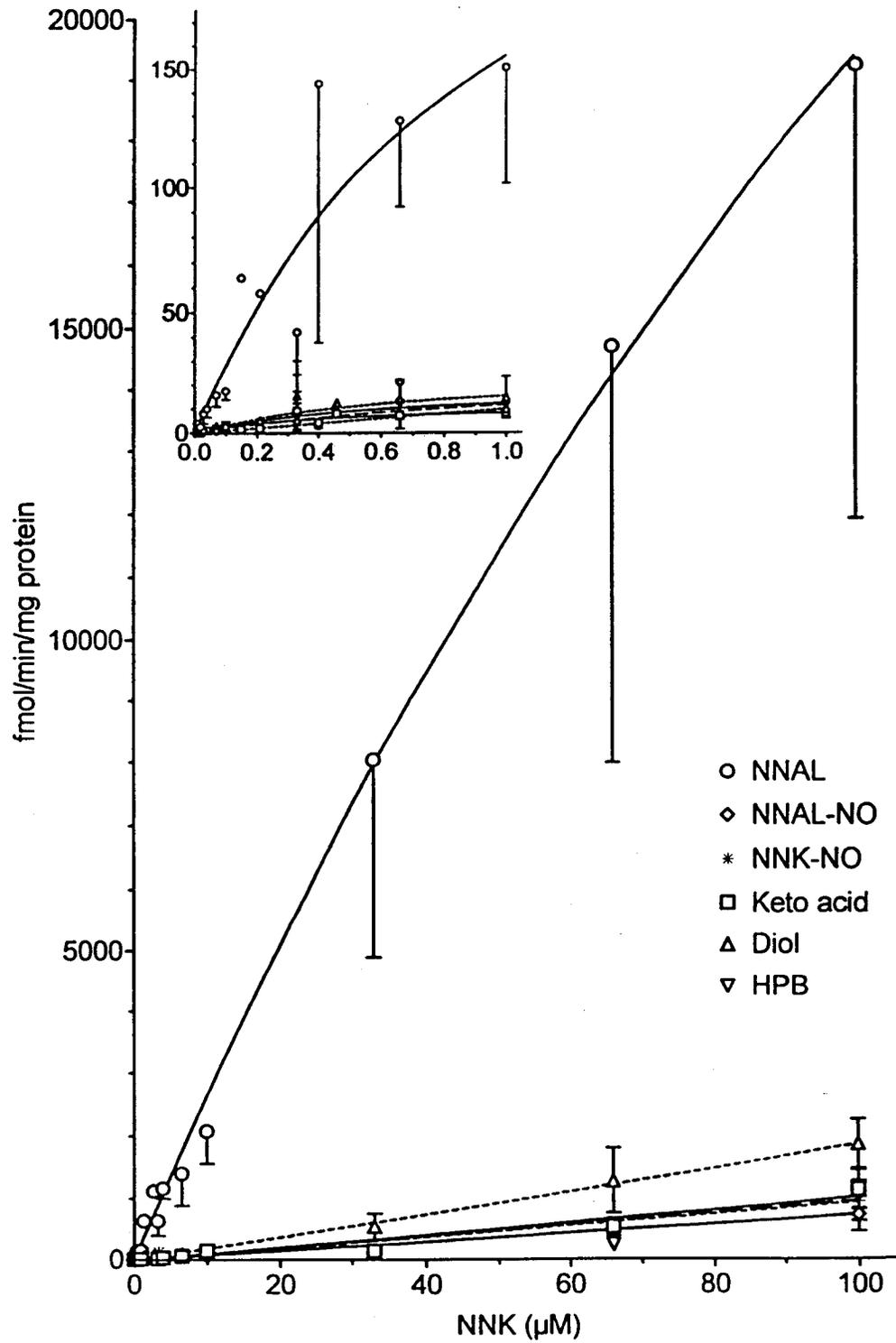
Kinetic parameters: K_m (μM), V_{max} (pmol/min/mg protein)

Metabolite	A/J Mouse		SG Hamster		F344 Rat		Human	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
0.01-1.0 μM NNK								
NNAL	--	--	No current data	No current data	26.0	4640	0.6	254
Keto acid	--	--			0.9	18	0.2	9.6
Keto alcohol	--	--			1.9	62	n.d.	n.d.
Hydroxy alcohol	--	--			n.d.	n.d.	--	--
0.01-100 μM NNK								
NNAL	174.0	4623			96.0	19220	43.6	6805
Keto acid	677.0	5818			154.0	1159	690.0	8036
Keto alcohol	141.0	2609			260.0	8357	12760	144500
Hydroxy alcohol	199.0	3153			16.2	253	n.d.	n.d.

Substrate range: 0.01-100.0 μM NNK

n.d., no detectable formation; --, Michaelis-Menton kinetics could not be fitted.

Figure 3. Metabolism of NNK by 7 different human lungs.



concentrations in human lung. These data suggest that HPB-releasing adducts derived from NNK are unlikely to be formed in the human lung and support data for their absence in human lung tissue (Blömeke et al., 1996).

Michaelis-Menton kinetics could not be fitted to NNK metabolism at low substrate concentrations in the A/J mouse liver. At high substrate concentrations (1.0-100 μM), significant metabolism of NNK by α -hydroxylation pathways occurs in addition to NNAL formation. NNK metabolism in the liver of the F344 rat suggested that hydroxy acid formation is the most favorable pathway for NNK metabolism; however, under conditions used in rat bioassay protocols, significant α -hydroxylation of NNK still occurs. At low physiological NNK substrate concentrations in the human liver, NNK metabolism is predicted to result primarily in the formation of NNAL.

In summary, these results obtained under identical experimental conditions provide further evidence that NNK metabolism in human lung and liver primarily yields NNAL. In contrast to this, NNK metabolism in lung and liver of the A/J mouse and F344 rat results in significant α -hydroxylation to DNA-reactive intermediates thought to be involved in NNK-induced tumorigenesis in these two organs.

The research program is predicted to be completed in April 1998.

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Appendix I. *In vitro* metabolism of NNK in tissues, cultured cells and microsomes from laboratory animals and man

Species	Tissue ¹	Conditions		Percentage formation of major metabolites ²							Keto acid ³	Hydroxy acid	Diol	Percentage α -hydroxylation ¹	Reference
		NNK (μ M)	Time (h)	NNAL	NNK-N-oxide	NNAL-N-oxide	Gluc	NNAL-	Keto alcohol	Keto					
A/J mouse	Lung (t)	4.7	4.0	1.2	49.8	0.8	--	27.1	19.5	0.8	0.8	47.4	Pepin et al., 1992		
A/J mouse	Lung (t)	4.7	0.8	0.6	53.7	1.3	--	27.6	13.4	1.3	2.1	42.3	Pepin et al., 1992		
A/J mouse	Lung (t)	2.36	2.0	1.6	53.9	0.6	--	29.7	12.4	0.4	1.4	42.5	Bouchard & Castonguay, 1993		
A/J mouse	Lung (t)	0.24	24	68.4	7.5	3.7	--	3.1	10.6	1.5	5.2	15.2	Castonguay et al., 1983a		
A/J mouse	Lung (m)	200.0	0.5	76.3	10.4	--	--	--	2.5	--	10.8	2.5	Peterson et al., 1991		
A/J mouse	Lung (m)	10.0	0.5	12.5	43.0	2.0	--	24.7	(17.1)	--	--	41.8	Smith et al., 1993		
A/J mouse	Lung (m)	10.0	0.5	16.3	31.8	--	--	49.7	2.2	--	--	51.9	Smith et al., 1990		
A/J mouse	Lung (m)	10.0	0.25	13.7	39.1	--	--	32.7	14.5	--	--	47.2	Lin et al., 1992		
A/J mouse	Lung (m)	10.0	0.25	17.8	42.2	--	--	26.2	(13.8)	--	--	40.0	Desai et al., 1995		
A/J mouse	Lung (m)	10.0	0.16	5.6	45.6	--	--	29.6	(19.2)	--	--	48.8	Hong et al., 1992		
A/J mouse	Lung (m)	--	0.6	29.4	21.7	--	--	48.7	--	--	--	48.7	Morse et al., 1995		
A/J mouse	Liver (m)	10.0	0.5	17.7	13.1	--	--	28.9	(40.3)	--	--	69.2	Hong et al., 1992		
A/J mouse	Liver (m)	10.0	0.25	53.3	--	--	--	16.3	30.4	--	--	46.7	Lin et al., 1992		
A/J mouse	Liver (m)	10.0	0.25	24.0	--	--	--	23.2	(52.8)	--	--	47.2	Desai et al., 1995		
A/J mouse	Liver (m)	10.0	0.16	26.6	4.7	3.1	--	24.4	(41.1)	--	--	65.5	Smith et al., 1993		
A/J mouse	Liver (m)	--	0.25	22.9	--	--	--	27.8	49.3	--	--	77.1	Morse et al., 1995		
A/J mouse	Intestine (t)	14.0	0.75	87.8	2.9	0.8	--	2.7	5.4	0.3	0.1	8.4	Pepin et al., 1990		
A/J mouse	Stomach (t)	14.0	2.0	67.2	8.0	0.3	--	5.7	18.2	0.3	0.3	24.2	Pepin et al., 1990		
NMRI (f)	Intestine (t)	1.0	2.0	4.8	28.9	8.1	--	2.4	44.9	10.8	--	58.1	Schulze et al., 1996		
NMRI (m)	Intestine (t)	1.0	2.0	7.1	29.5	4.7	--	4.2	50.6	3.9	--	58.7	Schulze et al., 1996		
F344 rat	Lung (m)	1.0	0.5	22.9	38.3	--	--	34.4	4.4	--	--	38.8	El-Bayoumy et al., 1996		
F344 rat	Liver (h)	5.0	2.0	41.7	6.1	0.5	--	6.4	35.4	9.0	0.9	50.8	Murphy & Coletta, 1993		
F344 rat	Liver (h)	1.0	18.0	11.4	2.8	7.9	12.5	3.7	44.7	17.0	--	65.4	Murphy et al., 1995		
F344 rat	Liver (m)	1.33	0.5	99.6	0.2	0.004	--	--	0.2	0.01	0.04	0.21	Hamilton & Teel, 1994		
F344 rat	Liver (m)	1.0	0.5	98.9	0.1	--	--	0.7	0.3	--	--	1.0	El-Bayoumy et al., 1996		
F344 rat	Esoph. (t)	40.0	24.0	91.3	4.5	--	--	0.7	3.5	--	--	4.2	Murphy et al., 1990b		
F344 rat	Nasal (t)	23.8	3.0	5.4	--	--	--	21.3	64.0	5.3	4.0	70.6	Brittebo et al., 1983		
F344 rat	Nasal (t)	23.8	24.0	4.9	--	--	--	5.6	69.9	11.2	8.4	86.7	Brittebo et al., 1983		
F344 rat	Oral (t)	1.0	24.0	19.5	51.0	2.0	--	16.3	11.2	--	--	27.5	Murphy et al., 1990b		
F344 rat	Oral (t)	1.0	24.0	26.2	36.3	8.8	--	6.9	20.7	0.6	0.5	28.2	Murphy et al., 1990b		

F344 rat	1.0	24.0	55.0	25.2	--	--	9.0	8.3	0.4	2.2	17.7	Murphy et al., 1991
F344 rat	1.0	24.0	49.9	21.5	5.5	--	9.1	11.5	0.7	1.8	21.3	Murphy & Heiblum, 1991
F344 rat	1.0	4.0	35.8	3.9	30.6	--	14.6	13.6	1.5	--	29.7	Murphy et al., 1990b
F344 rat	1.0	4.0	50.8	20.5	5.1	--	8.7	14.3	0.6	--	23.6	Murphy et al., 1990b
F344 rat	11.0	4.0	81.9	5.5	--	--	3.1	9.5	--	--	12.6	Murphy et al., 1990b
SD rat	10.0	0.5	28.6	24.4	0.5	--	46.0	0.5	--	--	46.5	Yoo et al., 1992
SD rat	0.47	0.33	25.7	46.3	--	--	15.5	12.5	--	--	28.0	Ardrès et al., 1996
SD rat	10.0	0.5	93.6	1.3	0.2	--	3.9	1.0	--	--	4.9	Yoo et al., 1992
SD rat	10.0	0.5	--	1.0	1.0	--	58.5	36.6	0.9	2.0	96.0	Hong et al., 1991
SD rat (f)	1.0	2.0	6.6	29.4	6.9	--	10.3	4.2	2.7	--	17.2	Schulze et al., 1996
SD rat (f)	1.0	2.0	78.2	11.2	1.1	--	3.2	6.0	0.4	--	9.6	Schulze et al., 1996
SG Hamster	4.2	3.0	20.4	32.3	1.8	--	17.9	25.5	0.3	1.8	43.7	Charest et al., 1989
SG Hamster	0.66	1.0	37.4	27.4	4.7	--	12.0	16.5	0.5	1.5	29.0	Tjälve & Castonguay, 1983
SG Hamster	0.24	3.0	22.1	33.2	5.5	--	17.7	20.7	0.7	--	39.1	Charest et al., 1989
SG Hamster	10.0	0.5	50.5	19.6	1.9	--	24.3	3.7	--	--	28.5	Jorquera et al., 1993
SG Hamster	--	0.5	13.0	16.5	7.2	--	40.6	9.8 ^s	--	12.9	50.4	Zhang et al., 1997
SG Hamster	0.66	1.0	86.0	1.2	3.6	--	--	2.9	3.1	3.2	6.0	Tjälve & Castonguay, 1983
SG Hamster	10.0	0.5	60.2	9.0	0.7	--	24.1	6.0	0.03	--	30.1	Jorquera et al., 1993
SG Hamster	8.0	0.5	44.5	8.7	3.2	--	35.5	4.0 ^s	--	4.1	59.5	Miller et al., 1994
SG Hamster	5.1	0.5	69.9	12.7	0.9	--	6.8	1.4 ^s	--	1.3	8.2	Miller et al., 1993
SG Hamster	5.1	0.5	67.7	8.3	4.6	--	12.0	6.1 ^s	0.1	1.2	18.2	Miller et al., 1996
SG Hamster	1.33	0.5	55.4	19.6	2.9	--	17.5	0.8 ^s	--	3.9	18.3	Hamilton & Teel, 1994
SG Hamster	1.0	1.0	62.7	2.4	--	--	30.6	3.3	0.1	0.9	34.0	Castonguay & Rossignol, 1992
SG Hamster	--	0.5	31.1	6.1	6.4	--	24.9	22.1 ^s	0.4	9.0	47.4	Zhang et al., 1997
SG Hamster	0.66	1.0	21.0	1.8	2.0	--	13.2	51.3	2.2	8.5	66.7	Charest et al., 1989
SG Hamster	0.66	1.0	31.1	11.5	--	--	18.2	38.0	--	1.2	56.2	Charest et al., 1989
SG Hamster	1.0	2.0	11.0	25.8	31.1	--	5.8	14.6	11.7	--	32.1	Schulze et al., 1996
Man	238.0	24.0	96.4	2.7	--	--	--	--	0.9	--	0.9	Castonguay et al., 1983b
Man	10.0	1.0	96.4	1.2	<0.2	--	<0.2	(1.4)	0.7	--	2.3	Smith et al., 1992
Man	10.0	0.17	96.1	--	--	--	-----	(3.0)	0.9	--	3.9	Smith et al., 1995
Man	3.0	0.17	83.5	1.0	--	--	7.5	7.0 ^s	--	1.0	14.5	Staretz et al., 1997a
Man	10.0	1.0	92.4	1.2	0.7	--	3.3	(1.5)	0.9	--	5.7	Smith et al., 1992
Man	238.0	24.0	99.9	--	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	238.0	24.0	95.4	4.5	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	238.0	24.0	99.2	0.1	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	6.0	24.0	94.8	0.7	1.5	--	0.2	0.5	1.8	0.6	2.5	Liu et al., 1993
Man	100.0	24.0	99.5	0.2	0.1	--	0.1	0.3	0.1	0.1	0.5	Liu et al., 1993
Man	6.0	24.0	94.7	1.2	0.4	--	0.8	2.1	0.4	0.5	3.3	Liu et al., 1993

Man	Oral ©	100.0	24.0	96.2	0.6	0.2	--	0.3	2.0	0.2	0.1	2.5	Liu et al., 1993
Man	Esophagus (t)	238.0	24.0	98.2	1.7	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	Kidney ©	2.3	50.0	99.8	0.03	--	--	--	0.05	0.06	0.03	0.11	Lacroix et al., 1992
Man	Trachea (t)	238.0	24.0	94.2	0.1	--	--	--	--	5.7	--	5.7	Castonguay et al., 1983b

1. Abbreviation in brackets: (t), tissue; ©, cultured cell; (h), hepatocyte; (m), microsome; --, not detected.

2. Calculated on the basis of total metabolites detected.

3. % α -hydroxylation calculated from the sum of keto alcohol, keto acid (keto aldehyde) and hydroxy acid; metabolic pathways known to produce reactive intermediates which bind to DNA.

4. Keto aldehyde measured instead of keto acid, value for keto aldehyde presented in brackets.

5. Joint determination of keto aldehyde and keto acid.

Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 4 -

**Commentary on the Results of the
IARC Multi-Center Study,
“Lung Cancer and Exposure to Environmental Tobacco Smoke,”
Published in the IARC Biennial (1996-1997) Report**

March 20, 1998

Commentary on the Results of the IARC Multi-Center Study, “Lung Cancer and Exposure to Environmental Tobacco Smoke,” Published in the IARC Biennial (1996-1997) Report

Results from the International Agency for Research on Cancer (IARC) multi-center epidemiologic study of environmental tobacco smoke (ETS) exposure and lung cancer risk were recently published for the first time in the IARC Biennial Report for 1996-1997 (IARC, 1997; see Tab A, section 3.7.2). This study, which began in 1988, represents the largest study in Europe and the second largest study ever conducted of its kind.

In 1986, IARC made the following comments regarding the epidemiological evidence for the reported association between ETS exposure and lung cancer (IARC, 1986): “Several epidemiological studies have reported an increased risk of lung cancer in nonsmoking spouses of smokers, although some others have not . . . The resulting errors could arguably have artifactually depressed or raised estimated risks, and, as a consequence, each is compatible either with an increase or with an absence of risk.” It was on the basis of that conclusion that IARC decided to undertake its multi-center study. The results, based on an analysis of 650 cases and 1,542 controls in 12 centers in 7 European countries, were as follows:

- RR for spousal exposure -- 1.16 (95% CI, 0.93-1.44)
- RR for workplace exposure -- 1.17 (95% CI, 0.94-1.45)
- RR for combined exposure -- 1.14 (95% CI, 0.88-1.47)

The report also states that there was no evidence of an association between lung cancer risk and ETS exposure during childhood.

The two immediate conclusions that can be drawn from these results are that both indices of ETS exposure, spousal and workplace, suggest a small but positive association with lung cancer, but neither association is statistically significant. What cannot be determined from the results as presented is the extent to which they have been corrected for known systematic biases -- in particular, misclassification of smoking habit and confounding by diet (spousal exposure only). The most recent meta-analysis carried out on the possible association of ETS exposure and lung cancer (Hackshaw et al., 1997) arrived at a combined relative risk of 1.24, which the authors estimated should have been reduced by 0.06 for misclassification of smoking habit and by 0.02 for dietary confounding. Although their suggested reduction is almost certainly inadequate, accepting even these values would yield a reduction of 33% for the spousal exposure risk estimate in the IARC study, resulting in a relative risk of 1.10, and a reduction of 25% for the workplace exposure estimate, resulting in a relative risk of 1.13. Although these values are, of course, still greater than 1.0, it is as likely that they suggest no association whatsoever, as it is likely that they suggest an association.

However, it should be noted that the results reported by IARC are in line with other studies and, in particular, in line with a number of meta-analyses. Therefore, clearly, a possible interpretation of these results is that they confirm an association between reports of ETS exposure and lung cancer, although the association is extremely weak. It is worth noting that the Introduction

to the brief text in the IARC Biennial Report states that ETS exposure is a “likely cause” of lung cancer. This is a far less conclusive statement than made by some other agencies and scientists.

There are several interesting points that were made in the discussion of these results in the IARC Biennial Report. The first deals with dose-response trends, where the claim is made that “several quantitative indicators of ETS exposure showed a dose-response relationship with lung cancer risk.” It is impossible to analyze this statement from the data provided in the summary; however, the fact that not all such indicators showed a dose-response relationship suggests that such an analysis would be of great importance.

Secondly, IARC states that: “A further study of non-smoking women in Moscow, Russian Federation, confirmed the results on ETS of the larger international investigation and suggested a role of environmental air pollution independent of the effect of ETS.” In actuality, the results of this study (Zaridze et al., 1998) “confirm” only the result for spousal exposure. The authors report no increase in lung cancer risk for workplace exposure; moreover, the reported dose-response effects were negative, whether years of exposure or number of cigarettes smoked by the spouse was used as the metameter of spousal exposure.

Lastly, the IARC summary states that: “In a separate exercise, the number of lung cancers occurring in the countries of the European Union that can be attributed to spousal ETS exposure was estimated to be about 800 among women and 300 among men.” The reader is likely to assume that the calculation referred to utilized the relative risk of 1.16 obtained from the IARC

multi-center study. However, in actuality the authors of the paper used a relative risk for spousal exposure of 1.30, almost two times the uncorrected IARC result (Trédaniel et al. 1997).

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<http://www.iarc.fr/>



Epidemiology

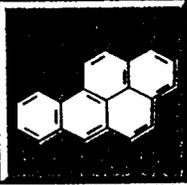
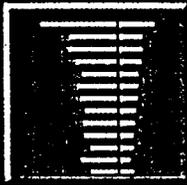
Pathology

Carcinogens

Genetics

Prevention

Detection



INTERNATIONAL AGENCY FOR RESEARCH ON CANCER



WORLD HEALTH
ORGANIZATION

BIENNIAL REPORT 1996/1997

Cancer Research for Cancer Control

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INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

BIENNIAL REPORT

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International Agency for Research on Cancer

Lyon, France

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3.7 Cancer of the lung

Lung cancer is the most frequent malignant neoplasm worldwide: tobacco smoking is responsible for most cases, and the control of smoking represents the most important approach to prevent lung cancer (see Section 2.4). Among the important research questions still to be answered are the contributions of other risk factors (occupation, diet, environmental pollution) in both smokers and non-smokers and the role of genetic predisposition: these questions are being addressed in a series of studies conducted in areas of high and low risk for lung cancer.

3.7.1

Case-control study of lung cancer in northern Thailand

D.M. Parkin and P. Pisani; in collaboration with P. Srivatanakul, Bangkok, Thailand; N. Martin, Chiang Mai, Thailand; V. Saensingkaew, Bangkok, Thailand; and T. Bishop, Leeds, UK

This study is investigating the reasons for the relatively high incidence of lung cancer, particularly in women, in northern Thailand. Age-standardized incidence rates in Lampang province are 41.8 per 10 000 in men and 20.1 per 10 000 in women. A case-control study comparing 196 cases of lung cancer with two groups of controls (217 hospital controls and 156 community controls drawn at random from the population of this province) was carried out from 1993 to 1995 and data analysis began in 1996.

Because one hypothesis under investigation is the role of air pollution from numerous coal-fired electricity generating plants, place of residence is an important variable of interest, linked to corresponding environmental measurements of arsenic and cadmium. Other factors investigated include tobacco habits, exposure to domestic smoke, and cooking practices. Blood samples from all subjects have been stored for analysis of

heavy metals and of metabolites and adducts of components of tobacco smoke. DNA is being extracted from white blood cells of cases and controls to study metabolic polymorphism at the GSTM1 and CYP1A1 loci.

3.7.2

Lung cancer and exposure to environmental tobacco smoke

P. Boffetta, P. Brennan, S. Lea and G. Ferro; in collaboration with W. Ahrens, Bremen, Germany; E. Benhamou and S. Benhamou, Villejuif, France; S.C. Darby, Oxford, UK; F. Forastiere and C. Fortes, Rome, Italy; C.A. González and A. Agudo, Barcelona, Spain; J. Trédaniel, Paris, France; S.K. Jindal, Chandigarh, India; K. H. Jöckel, Essen, Germany; A. Mendes, Lisbon, Portugal; F. Merletti, Turin, Italy; G. Pershagen and F. Nyberg, Stockholm, Sweden; R. Saracci, Pisa, Italy; L. Simonato, Padua, Italy; H. Wichmann, Munich, Germany; C. Winck, Porto, Portugal; and D. Zaridze, Moscow, Russian Federation

Environmental tobacco smoke (ETS) is a likely cause of lung cancer [27, 37], while evidence of an association with other neoplasms is inconclusive. However, the quantitative aspects of the association between ETS exposure and lung cancer risk are not yet well established, nor is the interaction between exposure to ETS and exposure to other carcinogens.

An IARC-coordinated international collaborative case-control study was aimed at investigating the relationship between exposure to ETS and to other environmental and occupational risk factors and the risk of lung cancer in subjects who have never smoked tobacco. A total of 650 cases and 1542 controls have been enrolled in 12 centres in seven European countries. Information on exposure to occupational carcinogens, urban air pollution, background radiation and dietary habits, as well as lifelong exposure to ETS, has been collected by personal interview of cases and controls. Self-reported (non-)smoking status was

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confirmed by interviews of relatives. The relative risk (RR) of lung cancer risk was 1.16 (95% CI 0.93-1.44) for exposure to ETS from the spouse, 1.17 (0.94-1.45) for workplace ETS exposure and 1.14 (0.88-1.47) for combined spousal and workplace exposure. Several quantitative indicators of ETS exposure showed a dose-response relationship with lung cancer risk; RRs were higher for squamous cell carcinoma and small cell carcinoma than for adenocarcinoma (Figure 20). There was no association between lung cancer risk and ETS exposure during childhood. Additional analyses are continuing on risk factors other than ETS.

A parallel study was conducted in Chandigarh, India, where ETS exposure comes mainly from bidi smoking. The statistical analysis will be completed in 1998. A further study of non-smoking women in Moscow, Russian Federation, confirmed the results on ETS of the larger international investigation and suggested a role of environmental air pollution independent of the effect of ETS [517]. In a separate exercise, the number of lung cancers occurring in the countries of the European Union that can be attributed to spousal ETS exposure was estimated to be about 800 among women and 300 among men [461].

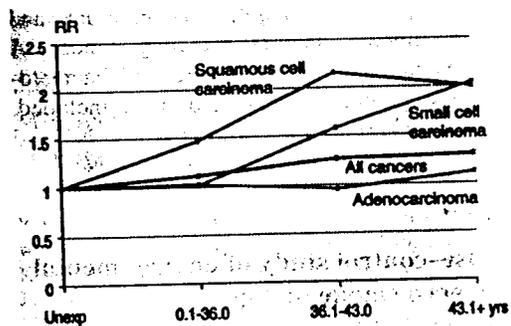


Figure 20. Relative risk of lung cancer by years of exposure to environmental tobacco smoke from spouse or workplace and by histological

3.7.3

Combined analysis of case-control studies of lung cancer in western Europe

P. Boffetta, P. Brennan and V. Gaborieau; in collaboration with W. Ahrens and H. Pohlabein, Bremen, Germany; E. Benhamou and S. Benhamou, Villejuif, France; S.C. Darby, Oxford, UK; F. Forastiere and C. Fortes, Rome, Italy; C.A. González and A. Agudo, Barcelona, Spain; K. H. Jöckel, Essen, Germany; F. Merletti, Turin, Italy; G. Pershagen and F. Nyberg, Stockholm, Sweden; R. Saracci, Pisa, Italy; J. Siemiatycki, Montreal, Canada; L. Simonato, Padua, Italy; and H. Wichmann, Munich, Germany

In parallel to the study on non-smokers described in Section 3.7.2, cases of lung cancer and controls have been enrolled in a series of studies in 10 centres in western Europe, irrespective of their smoking habits. Comparable information on tobacco smoking, exposure to occupational carcinogens and urban air pollution has been collected from about 9000 cases and 10 000 controls. The analysis focuses on detailed aspects of tobacco carcinogenesis that cannot be addressed in smaller studies, such as the effect of very light smoking, long-term quitting and smoking of products other than cigarettes (Figure 21). These analyses will be completed in 1998. In parallel, information on exposure to occupational carcinogens

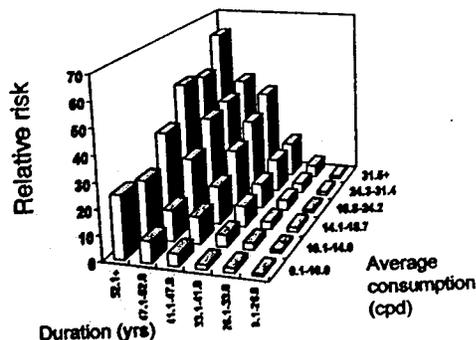


Figure 21. Relative risk of lung cancer by fine categories of cigarette consumption and duration of smoking. Reference category: never smokers.

and urban air pollution will be integrated into the common database.

3.7.4

Multicentric case-control study of lung cancer in central and eastern Europe

P. Brennan and P. Boffetta; in collaboration with E. Fabianova, Banska Bystrica, Slovakia; J. Fevotte, Lyon, France; A. Fletcher, London, UK; D. Mates, Bucharest, Romania; P. Rudnai, Budapest, Hungary; J. Siemiatycki, Montreal, Canada; N. Szeszenia-Dabrowska, Lodz, Poland; D.G. Zaridze, Moscow, Russian Federation; and W. Zatonski, Warsaw, Poland

Countries of central and eastern Europe have the highest incidence and mortality of lung cancer ever recorded. Air pollution is often blamed as the main contributor to this excess, but evidence for its role is limited. A study has been initiated in six areas of Hungary, Poland, Romania, the Russian Federation and Slovakia, to assess the relative contributions of tobacco smoking, occupational exposures and outdoor air pollution in lung carcinogenesis. Enrolment of a total of 3000 cases and a comparable number of controls has started. Special efforts are being made to assess past occupational exposures using detailed employment histories evaluated by panels of local experts. Blood samples will also be collected, to investigate polymorphisms of metabolic enzymes.

3.7.5

Case-control studies of lung cancer in Brazil, Uruguay and Argentina

P. Boffetta; in collaboration with E. de Stefani, Montevideo, Uruguay; E. Matos, Buenos Aires, Argentina; and V. Wunsch, São Paulo, Brazil

The urban areas of Brazil, Uruguay and Argentina have among the highest death rates in the Americas for cancer of all sites and of the lung in particular. Three similar studies have been designed to identify associations between environmental and occupational exposures and risk of lung cancer in São Paulo, Brazil, in Uruguay and in Buenos

Aires, Argentina, and to examine the synergistic effect of selected occupational exposures and tobacco smoking. The study in Uruguay confirmed the important role of known carcinogens, such as tobacco smoking and asbestos, and suggested an increased risk among workers of the meat industry and workers exposed to pesticides [86]; it is also addressing the risks for other cancer sites. The study in São Paulo suggested a smaller role than expected for occupational exposures, with increased risks in only a few categories, such as machinery and pottery workers. Data collection for the study in Argentina was completed in 1997 and analysis will be carried out in 1998.

3.7.6

Multicentric case-control study of lung cancer in India

P. Boffetta and R. Sankaranarayanan; in collaboration with M. K. Nair, Trivandrum, India; D.N. Rao, Bombay, India; and V. Shanta, Madras, India

Although the industrial population in India is very large and many hazardous industries are present, virtually no information exists on occupational risk factors for cancer. The presence of a network of well organized cancer registries is a favourable condition for conducting multicentric case-control studies, and therefore such a study has been started in Bombay, Trivandrum and Madras, to investigate occupational and environmental factors for lung cancer. A series of cases of lymphatic and haematopoietic neoplasms has also been included. Data collection was completed in 1997 and the analysis will be completed in 1998.

3.7.7

Case-control study of environmental tobacco smoke and genetic susceptibility to lung cancer

P. Boffetta, M. Lang, N. Malats, M. Friesen, S. Atawodi, S. Lea and J. Hall; in collaboration with W. Ahrens, Bremen, Germany; S. Benhamou, Villejuif, France; I. Brüske-Hohlfeld and H. Wichmann, Munich,

Germany; Forastiere, Poznan, Poland; Finland; A. Italy; G. Pe L. Simonat Russian Fe

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3.8.1

Multicentric study of laryngeal and urinary bladder cancer

P. Boffetta; in collaboration with E. Kogevinas, Janeiro, B Menezes, Canada; and

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Germany; V. Constantinescu, Bucharest, Romania; F. Forastiere and C. Fortes, Rome, Italy; B. Gabriel, Poznan, Poland; K. Husgafvel-Pursiainen, Helsinki, Finland; A. Menezes, Pelotas, Brazil; F. Merletti, Turin, Italy; G. Pershagen and F. Nyberg, Stockholm, Sweden; L. Simonato, Padua, Italy; and D.G. Zaridze, Moscow, Russian Federation

Among lung cancer cases, non-smokers have been exposed on average to lower levels of carcinogens than smokers; genetic susceptibility may play a greater role in risk of lung cancer in the former group of cases.

In ten centres from Brazil, France, Germany, Italy, Poland, Romania, the Russian Federation and Sweden, blood samples have been collected from about 150 non-smoking lung cancer cases, 150 smoking lung cancer

cases and 200 non-smoking control subjects, in order to determine (i) genetic polymorphism of glutathione *S*-transferase M1 and T1, (ii) the levels of the DNA repair enzyme *O*⁶-methylguanine-DNA methyltransferase, (iii) the formation of haemoglobin adducts with 4-hydroxy-1-(3-pyridyl)-1-butanone (a metabolite of tobacco-specific nitrosamines), and (iv) genetic alterations in the *p53* gene and *K-ras* mutations in lung neoplastic tissue of cases. Cases and controls have been interviewed about their smoking habits and exposure to environmental tobacco smoke. Enrolment of patients and laboratory analyses have been completed and statistical analysis will take place in 1998.

3.8 Head and neck cancer

Cancers of the head and neck comprise an important group of neoplasms that are showing increasing incidence in many parts of the world. Although alcohol drinking and tobacco smoking are established causes of these cancers, infection with the human papillomavirus may represent an additional important risk factor, as do some occupational exposures. In addition, patients with head and neck cancer are at increased risk of developing a second tobacco-related neoplasm, making them an important population in which to explore genetic susceptibility.

3.8.1

Multicentric case-control study of laryngeal cancer in Brazil, Argentina and Uruguay

P. Boffetta, P. Brennan and R. Herrero; in collaboration with E. de Stefani, Montevideo, Uruguay; M. Kogevinas, Barcelona, Spain; S. Koifman, Rio de Janeiro, Brazil; E. Matos, Buenos Aires, Argentina; A. Menezes, Pelotas, Brazil; J. Siemiatycki, Montreal, Canada; and V. Wunsch, São Paulo, Brazil

Argentina, Uruguay and southern Brazil have high incidence rates of laryngeal cancer, that do not seem to be explained only

by exposure to known carcinogens such as tobacco smoking and alcohol drinking. Following a series of studies of lung cancer (see Section 3.7.6), a multicentric study of laryngeal cancer has been initiated in three areas of Brazil (Rio de Janeiro, São Paulo and Pelotas and Porto Alegre), in Buenos Aires and in Montevideo. The study aims to identify occupational risk factors of this disease; additional aims are the assessment of the role of HPV infection, quantification of the contribution of tobacco smoking and alcohol drinking, and clarification of the role of other possible lifestyle risk factors, such as diet and mate drinking. Collection of interview data and biological samples started in 1997 and will be completed in 1999. In some of the centres, the study is being conducted in parallel with an investigation of the role of human papillomavirus infection in oral cancer (see Section 3.8.4).

3.8.2

Combined analysis of case-control studies of sinonasal cancer

P. Boffetta, E. Merler and D. Colin; in collaboration with R.B. Hayes, and L.A. Brinton, Bethesda, MD,

Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 5 -

**A Probabilistic Risk Analysis of
Lung Cancer Mortality
Associated with ETS Exposure**

March 20, 1998

Introduction

For the purpose of demonstrating the fragility of the United States Environmental Protection Agency's (U.S. EPA) attributable death calculations in its Risk Assessment on ETS (1992), we utilized U.S. EPA's relative risk point estimate. It is important to note that we do not believe that this point estimate is scientifically justified. In fact, the data indicate that one cannot distinguish the association between reported exposure to ETS and lung cancer described by the EPA from no association.

The statistical analysis provided by Philip Morris in this section examines the influence of just two of several possible modifications to some of the assumptions employed by the U.S. EPA in its Risk Assessment on ETS (1992). The technique of Monte Carlo simulation is used to construct a probabilistic model of estimated mortality purportedly associated with ETS exposure; it emphasizes the uncertainty associated with the single estimate reported by the U.S. EPA. The reduction in the number of so-called attributable deaths that follows from changes in only two factors reinforces our position that the U.S. EPA failed to utilize reasonable statistical assumptions in its estimation of the number of attributable deaths in its report.

A Probabilistic Risk Analysis of Lung Cancer Mortality Associated with ETS Exposure

The U.S. EPA's (1992) estimates of female and male annual lung cancer mortality in nonsmokers (never smokers plus former smokers who have quit for 5+ years) purportedly

attributable to ETS sources for the United States are shown in **Table 1**. EPA's total estimate of 3,060 deaths among nonsmokers attributable to ETS exposure has two main components: (i) 2,200 deaths attributed to "Background" (non-spousal) exposure to ETS, primarily in the workplace and other away-from-home settings, and (ii) 860 deaths attributed to "Spousal" exposure to ETS from a spouse who smokes.

The estimated mortality associated with Background and Spousal ETS exposure is a function of two key parameters: (i) the Relative Risk (RR) of lung cancer for nonsmokers exposed to spousal ETS relative to nonsmokers not exposed to spousal ETS (but who are exposed to background ETS), and (ii) the Z-factor, which is the ratio between the mean dose level in the "exposed" (spousal ETS) group and the mean dose level in the "unexposed" (non-spousal or background ETS) group. The value of Relative Risk assumed by the EPA is $RR = 1.19$ (90% CI: 1.04-1.35), based on EPA's meta-analysis of 11 U.S. epidemiological studies of never-smoking females. The RRs from the 11 studies were corrected for smoker misclassification prior to the meta-analysis using a 1.09% misclassification rate. However, the RRs were not corrected for other likely sources of bias, such as confounding due to dietary and other lifestyle factors inherent in the spousal smoking design, as well as recall bias. The value of the Z-factor assumed by EPA is $Z = 1.75$, and is based on U.S. urinary cotinine studies cited by EPA.

As stated in EPA's report *Respiratory Health Effects of Passive Smoking* (EPA, 1992), the estimated mortality attributed to Background ETS exposure is proportional to $(RR - 1) / (Z - RR)$, and can be computed as

Table 1. U.S. EPA's estimates of annual lung cancer mortality.

Estimated annual lung cancer mortality						
Smoking status	Sex	Exposed to spousal ETS	Population (in millions)	Background ETS	Spousal ETS	Total ETS
Never-Smoker	F	No	12.92	410		410
Never-Smoker	F	Yes	19.38	620	470	1090
Never-Smoker	M	No	9.93	320		320
Never-Smoker	M	Yes	3.13	100	80	180
Former Smoker	F	No	2.0	60		60
Former Smoker	F	Yes	6.7	210	160	370
Former Smoker	M	No	8.8	280		280
Former Smoker	M	Yes	6.2	200	150	350
TOTAL			69.07	2200 (71.9%)	860 (28.1%)	3060

$$\text{Estimated Mortality Attributed to Background ETS} = 6484.21 \times \frac{\text{RR} - 1}{Z - \text{RR}}$$

where 6484.21 is a proportionality constant such that EPA's estimate of 2,200 is obtained when RR = 1.19 and Z = 1.75. Similarly, the estimated mortality attributed to Spousal ETS exposure is proportional to (RR - 1) (Z - 1) / (Z - RR), and can be computed as

$$\text{Estimated Mortality Attributed to Spousal ETS} = 3379.65 \times \frac{(\text{RR}-1)(Z-1)}{Z - \text{RR}}$$

where 3379.65 is a proportionality constant such as that EPA's estimate of 860 is obtained when RR = 1.19 and Z = 1.75.

Based on the above formulas, the estimated mortality attributed to Background, Spousal, and Total (Background + Spousal) ETS exposure as a function of the assumed value of Z is shown in **Figures 1, 2, and 3** for values of RR equal to 1.19, 1.15, and 1.10, respectively.

As shown by Figures 1, 2, and 3, the estimated mortality is very sensitive to the assumed values of RR and Z, both of which are subject to considerable uncertainty. A Monte Carlo analysis was performed to help understand the distribution of possible values of the mortality estimates as a function of the uncertainty in RR and Z. For this analysis, the uncertainty in RR incorporates both the uncertainty in the smoker misclassification rate as well as the sampling distribution for the "true" Relative Risk. The uncertainty in the smoker misclassification rate is modeled as a compound distribution, consisting of a uniform distribution from 1% to 3% and a

EPA Estimate of Total (Spousal + Background) ETS-Attributable Lung Cancer Deaths
By Z-Factor

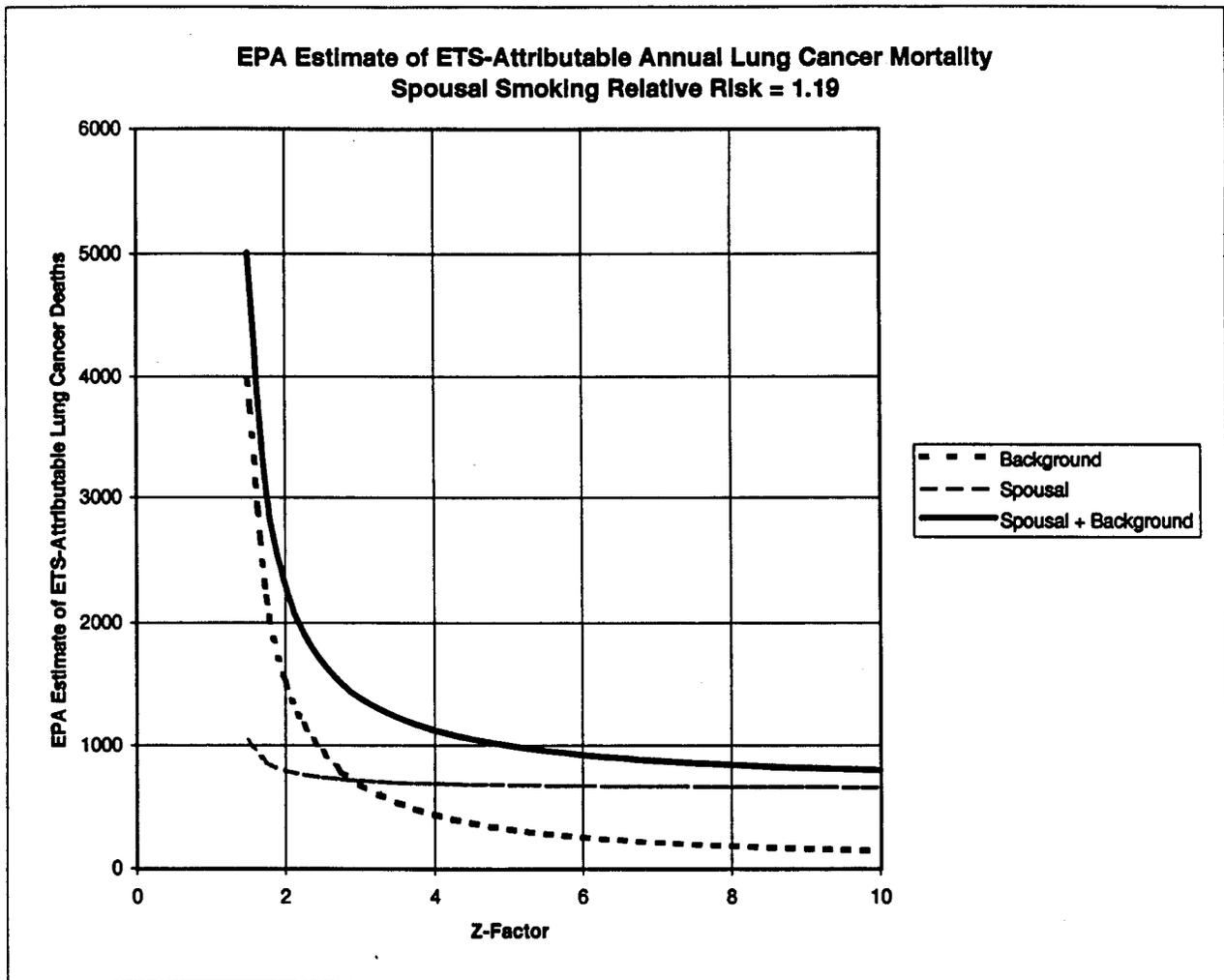


Figure 1.

EPA Estimate of Total (Spousal + Background) ETS-Attributable Lung Cancer Deaths
By Z-Factor

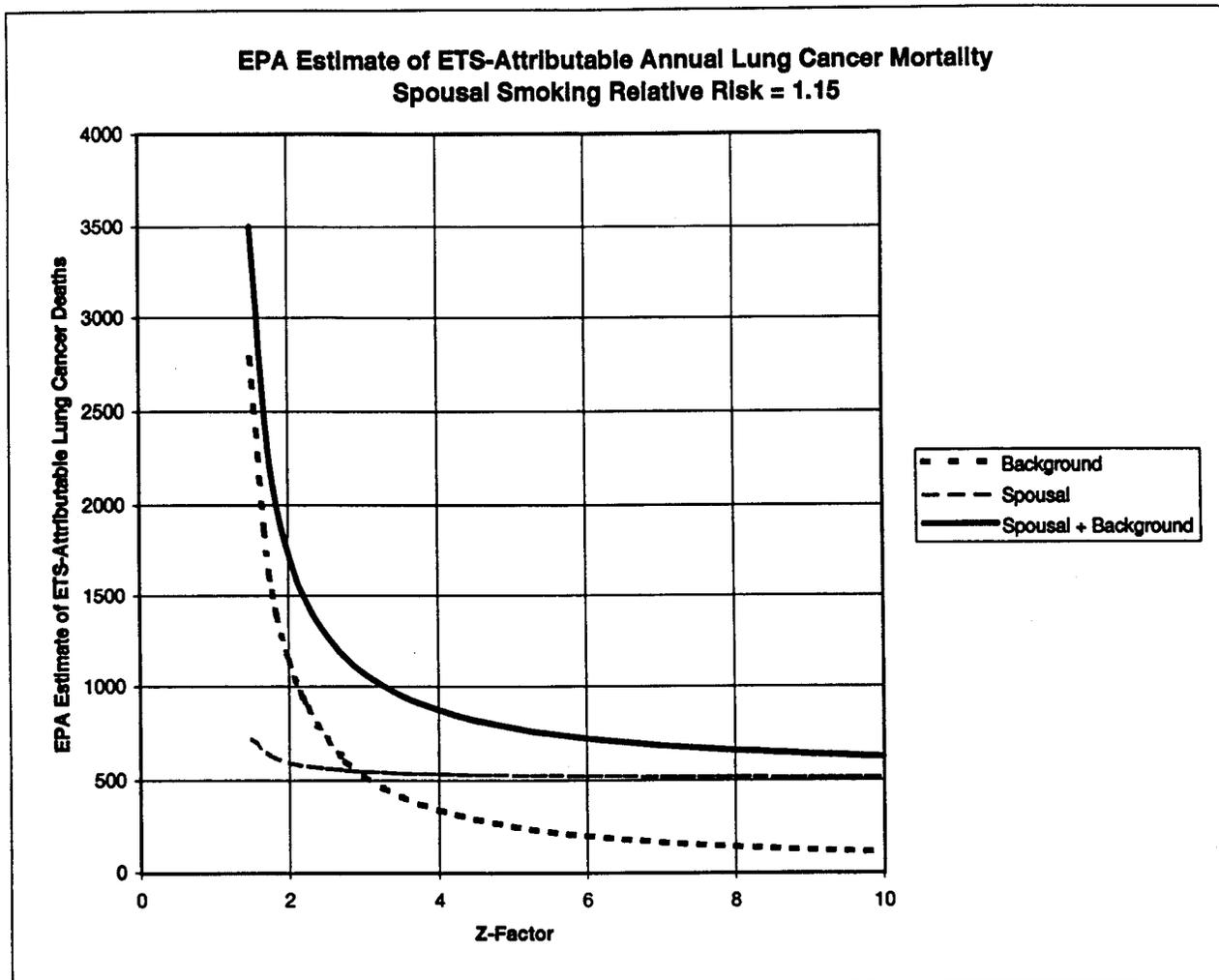


Figure 2.

EPA Estimate of Total (Spousal + Background) ETS-Attributable Lung Cancer Deaths
By Z-Factor

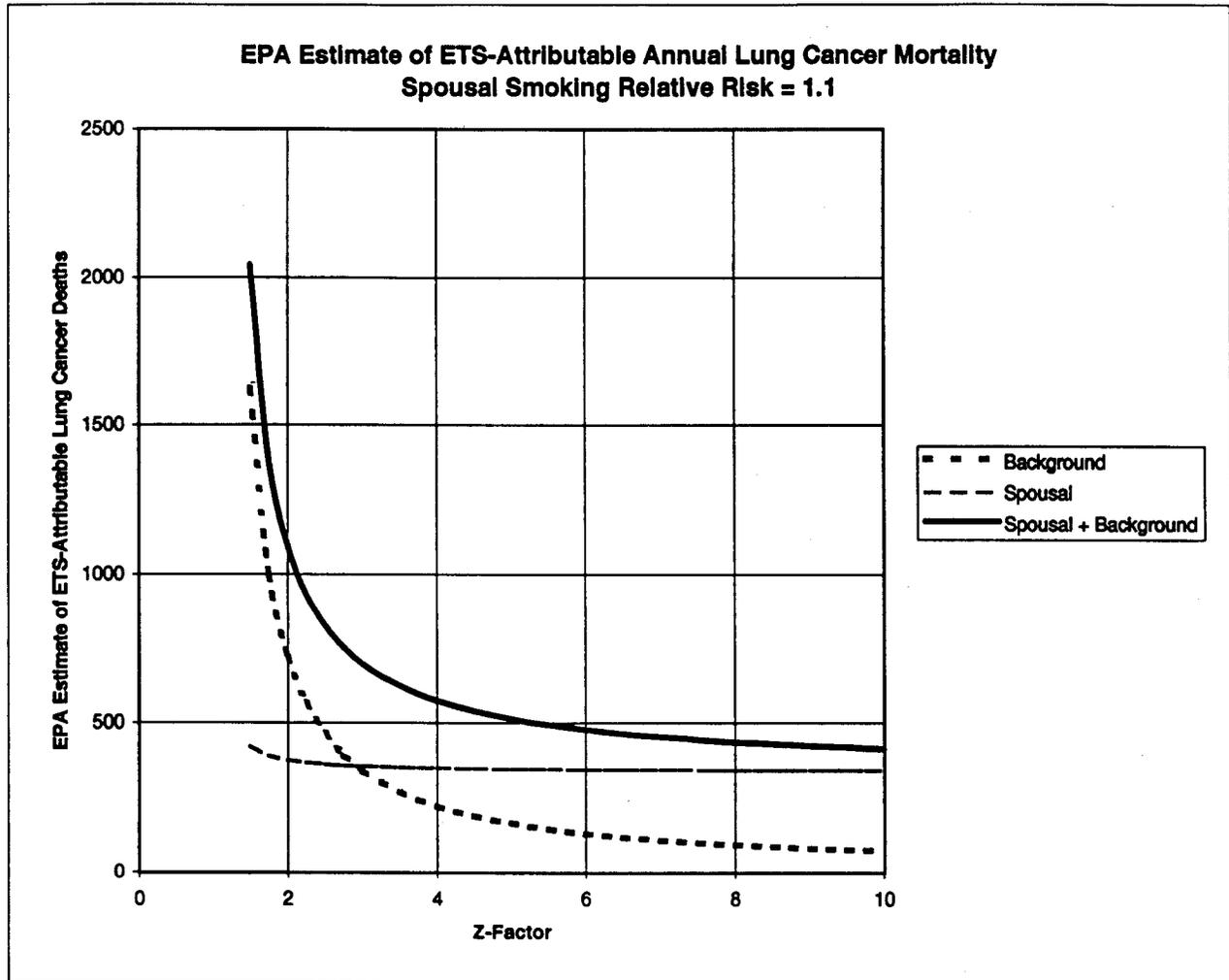


Figure 3.

decreasing triangular distribution from 3% to 5%. Based on results by Ogden et al. (1997), the uncertainty in the smoker misclassification rate is simulated by using the distribution in **Figure 4** for the geometric mean of the Relative Risk distribution. The value of the “true” Relative Risk is estimated from a lognormal distribution with the randomly selected value of the geometric mean and a geometric standard deviation equal to 1.0825 (determined from the 90% confidence interval 1.04-1.35 for the “true” Relative Risk).

The uncertainty in the value of the Z-factor is represented by a compound distribution, consisting of a uniform distribution from 1.75 to 10, followed by a declining triangular distribution from 10 to 20. This distribution is shown in **Figure 5**.

Based on the preceding uncertainty assumptions for Relative Risk and Z-factor, the Monte Carlo analysis of estimated mortality was performed for 10,000 iterations using Crystal Ball® Version 4.0. Again, we reaffirm our strong view that the Relative Risk point estimate reported by U.S. EPA is scientifically unjustified and that our use of this value is solely illustrative. With this in mind, the resulting simulated distribution for mortality associated with Total (Background + Spousal) ETS exposure is shown in **Figure 6**. The mean and median of this distribution are 804 and 702 respectively, which are far below the EPA’s estimate of 3,060. In fact, 3,060 is at the 98.7th percentile of this distribution.

In this simulation, uncertainty in the Relative Risk and Z-factor are represented by independent probability distributions. As stated in the EPA’s report, however, the parameters RR

Figure 4.

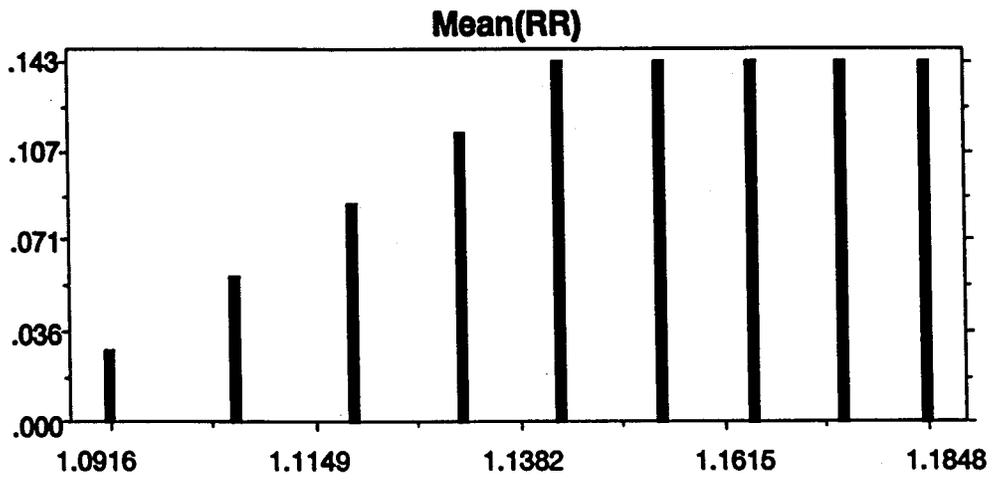


Figure 5.

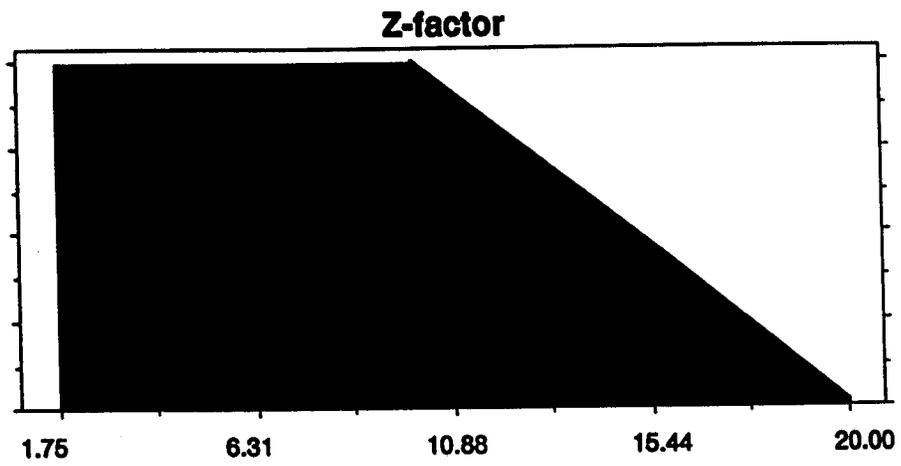
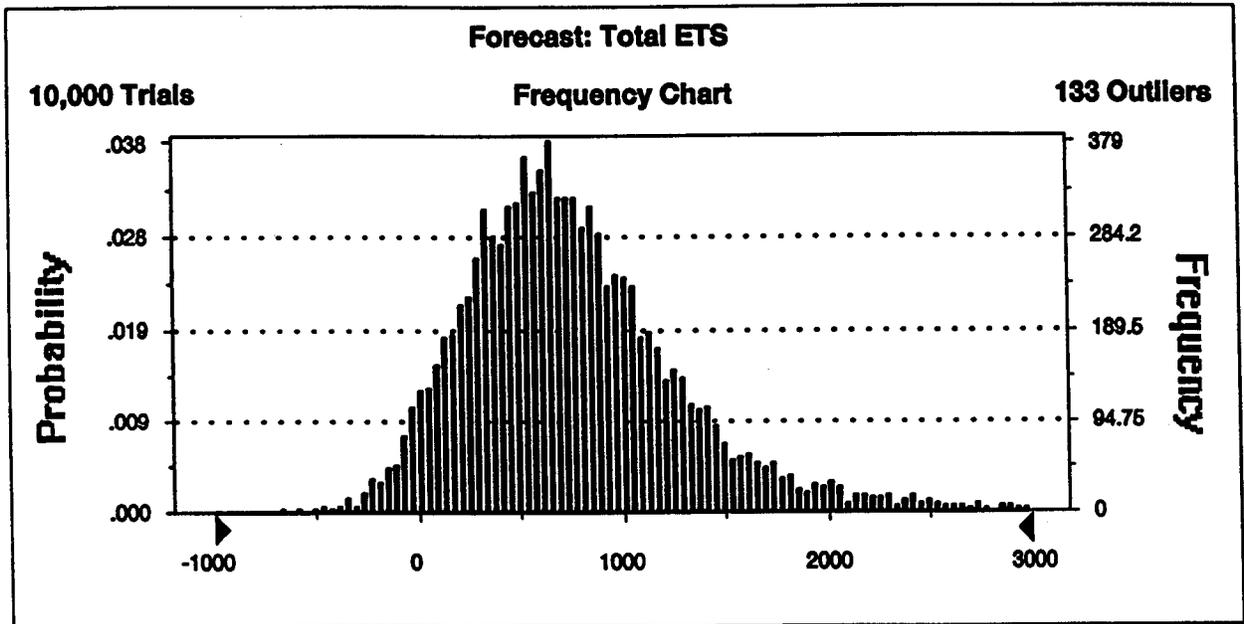


Figure 6.



and Z are not actually independent, but would be expected to covary in the same direction (to be positively correlated). For example, if the contributions of background to total ETS exposure decrease, Z would increase, and the observable relative risk from spousal exposure would tend to increase as well. Sensitivity analyses reveal that the effect of increasing correlation between RR and Z decreases the mean and standard deviation of the resulting simulated distribution of estimated mortality. Hence, estimates of mortality as high as 3,060 are even less likely than represented by the distribution in **Figure 6**.

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- Appendix 6 -

**Measurement of Cotinine Concentration in
Biological Fluids and Use of the Results in
Epidemiological and Exposure Studies**

March 20, 1998

Introduction

There are two reasons that a biomarker for tobacco use or environmental tobacco smoke (ETS) exposure is of considerable interest. The first is to determine whether an individual is a smoker or nonsmoker. The second is to attempt to quantify levels of exposure to ETS for nonsmokers. To answer the first question is relatively straightforward, since in principle the difference between a given biomarker for a smoker and a nonsmoker should be large. The second question, however, requires one to attempt to differentiate between two small and similar values. As a result, the analytical criteria for these two applications are very different, and require consideration of different methodologies. In addition to the analytical concerns, the potential impact of interpersonal variation in metabolic rates and pathways contributes confusion. Add the choice of saliva, serum, or urine as the sample matrix, and it is easy to see why a complex set of measurement and data interpretation uncertainties has evolved. Some of these issues will be addressed in this discussion.

Cotinine is a carbon-oxidation metabolite of nicotine. It is found at measurable levels in saliva, serum and urine of subjects who smoke, who use other forms of tobacco, or who have been exposed to ETS at sufficient levels (Benowitz, 1996). Cotinine meets many of the criteria proposed by the National Research Council (NRC) for an ETS biomarker (NRC, 1986), but it must be kept in mind that cotinine is derived from nicotine. Although cotinine may serve as a biomarker for nicotine intake, it may not serve as well as a biomarker for ETS exposure. Furthermore, cotinine has a biological half-life of approximately 20 hours and can only represent nicotine intake within an

interval of a few half-lives. While a number of alternative biomarkers have been proposed, most of these have been devaluated relative to cotinine (Benowitz, 1996, Table 5). In the search for a biomarker for tobacco use or ETS exposure, by a process of elimination cotinine appears well on the way to becoming the compound of choice for many researchers (Benowitz, 1996).

Analytical methods may be described as selective or as specific. Although many analytical methods exist for specific detection and quantification of cotinine, most of these are based on instrumental methods of analysis (Benowitz, 1996, Table 4). Frequently, these methods are considered too expensive, too labor intensive, or to require special skills beyond those frequently available for epidemiological or exposure studies. Radioimmunoassay (RIA) methods for cotinine were developed and widely applied in the scientific community that conducts various epidemiological and exposure investigations (Langone et al., 1973). RIA methods are rapid, economical and require few special analytical skills, but they are selective rather than specific for chemical compounds.

Selectivity was likely thought to be an adequate criterion for cotinine. Until approximately 1987, cotinine was considered to be the major, if not the only, known nicotine metabolite of analytically significant concentration in the biological fluids of interest. Although N-oxidation metabolites of nicotine were known, they were found only at low concentrations (Turner, 1969). After 1987, the presence of significant concentrations of *trans*-3-hydroxycotinine (Neurath and Pein, 1987), and glucuronide conjugates of nicotine, cotinine and *trans*-3-hydroxycotinine (Curvall et al., 1989) in urine were recognized. Although cotinine appears to be the major nicotine

metabolite in saliva and serum (Curvall et al., 1990), *trans*-3-hydroxycotinine (Byrd et al., 1994) or its glucuronide conjugate (Andersson et al., 1997) is the most prevalent in urine. Actually, cotinine constitutes only 10-15% of the nicotine metabolites. At least seventeen (17) metabolites of nicotine have been identified in urine (Kyerematen et al., 1987). Many of these have been quantified (Byrd et al., 1994; Andersson et al., 1997).

Cotinine as a Biomarker for Smoker Classification

The evolution of cotinine as a biomarker began as a means to discern smokers from nonsmokers. The levels of cotinine in saliva, serum and urine of a smoker of >20 cigarettes per day is relatively easy to distinguish from that of a nonsmoker using a variety of analytical methods including RIA. In large epidemiological studies for which cost, labor, time and other issues are important, saliva samples are the easiest to obtain. It has been reported that analytical determinations of cotinine in saliva provide essentially the same information as similar determinations in serum (Curvall et al., 1990). A mean saliva to serum ratio of cotinine concentration of 1.2 has been reported (Curvall et al., 1990). Although further study is needed, at this time both matrices are thought to be relatively free of other analytically significant metabolites of nicotine. The principal reason that nicotine is not used as a biomarker for its uptake is that the half-life of nicotine in biological fluids is only approximately 2 hours (Benowitz, 1996). Measurement of nicotine would not give a representative estimate of nicotine intake during the previous few days.

In epidemiological or exposure studies, classification of an individual as a smoker or nonsmoker is critical to the results, especially in the interpretation of data at low relative risk (RR) levels such as those reported with regard to ETS exposure (Jenkins et al., 1996). Wide ranges of smoking status misclassification have been suggested based on questionnaire data (Riboli et al., 1995), and misclassification of 2 -5% smokers claiming to be nonsmokers is not uncommon (Jenkins et al., 1996; Phillips et al., 1996). RR values below approximately 2.0 are considered weak associations (Greenberg, 1986). In the area of ETS epidemiology, low RR values, as for example 1.2, could be significantly influenced by smoking status misclassification of less than 5%.

Utilization of cotinine as a biomarker for smoker classification is less of an analytical issue than a policy issue. Interpersonal variability in metabolic processes, lifestyle, and other factors creates variations in cotinine levels that make it very difficult to distinguish an occasional smoker from a nonsmoker with recent exposure to high levels of ETS (Phillips et al., 1996). An additional issue that is rarely addressed in the literature is "intra-personal" variability. For example, researchers who conduct exposure studies infrequently collect more than one, possibly two, samples from an individual subject. Ranges of exposure that result from daily lifestyle variations are not considered. This variability may well be much greater than the analytical variability.

As a result, uncertainty exists over the cotinine concentration that unambiguously differentiates a smoker from a nonsmoker. At the present time, somewhat empirical decisions are made. These decisions may be influenced by the goal of the investigation. It has been suggested that cotinine concentrations of 10-50 ng/mL in saliva or serum, or 50 nanogram cotinine per milligram

creatinine in urine (cotinine-creatinine ratio: CCR) are levels below which a subject is considered to be a nonsmoker and above which is classified as a smoker. Conflicts with questionnaire data arise and are occasionally resolved by dismissing the subject from the study. The latter decision is prudent for experimental purity, but could eliminate an important segment of the total population.

The complex distribution of cotinine species in urine would recommend the use of serum or saliva for smoking status classification. Pirkle et al. (1996) have determined serum cotinine levels for over 4,000 subjects spanning wide age groups, smoking status and ETS exposure. Their results show a finite percentage of the population with serum cotinine levels below 10 ng/mL that report tobacco use, and above 10 ng/mL that report no tobacco use or ETS exposure at home or at work. Three regions of classification should be considered: smokers, nonsmokers and indeterminate. The overlap is a combined result of interpersonal metabolic variances, variation in the degree of ETS exposure in nonsmokers, inaccurate questionnaire-derived data and analytical variance. These levels of uncertainty not only have potential for impact on conclusions drawn from scientific studies, but on decisions regarding individuals such as classification for other purposes.

In summary, when an individual is clearly a smoker, or clearly a nonsmoker with moderate ETS exposure, cotinine measurements in saliva, serum or urine may be capable of distinguishing between the two. To determine the classification of occasional smokers or nonsmokers with significant ETS exposure is not an analytical issue, but one of establishing a decision point. Establishing this point is complicated by the lack of a clear definition of a smoker. If the cotinine level used to classify a subject as a smoker is too high, smokers may be erroneously

classified as nonsmokers. One study (Saracci and Riboli, 1989) that classified as smokers subjects with urinary levels of cotinine above 50 ng/mg creatinine, later reported that of the 47 subjects that were excluded from the study as smokers, only 20 (1.5% of the total study population) can be considered to be smokers (Riboli et al., 1995). The authors suggested that a cutoff level of 150 ng/mg should be used to avoid exclusion of nonsmokers with high ETS exposure. The position could be taken that a level lower than 50 ng/mg is more realistic to identify light smokers who are deceivers in questionnaire responses. This example clearly demonstrates that a single point value used for all subjects is woefully inadequate for such an important decision. Selection of the point value criteria may be driven inadvertently by the goal of the study.

Cotinine as a Biomarker for ETS Exposure

The analytical chemistry issues for the use of cotinine as a biomarker for classification of smoking status are minor compared to those associated with its use for estimating ETS exposure-dose relationships. The evolution of the use of cotinine levels from a method to make a binary decision (smoker or nonsmoker) to implied quantification of ETS exposure was a slow but relentless process. Cotinine concentrations in the case of regular smokers are normally 2-3 orders of magnitude greater than of the limit of quantification (LOQ) of analytical methods that have been applied. Even the marginal region of 1-5 ng/mL in saliva, serum and urine are adequately measurable using RIA and chromatographic methods.

Especially in urine samples, analytical and interpersonal metabolic considerations may become paramount. These factors, coupled with what may be considered a propensity toward analytical expediency in epidemiological ETS exposure studies, may lead to opportunities for contradictory conclusions. Recently, prominent professors of epidemiology expressed the view that contradictory findings in epidemiological research are “common” (statements of Trichopoulos et al. 1997). It is possible that some of these contradictions stem from inadequate experimental design, insufficient number of subjects, or from errors in the analytical data upon which decisions are based.

As mentioned above, saliva, serum and urine have all been used as matrices for the determination of nicotine and its metabolites. Because urine has been used in a large number of epidemiological studies, and because that matrix appears to contain the largest number of metabolites, it will be considered first.

Metabolic Products of Nicotine in Urine

At least 17 metabolic products of nicotine have been identified in human urine (Kyerematen et al., 1987). Of these, cotinine has been the focal component. Analytical methodologies have been developed that may be applied reasonably to those compounds shown in **Table 1**, along with some of their glucuronide conjugates. The remaining metabolites require more involved analytical methods. **Table 2** shows the results of two independent studies that measured in human urine the compounds shown in **Table 1**. The results are reported as percent of the total found based on nicotine equivalents of each compound, and the cumulative total if the compounds

Table 1. Nicotine and metabolites that have been determined analytically.

Compound	Abbreviation
Nicotine	NIC
Nicotine glucuronide conjugate	NIC-G
Cotinine	COT
Cotinine glucuronide conjugate	COT-G
<i>trans</i> -3'-Hydroxycotinine	3HC
<i>trans</i> -3'-hydroxycotinine glucuronide conjugate	3HC-G
Nicotine-N'-oxide	NNO
Cotinine-N-oxide	CNO
Demethylnicotine	DMC

**Table 2. Distribution of nicotine and metabolites in two studies.
(Each compound was converted to an equivalent amount of nicotine.)**

NIC + metabolites	% of total found ¹	cumulative % total	% of total found ²	cumulative % total
3HC	35	35	36	36
COT-G	17	52	14	50
COT	13	65	9	59
NIC	10	75	9	68
3HC-G	9	84	23	91
NIC-G	3	87	5	96
NNO	7	94	3	99
CNO	4	98	1	100
DMC	2	100	Not Determined	
(Smokers) N =	11		91	
PERCENT N-OXIDE	11		4	

-
1. Byrd et al. (1994) (SD values are 3-8%, taken from Table I, RSD values calculated to be 21-75%).
 2. Andersson et al. (1997) (SD values are 0.9-10.6 taken from Table 4, RSD values calculated to be 28-100%).

are taken into account sequentially. There is general agreement between these mean values reported in the two studies, although a significant difference was found between the distribution of the glucuronide conjugates, especially the 3HC-G. The variance of the mean of the percentage of each metabolite is somewhat higher in the study by Andersson et al. (1997) than in the one by Byrd et al. (1994).

It is clear from Table 2 that free (i.e., not conjugated) cotinine accounts for only about 10% of the total nicotine metabolites analytically detectable in human urine. This observation requires a number of considerations:

1. Whether a selective (i.e., RIA) or specific (i.e., GC/MS) analytical method is used for cotinine determination, only about 10% of the nicotine equivalent as cotinine will be measured unless an enzyme-catalyzed hydrolysis is conducted to free the conjugated cotinine. Even then only a portion of the nicotine metabolites is measured.
2. In the case of RIA, a cross reactivity of approximately 34% for *trans*-3'-hydroxycotinine (3-HC) in urine samples has been reported (Zuccaro et al., 1997). Multiple regression of data presented by Zuccaro et al. (1997) yields an expression for cotinine concentration determined by RIA ($[COT]_{RIA}$) versus cotinine concentration ($[COT]$) and *trans*-3'-hydroxycotinine ($[3-HC]$) determined by HPLC as follows (concentrations in $\mu g/L$):

$$[COT]_{RIA} = 0.97 \pm (0.63) [COT] + 0.29 (\pm 0.14) [3-HC] + 557 (\pm 371)$$

Interestingly, both the coefficient of variation and the “p” value for the coefficient for 3-HC are smaller than that for COT. This suggests that the results from RIA determinations as used by Zuccaro et al. are more strongly correlated to the [3-HC] than to [COT], although more selective for cotinine.

The results of both studies represented in **Table 2** indicate that the mean *trans*-3'-hydroxycotinine is about three times the concentration of cotinine in urine. RIA would yield a result that is a combination of cotinine and cross reactivity with *trans*-3'-hydroxycotinine. The analytical bias can be as high as a factor of two, and leads to considerable confusion when RIA results are compared to GC or GC/MS results.

3. If enzymatic hydrolysis of the conjugates is included in the analytical step, the resulting nicotine, cotinine and *trans*-3'-hydroxycotinine concentrations determined by chromatography could account for approximately 85-90% of the total nicotine and its metabolites found in urine samples. This can be concluded from the cumulative total columns in **Table 2**. The only reliable approach to relate nicotine dose (and, thereby, uptake) to nicotine metabolites in urine for individuals is to perform a complete suite of metabolite determinations to account for pharmacokinetic differences.
4. The results in **Table 2** represent mean values that are reasonably consistent between the two studies. However, the variation of each mean can be up to 100%. Some of this distribution is analytical error; some of it is interpersonal variation. Although correlation between means

in different matrices has been established (Benowitz, 1996), no controlled experiments are known to determine the variance between individual values either on an interpersonal or intrapersonal level.

In summary, with frequently used analytical approaches for determination of cotinine in urine, only about 10% of the total nicotine is accounted for in the analysis. When RIA is used, the representation as a cotinine determination may be in error by as much as a factor of two. Approximately 25-35% of the metabolites is present as conjugates not detected unless enzymatic hydrolysis is conducted. Interpersonal variation is an important issue dealing not only with nicotine exposure, but also with metabolic rates and distribution among the various metabolites and their conjugates. When only 10% of the metabolite products of nicotine are used, it is not unexpected that large variations in results will be obtained.

Finally, urine volume is a variable associated with many factors including liquid consumption. It is possible that careful management of 24-hour urine sampling can be used successfully (Benowitz et al., 1997). Unfortunately, many studies related to ETS exposure make determinations using only one sample. Use of the cotinine-creatinine ratio (CCR) to adjust for urine output has limitations because of the variables that influence creatinine output.

Metabolic Products of Nicotine in Serum and Saliva

Little has been reported in the literature concerning nicotine metabolites other than cotinine in blood. From the point of view of analytical significance, cotinine appears to be the most important nicotine metabolite in serum. The conversion of nicotine to cotinine in blood by the liver of smokers has been related to daily nicotine intake as $0.08 \text{ [(mg/24 hr)/ng/mL]}$, with a coefficient of variation of 21.9% (Benner et al., 1989). Thus, a serum cotinine level of 250 ng/mL corresponds to a daily intake of 20 ± 4 mg of nicotine. It was suggested that this factor also applies to nonsmokers (Benowitz, 1996).

Benowitz (1996) has described a calculation that suggests a urine to blood ratio of 6, but cautions that the interpersonal variability described previously would contribute to variability in this ratio. There are insufficient data to estimate the interpersonal variability. The ratio predicted by Benowitz has had some experimental verification with a urine to blood cotinine ratio of 5 reported (Jarvis et al., 1984). Benowitz (1996) also reports that saliva to blood ratios are 1.1-1.4, leading to an essentially interchangeable use of saliva and serum cotinine data. These conversions have been summarized in **Table 3**.

Using the serum to daily nicotine intake conversion given above, and data from a large study (Pirkle et al., 1996, Figure 2), the approximate daily intake for nonsmoking subjects with no reported ETS exposure, reported ETS exposure and reported smokers is 0.024, 0.08, and 40 mg, respectively. Using a similar approach to a different set of data, a group of 91 smokers with mean

Table 3. Conversion factors for cotinine.

To convert	Multiply by
Cotinine in serum to cotinine in urine ng/mL → ng/mL	5-6
Cotinine in serum to cotinine in saliva ng/mL → ng/mL	1.1-1.4
Cotinine in serum to daily (24 hr) nicotine intake ng/mL → mg/day	0.08±0.18

saliva cotinine concentrations of 261.1 (± 116.2) ng/mL would convert to a daily intake of 20.9 (± 3.8) mg of nicotine, which may be compared to the experimentally determined 19.8 (± 6.3) (Andersson et al., 1997). Other examples reinforce the evidence that serum or saliva cotinine levels can be used to estimate the mean values of nicotine intake, but the variance about those estimates for individuals may be significant, as described below. Furthermore, most of the available data are based on single samples from each individual, and very little is known about intra-individual variation.

Use of Cotinine as a Nicotine Biomarker in Exposure Studies

In a large study by Jenkins et al. (1996), saliva samples for cotinine measurements were taken for nonsmoker subjects in 16 cities in the United States the day before and the day after personal monitors were used to measure ETS parameters, including nicotine, in workplace and non-workplace environments. Four cell types were defined depending on smoking taking place away from work or in the workplace. A strong correlation ($R^2 = 0.991$) was found between the median 24-hr time weighted average (TWA) nicotine exposure and the median average salivary cotinine level on a cell-by-cell basis. Salivary cotinine levels were not well correlated with individual 24-hr TWA nicotine levels ($R^2=0.105$). Based on an earlier study discussed above (Saracci and Riboli, 1989), nonsmokers not exposed to ETS had an apparent estimated daily intake of 0.024 mg of nicotine, and nonsmokers exposed to ETS 0.08 mg. These compare favorably with similar calculations using the data of the study by Jenkins et al. (1996). For the cell with nonsmoking home and nonsmoking workplace, the calculated daily intake is 0.013 mg (vs. 0.024), whereas, for the subjects in the cell with smoking away from work but not at work, an estimated daily intake of 0.07

mg (vs. 0.08) is calculated. In the most extreme case of ETS exposure both at work and away from work, a daily nicotine intake of 0.16 mg is estimated. These results appear to be consistent findings, although the Jenkins data were less so when salivary cotinine levels were below 2 ng/mL. All comparisons are based on mean or median values and may not be valid for individuals.

Although salivary cotinine measurements have been used as a biomarker for nicotine exposure for means (or medians) of large numbers of subjects from within given exposure groups, a wide range of levels of salivary cotinine is found for individual smokers and nonsmokers alike that does not correlate well to their nicotine exposure levels (Saracci and Riboli, 1989). Because of the consistent relationships between cotinine in saliva, serum and urine, it is concluded that none of these matrices can be reliably applied to individual subjects using a single measurement. As stated above, analytically valid measurements of cotinine in any of these matrices can distinguish a smoker of 20 cigarettes per day from a nonsmoker who is not exposed to ETS. Distinction between a light smoker and a nonsmoker exposed to high ETS levels is very problematic. Kemmeren et al. (1994) have used a statistical equation shown below, and based on expressions for “t” values, to predict the number of replicate measurements needed to estimate the “habitual” serum cotinine level of a subject within a selected percentage of the “true value.” The expression used is

$$k_{0.95} = (1.96 \times CV/D)^2$$

in which “k” is the number of measurements needed to estimate the habitual cotinine level within a certain percentage “D” of the intra-personal variation “CV” at a 95% confidence level. The intra-

personal coefficient of variation (CV) was estimated to be 16.1%, although there is little supporting evidence for this value. Using this value, one calculates that a single sample can estimate the “habitual” cotinine concentration to within approximately $\pm 32\%$. Ten (10) replicate samplings would be required to obtain a measure within $\pm 10\%$ of the “habitual” value. The issue of the time period over which sampling and measurements were or should be made was discussed briefly by Kemmeren et al. (1994), who observed that samples should be taken over a longer period of time than just a few days.

In summary, it does not seem appropriate to debate a point value for a cutoff to distinguish smokers from nonsmokers based on a single measurement of salivary cotinine. In this case, the intra-personal variation in the concentration will contribute significantly to the cotinine level, and less is known about the interpersonal variation with a fixed uptake of nicotine. These pharmacokinetic and lifestyle factors must be given more consideration when attempting to use a point value for decision making.

Salivary Cotinine Concentration Related to Air Quality Measurements

Benowitz (1996) has discussed relationships between cotinine measurements and nicotine intake. The relationships proposed have been described earlier and provide some opportunity to assess nicotine intake on a daily average. Three ETS exposure studies involving subjects from 16 cities in the United States (Jenkins et al., 1996), Stockholm, which has exceptionally low ETS (nicotine) levels (Phillips et al., 1996), and Barcelona, which tends to have

relatively high levels of ETS both in the workplace and at home (Phillips et al., 1997), provide an opportunity to consider such relationships. In all three studies, salivary cotinine measurements were made using the RIA method both prior to and after the subjects' breathing zones were sampled using personal monitors. In the case of working subjects, dual personal monitors were used for workplace and away from workplace environments. The data obtained from the monitor samples were used to calculate 24-hour time weighted average (TWA) nicotine concentrations.

Figure 1 shows a plot of the median salivary cotinine concentration versus the 24-hr TWA nicotine in air concentration for different subject categories for all three studies. The median was used because at very low levels near analytical detection limits it is generally a more representative value than the mean. In most cases, at higher concentrations the mean and median were relatively close in value. One clear outlier point is observed in the results shown in **Figure 1**. This point represents non-working housewives or househusbands living in a nonsmoking home environment in Stockholm (Phillips et al., 1996). That datum was not included in the regression. No explanation was provided for this result, which represents by far the highest nicotine and cotinine levels found in the Stockholm study, and is almost 10-fold higher than that found for homes of workers with smoking environments. Only nine (9) subjects were in this group, and it is possible that the values obtained were not representative. There could also have been unknown extraneous sources of nicotine.

After removing the datum discussed above, the remaining data shown in **Figure 1** yield the following linear regression.

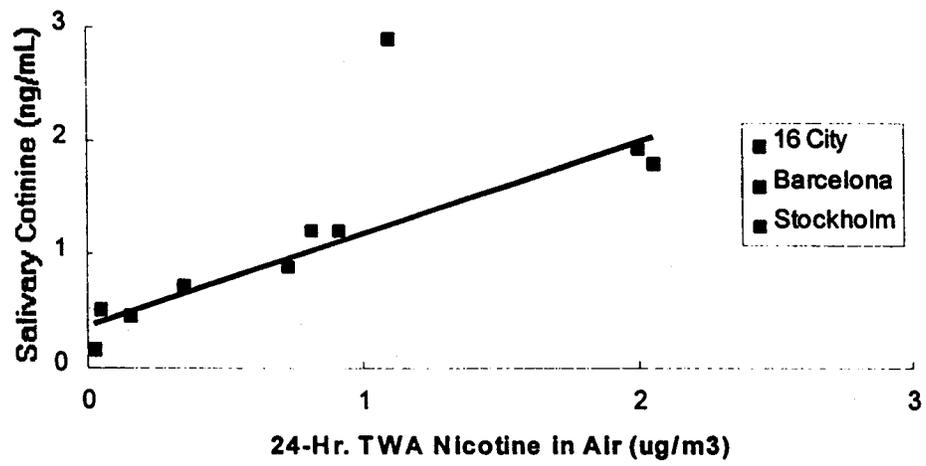


Figure 1. Plot of Salivary Cotinine Concentration versus 24-hr. TWA Nicotine Concentration Exposure in Nonsmokers

$$\text{Salivary Cotinine (ng/mL)} = 0.76(\pm 0.07) * \text{Nicotine } (\mu\text{g/m}^3) + 0.38(\pm 0.07)$$

For both the coefficient and intercept, $p < 0.001$. However, again it should be emphasized that this correlation is for median values of groups of subjects ranging from as few as 9 to over 100. Individual variation is expected to be large, but the data were not made available in the publications. Furthermore, geographic location appears to play a significant role (Jenkins et al., 1996; Phillips et al., 1996; Phillips et al., 1997). Thus, there is no evidence that salivary cotinine concentration can be used to estimate ETS exposure for an individual using such a correlation.

The intercept in the regression of the data shown in **Figure 1** is different from zero with statistical significance. The implication of this intercept is that the mean salivary nicotine concentration in all groups has a base level that is not derived from nicotine in ETS. Using the factor shown in **Table 3**, a daily intake of $30 \mu\text{g}/24 \text{ hr}$ from an additional source is estimated. It is tempting to suggest that there are sources of cotinine in saliva of nonsmokers other than that from ETS exposure. Dietary sources have been suggested (Castro and Monji, 1986; Sheen, 1988; Davis et al., 1991; Domino et al., 1993; Domino, 1995). There are still open questions concerning potential cross-reactivity and other issues related to the RIA method of analysis, so that the potential for an artifact cannot be ruled out.

A factor that contributes to the intercept described above is the data analysis policy used. In the indoor air studies mentioned above (Jenkins et al., 1996; Phillips et al., 1996; Phillips et al., 1997), if the concentrations of salivary cotinine were found to be below the limit of

quantification (LOQ) or limit of detection (LLD), the values were empirically set equal to one-half of the LLD or LOQ. Depending on a number of factors and the criteria used to establish the LLD or LOQ, this means that in some cases up to 60% of the data was set to 0.5 ng/mL for salivary cotinine. With so few data points, a linear regression with an intercept near 0.5 ng/mL appears reasonable. However, one does not know what the values may be if they are below the LLD or LOQ. The analytical method used by Pirkle et al. (Pirkle et al., 1996; Bernert et al., 1997) has a reported detection limit of 50 ng/L (0.05 ng/mL). Based on estimates calculated from data shown in Figure 2 in Pirkle et al. (1996), approximately 50 % of the data for nonsmokers is found in the region below 0.5 ng/mL, further bringing into question the data treatment used in exposure studies.

Other Factors that Affect Cotinine Concentration in Biological Fluids

From the data discussed so far, it appears that salivary and serum cotinine concentrations may be correlated to exposure to nicotine in ETS for nonsmokers. Almost all exposure studies have been conducted with mature adults >17 years of age. However, a number of studies have suggested that cotinine levels for a given exposure are higher in children and blacks (Zuccaro et al., 1997; Wagenknecht et al., 1993; Pattishall et al., 1985). In one of the few studies to attempt to use multivariate methods of analysis of exposure data, Pirkle et al. (1996) performed multiple regression of log serum cotinine concentration versus such parameters as age, ethnic background, number of smokers in the house, size of household, number of rooms in the house, etc. Dietary intake was considered in the form of consumption of bell peppers and found to be of

marginal statistical significance ($0.001 < p < 0.05$) in children 4-11 years of age, but not significant in adults. The regression coefficients that were obtained had a wide range of statistical reliability.

As an illustrative example, **Figure 2** shows a plot of serum cotinine concentrations calculated using regression coefficients from Pirkle versus age for white and black children living in a 5-room house, with 3 members in the household, with 1 smoker, and after consuming 20 g of bell pepper. There are two observations from **Figure 2**: all other factors being equal, young children show cotinine levels higher than adolescents, and black children have higher cotinine levels than whites. The statistical significance of many of the regression coefficients was marginal, but a trend is suggested.

In a 1984 report, Greenberg et al. determined urinary and salivary cotinine levels in infants and reported urinary cotinine to be the most reliable indicator of exposure. Median levels of cotinine in urine were 351 ng and 4 ng of cotinine/mg creatinine for ETS exposed and not exposed, respectively. From these data, Van Vunakis et al. (1987) have estimated that on a creatinine basis, urinary cotinine levels in infants are approximately 60 times greater than those found in adult males. Based on urine volume, the concentration of cotinine in the urine of infants is 8 and 1.6 times as much as the ETS-exposed and highly exposed groups, respectively. Weaver et al. (1996) determined the urinary cotinine levels of 79 inner-city children and found a mean of 54.7 ± 45.6 ng/mL with a range of 1-244 ng/mL.

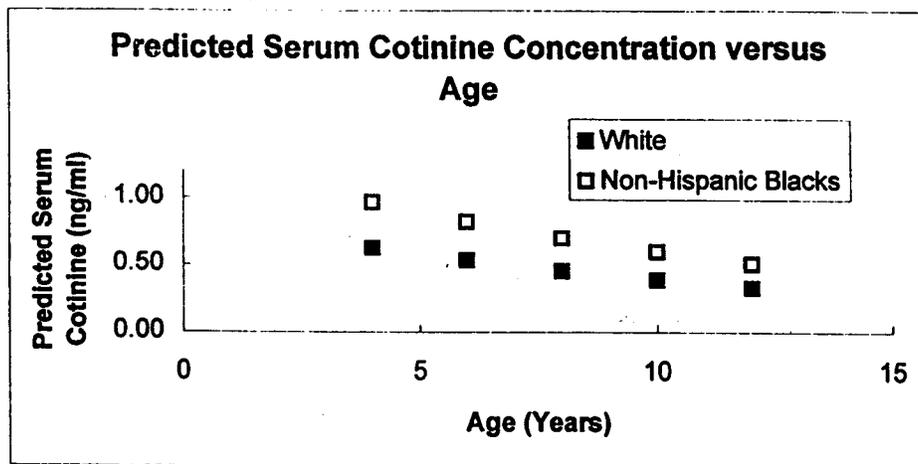


Figure 2. Use of Multivariate Analysis of NHANES III[®] Data to Illustrate the Effect of Age and Ethnicity (NHANES III = Third National Health and Nutrition Examination Survey)

Clearly, the age and ethnic background of young children must be taken into account when the cotinine levels of this group are used to infer levels of exposure to ETS. If this factor is not taken into consideration, cotinine levels found in exposure studies can be misinterpreted as excessive exposure.

Conclusions

Cotinine is not a perfect biomarker for nicotine exposure. Issues such as interpersonal variation in metabolism, non-tobacco sources of nicotine, and how well nicotine represents ETS exposure plague all biomarkers. For the present, cotinine appears to be the most reliable biomarker for estimating day-to-day exposure to tobacco smoke provided that it is applied only to mean (or median) values of large numbers of subjects.

There is much room to improve upon the use of nicotine metabolites as biomarkers for nicotine intake. Cotinine in serum and saliva appear to be equally advantageous over the use of urine samples; principally because of the limited number of analytically significant metabolites in these media. This is especially true if RIA methods are used, because of the potential for cross-reactivity of metabolites other than cotinine. Further, because cotinine represents 10-15% of the total nicotine metabolite in urine, variation in the larger concentrations of the other metabolites could have significant impact on the use of cotinine only in relating the result to nicotine intake.

A viable analytical method that has a detection limit much below that of RIA and gas chromatography is needed. The method must be rugged and capable of processing large numbers of samples economically. Such a method has been developed for cotinine in serum based on liquid chromatography combined with atmospheric pressure ionization tandem mass spectrometry (LC/API/MS/MS) (Wagenknecht et al., 1993). Work is underway to apply the method to saliva samples. Initial capital outlay is high, but the ruggedness and throughput capabilities of the method are excellent.

However, saliva and serum have their own limitation as a sample matrix. Only intensive or concentration measurements may be made. To obtain data on the equivalent of nicotine eliminated over a period of time, total nicotine metabolite excretion by collecting urine samples has a number of advantages. **Table 2** shows that approximately 90% of the total nicotine metabolite in urine could potentially be estimated by that approach. Therefore, a method using enzymatic hydrolysis of the glucuronide conjugates of nicotine and its metabolites in urine, with the subsequent determination of nicotine, cotinine and *trans*-3-hydroxycotinine is required. This method should have a detection limit to permit quantitative data to be obtained for subjects with the lowest level of nicotine exposure. It is likely that modification of existing procedures coupled with LC/API/MS/MS could meet these needs.

What Is Philip Morris Doing About Cotinine

Until an even more appropriate biomarker for nicotine intake is found, cotinine is currently the one of choice. The issue is not cotinine *per se*, but the limitations and misinterpretations associated with measurements and data. Several factors need to be considered.

Some of these are:

1. Which biological fluid is overall the most appropriate as the measurement medium?
2. What are the recommended procedures and protocols for the acquisition, transportation, preservation and storage of these samples?
3. Which nicotine metabolites should one measure to obtain improved and appropriate accuracy in the estimation of nicotine intake?
4. What analytical method is best suited to the task?
5. How should data be analyzed?
6. How can the proliferation of expedient but questionable analytical practices be contained?

A number of these issues are being addressed directly or indirectly by Philip Morris-supported programs.

1. *Which biological fluid is overall the most appropriate as the measurement medium?*

Each of the principal biological fluids used for biomarker measurements, serum, saliva and urine, has advantages and disadvantages as described above. The analytical methods that may be applicable also have a range of strong and weak points. In 1986, a group of experts met to discuss these issues in detail (Watts et al., 1990). This group concluded that “estimation based on urinary cotinine excretion would be less reliable than estimation based on plasma or salivary levels.” They also found that “good correlations were reported between saliva and blood for results from the same subject,” as found by others (Curvall et al., 1989). The number of nicotine metabolites in urine, the interpersonal variation, and the issues of using the CCR method that result from creatinine concentration variation, all counter the relative convenience of collecting urine samples.

Following the recommendations given above, Philip Morris is participating in a program to carefully investigate the use of saliva as the sample medium of choice. Although urine has considerable interest from a pharmacological perspective, as a sample medium used strictly to estimate nicotine exposure, either serum or saliva appears to have the advantage. Work is underway to establish appropriate sampling protocols and analytical methodologies for

saliva. An excellent method for cotinine determinations in serum was recently published (Bernert et al., 1997).

2. *What are the recommended procedures and protocols for the acquisition, transportation, preservation and storage of these samples?*

A number of recommendations for sample acquisition and handling for saliva have been reported. The subject is too detailed to be reviewed here. Philip Morris is participating in a study to evaluate a number of factors in the subject preparation, sample collection, sample preservation, sample transportation and storage for saliva. A method and procedures document is planned.

3. *Which nicotine metabolites should one measure to obtain improved and appropriate accuracy in the estimation of nicotine intake?*

For a variety of reasons, the recommendations reported above (Watts et al., 1990) have not been adopted by many researchers who conduct epidemiological investigations, particularly with respect to the use of urine as the sample medium. If nicotine metabolites are to be used in urine, improved analytical methods are required for acceptable results because of the number and variation in the nicotine metabolites. Philip Morris is conducting a statistical analysis of existing data to address this issue. The outcome of this analysis will be an

opportunity to define the analytical needs to minimize variations resulting from the complexities of a mixture of metabolites.

4. *What analytical method is best suited to the task?*

Philip Morris is participating in the development of an analytical method for cotinine and possibly *trans*-3-hydroxycotinine in saliva. For cotinine at least, the lower limit of quantification (LOQ) is expected to be approximately 0.05 ng/mL using conservative statistical policies. This may be compared with the current approximately 0.5-1.0 ng/mL using RIA methods. The method will have relatively high initial capital costs, but is capable of a throughput of over 100 samples per day. Under consideration is the participation in the development of an alternative method for urine that will involve enzymatic hydrolysis of the glucuronide conjugates of nicotine, cotinine and *trans*-3-hydroxycotinine with the subsequent determination of these three metabolites.

5. *How should data be analyzed?*

There are two issues with data analysis relative to cotinine that are present in the literature.

- a.) In exposure studies, cotinine levels below the limit of quantification (LOQ) of the analytical method used are empirically set equal to 50% of the LOQ. This has the negative impact that regardless of how much ETS exposure is reduced, a finite

cotinine level is guaranteed. This can result in a misuse of values below an LOQ. As ETS exposure is reduced, new analytical methods are required to demonstrate reduction in nicotine intake.

- b.) Smoker-nonsmoker discrimination is based on the use of a point value “cut-off” approach. This assumes that anyone below a set value of cotinine level is a nonsmoker, and anyone above that value is a smoker. This is not consistent with biological diversity. New methods of distributive data analysis that permit incorporation of a variety of factors to predict the probability of a single result are needed. Philip Morris is participating in the evaluation of such methods of data analysis.

6. *How can the proliferation of expedient but questionable analytical practices be contained?*

For a variety of reasons, often commercial, new quick and easy methods for the detection and alleged quantification of cotinine or other biomarker for nicotine appear in the literature, in patents or in the marketplace. These devices offer quick, simple and near-patient determinations that may be used for various purposes, such as establishment of smoking status for insurance applications. The complexities described above all but preclude successful use of such simple, nonselective “dip-stick” tests. One such device is known as NicCheck™ on the market and is FDA approved for physician use for detection of smokers.

Philip Morris is participating in an investigation of this device to ascertain its reliability, especially against potential false positive results.

Appendices

The following papers are appended to this section:

Schepers, G., Demetriou, D., Rustemeier, K., Voncken, P., and Diehl, B., Nicotine phase 2 metabolites in human urine -- structure of metabolically formed *trans*-3'-hydroxycotinine glucuronide, *Med. Sci Res.* 20: 863-865 (1992). [Tab A]

Schepers, G., Rustemeier, K., Walk, R.A., and Hackenberg, U., Metabolism of S-nicotine in noninduced and Aroclor-induced rats, *Eur. J. Drug Metab. and Pharmacokin.* 18: 187-197 (1993). [Tab B]

Schepers, G., and Walk, R.A., Cotinine determination by immunoassays may be influenced by other nicotine metabolites, *Arch. Toxicol.* 62: 395-397 (1988). [Tab C]

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Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 7 -

**Benzo[a]pyrene:
Environmental Distribution and
Human Exposure**

March 20, 1998

Benzo[a]pyrene: Environmental Distribution and Human Exposure

Incomplete combustion of organic matter represents the major source of polynuclear aromatic compounds (PAH) in the environment. PAHs are found at detectable concentrations in air, water, and soil samples of all types. Concentrations are typically small, in the order of $\mu\text{g}/\text{kg}$ or ng/m^3 . Since PAHs are highly lipophilic, they accumulate in organic fatty material and therefore have the potential to concentrate in the food chain.

Of the numerous PAHs, one compound that has perhaps received the most attention is benzo[a]pyrene (BaP). It is the focus of this discussion. The International Agency for Research on Cancer (IARC) has classified BaP as *probably carcinogenic to humans -- IARC Overall Evaluation 2A* (IARC, 1983, 1986a, 1986b). BaP has been identified in both mainstream and sidestream smoke from cigarettes, cigars, and pipes; marijuana smoke; and smoke-polluted environments (IARC, 1986b). Non-occupational inhalation exposure to BaP is primarily from tobacco smoke and urban air. However, Hattemer-Frey and Travis (1991) estimate that inhalation accounts for only 2% of the total daily intake of BaP. The focus of this paper therefore concerns the environmental distribution of BaP and human exposure to BaP.

Atmosphere: Emission Sources of BaP

Osborne and Crosby (1987) cite the principal sources of BaP in the atmosphere as (1) coal- and oil-fired power stations, (2) domestic heating, (3) miscellaneous industrial processes, (4) vehicle exhausts, and (5) cigarette smoke, forest fires and volcanic activity. The yearly global emission of BaP is estimated to be about 5,000 tons, with the greatest contribution coming from coal combustion. BaP emissions in the U.S. have been estimated to be 1,260 tons/year, accounting for approximately 25% of the worldwide total (Grimmer, 1979).

As can be seen in **Table 1**, the major emission sources in the U.S. are heating and refuse burning. The percentages in **Table 1** are derived from a table presented by Grimmer (1979), reproduced herein as **Table 2**.

Since Osborne and Crosby (1987) cited cigarettes as a principal source of BaP emissions, an estimate was calculated of the tons emitted in sidestream smoke/year. This estimate is based on cigarette consumption/year in the U.S. (Tobacco Manufacturers Association, 1997), and uses the value of 147 ng/cig BaP in sidestream smoke (SS) (based on values for the 1R4F reference cigarette cited in R.J. Reynolds, 1988). **Figure 1** shows that for the years 1983-1996, the estimated emission of BaP in sidestream smoke to the atmosphere is less than 0.099 tons/year, which calculates to be less than 0.007% of the total estimated emissions in the U.S. Thus, SS is certainly not a major contributor to BaP in the atmosphere, compared to other sources.

Table 1. Percentage by source of estimated BaP emission in the United States.

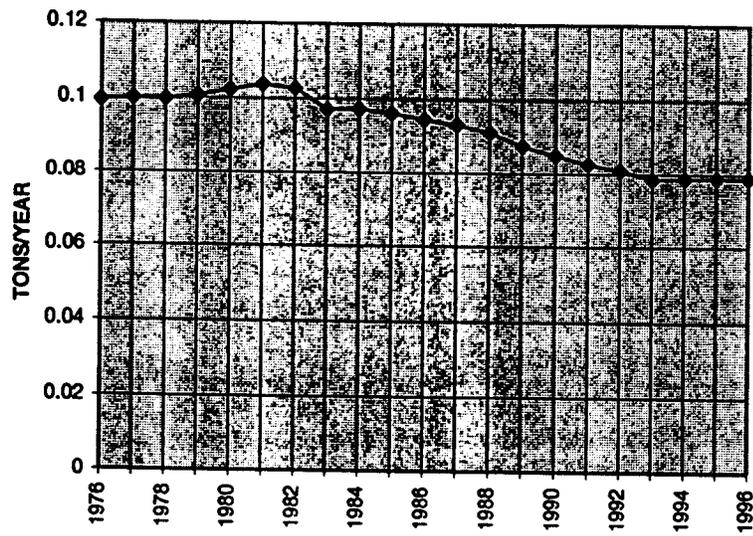
Emission source	Percentage
Automotive exhaust	1.7
Heating	38
Refuse burning	45
Industrial plants	16

Table 2. Estimated B(a)P emissions in the United States, after Grimmer (1979).

Source	Tons/year	Total
Vehicle exhaust		
Gas-powered cars	10	
Gas-powered trucks	12	
Diesel fuel-powered trucks and buses	0.4	
		22.4
Heating		
Coal		
Hand-stoked residential furnaces	420	
Intermediate units	10	
Coal-fired steam power plants	1	
Oil		
Low-pressure air atomizer and others	2	
Gas	2	
Wood	40	
		475
Refuse burning		
Commercial, residential, institutional and apartments	33	
Open burning		
Forest and agricultural	140	
Vehicle disposal	50	
Coal refuse fires	340	
		563
Industrial plants		
Petroleum cracking	6	
Asphalt air-blowing	<1	
Coke production	192	
		200
Total (all sources)		1260

Figure 1.

**ESTIMATED BaP EMITTED INTO ATMOSPHERE
FROM SIDESTREAM SMOKE**



Occurrence of BaP in Air

The concentration of BaP in ambient air is dependent on a number of factors:

- 1) Season -- generally highest in winter and lowest in summer;
- 2) Source of emission -- industrial and transportation;
- 3) Meteorological factors;
- 4) Urban vs. rural settings; and
- 5) Geographic location (Europe vs. U.S.).

Table 3 and **Table 4** (after Pucknat, 1981) illustrates some of these factors; they are cited in the literature as being used for various calculations. The U.S. average for urban sites for the 5-year period 1966-1970 is about 2.0 ng/m³ (Pucknat 1981, p. 85). The BaP concentration range in urban air of U.S. cities as determined by various authors in recent years (published during the period 1971-1977) is 0.13 to 3.2 ng/m³ (Pucknat, 1981, p. 169). As can be seen in **Table 5**, BaP levels in European countries have historically been much higher than those reported in the U.S.; there is also a wide variation from winter to summer.

Pucknat (1981) cites a paper within a paper which reports a "safe" lifetime BaP dose for human lungs as 4.3 mg. On the basis of this value, he then states that the concentration of atmospheric BaP should not exceed 120 ng/m³. A standard BaP concentration for industrial workers was determined to be 200 ng/m³. (OSHA Workplace Exposure Limit (PEL) for coal tar pitch

Table 3. Average BaP concentrations (ng/m³) in U.S. urban and rural areas (after Pucknat (1981), Table 5.14, p. 168).

	1966	1970	1976
Urban	3.2	2.1	0.5
Rural	0.4	0.2	0.1

Table 4. Summer-winter average of ambient BaP concentrations (ng/m³) in the air of selected cities (after Pucknat (1981), p. 169).

City	BaP (ng/m³)
Atlanta	4.5
Birmingham	15.7
Detroit	18.5
Los Angeles	2.9
Nashville	13.2
New Orleans	3.1
San Francisco	1.3

Table 5. Atmospheric benzo[a]pyrene concentrations (ng/m³) for various locations around the world in summer and winter (after Osborne and Crosby (1987), Table 17.1, page 302).

Location	Winter	Summer	Year
Sydney	8	0.8	1962-63
Liege, Belgium	110	15	1958-62
Ontario, Canada	15-20	1.2-18.5	1961-62
Prague	122	19	1964
Copenhagen	17	5	1956
Helsinki	5	22	1962-63
Paris	300-500		1958
Budapest	1000	32	1968
Teheran	6	0.6	1971
Belfast	51	9	1961-62
Milan	610	3	1958-60
Amsterdam	22	2	1968
	18	2	1969
	5		1970
	8		1971
Oslo	15	1	1956
Poland	130	30	1966-67
Madrid	120	0	1969-70
Stockholm	10	1	1960

volatiles of 0.2 mg/m³ averaged over an 8-hour workshift (Final Rule, January 1989); NIOSH recommended airborne exposure limit for coal tar pitch volatiles of 0.1 mg/m³ over a 10-hour workshift; ACGIH recommendation that worker exposures, by all routes, be controlled to levels as low as can be reasonably achieved; New Jersey Hazardous Substance Fact Sheet -- Benzo(a)Pyrene -- Micromedex, Inc., 1974-1998.)

Water: Sources and Occurrences

According to a National Academy of Sciences (NAS) report (*Petroleum in the Marine Environment*, NAS 1975), about 6 million tons of petroleum hydrocarbons enter the oceans annually; the major contributors are marine transportation and runoff (urban and river). Other sources of PAHs in the oceans are coastal refineries, industrial and domestic waste, natural seeps, and atmospheric fallout. BaP levels found in water are shown in **Table 6** (after Osborne and Crosby, Table 17.5, p. 307).

As one can see, the levels vary significantly depending upon the sampling location and the type of water, but in general they are rather low. This is not unexpected since PAH compounds in solution are readily adsorbed on to the surface of dust, soil or other insoluble particles. These particles will fall slowly to the bottom, and thus PAH compounds are removed from solution. The levels of BaP reported in sediments on the other hand can be rather high in the order of $\mu\text{g}/\text{kg}$ or even mg/kg of dry sample.

Table 6. BaP levels in water.

Sample	Country	BaP (ng/L)
Tap water	FRG	0.25 - 9
Tap water	USA	0.2 - 1.6
Groundwater	FRG	1 - 10
Groundwater	USA	0.2
Rainwater	FRG	4 - 80
Reservoirs	UK	0.7 - 3.8
Well water	UK	0.2 - 0.6
Well water	FRG	2 - 15
Lake Erie	USA	0.3
River Rhine at Mainz	FRG	50 - 110
River Rhine at Koblenz	FRG	10 - 60
River Thames	UK	170 - 280
River Thames	UK	4.2 - 430
River Trent	UK	5.3 - 504
River Severn	UK	1.5 - 48
Ohio River	USA	5.6
Delaware River	USA	41.1
Motorway run-off	UK	570
Domestic effluent	FRG	38
Human urine		1300
Sewage sludge	FRG	1.7 (mg/kg)

Soil: Sources and Occurrences

The majority of investigations of PAHs in soils have been carried out by Soviet investigators between 1967-1977; these papers only reported the BaP content (Osborne and Crosby, 1987). The concentrations of BaP measured in the U.S.S.R. ranged from 0.0008 mg/kg to 200 mg/kg, with the maximum value found in the vicinity of an oil refinery. Similarly high concentrations (650 mg/kg) were measured in the area of a carbon black factory. In samples of sandy and forest soil collected in West Germany, considerably lower concentrations of BaP, ranging from 0.001 to 0.0004 mg/kg, were found. The contamination of soil can be attributed almost exclusively to emissions from combustion processes. In the majority of surface soil samples taken in Iceland, where hardly any fossil fuels are burnt, the most commonly found PAHs were not detected (detection limit for BaP, e.g., 0.02 $\mu\text{g}/\text{kg}$ soil). Soil samples taken at the Reykjavik, Iceland airport, however, were extremely contaminated, with BaP concentrations reaching 0.785 mg/kg.

“Human Exposure to Benzo(a)pyrene”

Hattermer-Frey and Travis (1991) used a multimedia transport model to evaluate environmental partitioning of BaP. Measured and predicted environmental concentrations were used to estimate the accumulation of BaP in the food chain and the subsequent extent of human exposure from inhalation and ingestion. Their results showed that the food chain is the dominant pathway of human exposure, accounting for about 97% of the total daily intake of BaP. See **Table 7**.

Table 7. Pathways of human exposure to B(a)P (after Hattemer-Frey and Travis, 1991).

Source	Daily intake ($\mu\text{g}/\text{day}$)	% of total daily intake
Food (total)	2.1	97
Inhalation	0.05	2
Water	0.01	1
TOTAL	2.16	100

This value of approximately 2.2 $\mu\text{g}/\text{day}$ average daily intake of BaP is in agreement with other values reported in the literature (e.g., Suess, 1976). Hattemer-Frey and Travis (1991) then went on to discuss human exposure to BaP from smoking and indoor air pollution, referencing a paper by Butler and Crossley (1979) that reportedly estimated that one cigarette delivers approximately 39 ng of BaP. Further, Hattemer-Frey and Travis used in their calculations an estimate that the average smoker smokes 20 cigarettes per day. Based on these calculations, they suggested that the smoker receives an additional 780 ng/day (0.78 $\mu\text{g}/\text{day}$) BaP from smoking. Additionally, they again referenced Butler and Crossley (1979), who reported that concentrations of BaP measured indoors (2.2 ng/m^3) were comparable to outdoor air concentrations (2.5 ng/m^3); thus, indoor activities would not substantially increase the BaP intake, since inhalation is not a major pathway of human exposure to BaP.

Mainstream smoke concentrations of 9.2 ng BaP/cigarette have been reported for the Kentucky Reference Cigarette 1R4F (R.J. Reynolds, 1988). The value for a filtered cigarette could be rounded to 10 ng BaP/cigarette. Thus, an average (1 pack/day) smoker of filtered cigarettes would be exposed to an additional 0.2 $\mu\text{g}/\text{day}$ of BaP, a level which is approximately 4 times the estimated daily intake of BaP by inhalation. [See Table 8] However, one should keep in mind that more than 90% of the daily intake of BaP is derived from other sources, primarily food.

This can be examined from the perspective of the daily lung burden as described by Chen and Thilly (1996). Instead of using the value of 10 m^3/day for breathing, the more widely used value of 20 m^3/day will be chosen. The value of 2.5 ng BaP/ m^3 as the “urban air concentration” will

Table 8. Estimated exposure of a smoker to BaP (ng/day).

	Cigarettes/day		
	10	20	40
1R4F filtered cigarette	100	200	400

be used, which, as shown in **Table 9**, is probably an over-estimation of the urban air BaP concentration in the 1990s, if the downward trend has continued.

Using the formula (breathing rate/day X urban air BaP level) = ng BaP by inhalation/day, for a breathing rate of 20 m³/day X 2.5 ng BaP/m³ = 50 ng BaP/day or 0.05 µg/day by inhalation/day. The level of 0.05 ug/day by inhalation corresponds to what Hattemer-Frey and Travis reported [See **Table 7**].

Examining BaP Exposure from ETS or Room-Aged Sidestream Smoke (RASS)

Attributing levels of PAH (BaP) in the air of restaurants, public rooms, etc., to ETS is difficult since other sources may be present, and other factors, such as ventilation rates, number of smokers, etc., may confound the issue. However, Grimmer (1983), under controlled conditions, reported 22 ng/m³ BaP where cigarettes were being smoked and less than 3 ng/m³ where no cigarettes were being smoked. He calculated that ETS contributed about 7 times the background BaP level. Grimmer states that “the measured concentrations of 22 ng BaP per cubic meter has to be considered as a maximum BaP concentration attainable by smoking. In practice nobody would tolerate this concentration” of smoke due to eye irritation, etc.

A 12-month inhalation study in rats using room-aged sidestream smoke (RASS) (INBIFO, data enclosed) reports the following concentrations of BaP in RASS from 1R4F cigarettes: 0.13 µg/m³ (upper limit) for the whole-body 12-month exposure group, and 0.121 µg/m³ (upper

Table 9. Average BaP concentrations (ng/m³) in U.S. urban and rural areas during 1966-1977 (after Pucknat 1981, Table 5.14, p. 168).

Location	Year		
	1966	1970	1976
Urban	3.2	2.1	0.5
Rural	0.4	0.2	0.1

limit) for the head-only 12-month exposure group (Hausmann et al., 1998). Again, if we consider urban ambient air to contain an average of 2.5 ng/m^3 BaP, one can see that the rats in this study were exposed to approximately 50 times the level of BaP found in ambient air. The RASS concentrations in this study were approximately 100-fold higher than the maximum of the average concentrations of respiratory suspended particles (RSP) reportedly attributable to ETS (Guerin et al., 1992; U.S. EPA, 1992; Jenkins et al., 1996). Thus, if one assumes that BaP, which is in the particulate phase, tracks with RSP, then $0.13 \text{ } \mu\text{g/m}^3$ would correspond to $0.0013 \text{ } \mu\text{g/m}^3$. From the perspective of human exposure, this level of exposure would be equivalent to $0.0013 \text{ } \mu\text{g/m}^3 \times 20 \text{ m}^3/\text{day} = 0.026 \text{ } \mu\text{g/}$ or 26 ng/day , which is about half the daily level by inhalation estimated by Hattemer-Frey and Travis.

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Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 8 -

**Section on Carcinogenesis from
Philip Morris U.S.A. Comments on
Health Effects of Exposure to Environmental Tobacco Smoke
Final Draft for Scientific, Public and SRP Review
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
May 5, 1997**

March 20, 1998

COMMENTS ON:

Health Effects of Exposure to Environmental Tobacco Smoke

Final Draft for Scientific, Public and SRP Review

**Office of Environmental Health Hazard Assessment
California Environmental Protection Agency**

PHILIP MORRIS U.S.A.

May 5, 1997

COMMENTS ON CHAPTER 7 – CARCINOGENIC EFFECTS

Summary Overview

Based on Chapter 7, “Carcinogenic Effects,” OEHHA contends that (1) the data support a causal relationship between ETS exposure and lung cancer, (2) the data support a causal relationship between ETS exposure and cancer of the nasal sinus and (3) the data are “suggestive” of a causal relationship between ETS exposure and cervical cancer. Philip Morris herein provides comments on those three conclusions. Given the limited time made available for public comments, Philip Morris has not been able to address in detail the other cancer endpoints discussed by OEHHA; this does not mean that the Company endorses OEHHA’s conclusions or the reasoning described therein, or that Philip Morris believes that OEHHA’s review is complete, impartial, or appropriate. For instance, Philip Morris notes that OEHHA has apparently not considered a number of potentially relevant references that could contribute to its discussion of non-lung cancers; thus, the review in Chapter 7 is incomplete on this basis alone. A list of those references is provided as **Attachment A** to this comment.

Section 7.2 – ETS and Lung Cancer

Appendix A of the Final Draft purports to be a “Summary of Public Comments and Responses” received on previously released draft chapters of the document. Pages A-2 and A-3 are described as a “List of Those Commenting”; however, this list is incomplete. In particular, the Comment of Philip Morris U.S.A. on the January 1996 External Review Draft, *Carcinogenic Effects of Exposure to Environmental Tobacco Smoke -- Excerpt: ETS and Lung Cancer*, is not included on the list. In so omitting the Comment of Philip Morris, OEHHA has failed to respond to the many issues raised therein, as it is required to do. Moreover, the essence of the discussion on lung cancer in the Final Draft is unchanged from the January 1996 External Review Draft. Therefore, the Comment already submitted by Philip Morris on the lung cancer claims made by OEHHA is still relevant. That Comment is resubmitted as **Attachment B** to this submission.

In the Comment on lung cancer already submitted by Philip Morris, a number of articles overlooked by OEHHA in its previous draft were referenced; however, OEHHA has failed to incorporate most of those references into the Final Draft document. **Attachment C** to this Section of this Comment lists those articles. It is incumbent upon OEHHA to address the important issues raised by these articles.

In the year since Philip Morris submitted the Comment that OEHHA ignored, additional relevant literature has accumulated in the area of ETS exposure and lung cancer. **Attachment D** to this Comment lists new lung cancer literature, which raises a number of important

issues relevant to OEHHA's claims. OEHHA should review this literature and incorporate it into the ETS assessment.

Section 7.3.1 -- Nasal Sinus Cancer

OEHHA Claim: Nasal sinus cancer is listed in Table ES.1 (p. ES-2) under “effects *causally* associated with ETS exposure.” [emphasis added]

Response:

- Nowhere does OEHHA indicate how it reached a conclusion of “causality.” In fact, OEHHA’s statements in the text of the document do not even mention a causal relationship.
- The reader of the OEHHA document is led to assume that the conclusion of “causality” is scientifically justified -- because it appears in the Executive Summary of the document -- when, in actuality, OEHHA provides *no* rationale at all for such a conclusion, and when the Executive Summary states a conclusion different from the conclusions stated in the text of the document.
- In the text, OEHHA states: “Of the studies examining the effect of ETS exposure on nasal sinus cancers, all three show consistent associations, presenting strong evidence that ETS exposure increases the risk of nasal sinus cancers in nonsmoking adults” (p. ES-7) and “Existing studies consistently show a significant positive association between exposure to ETS and nasal sinus cancer in nonsmokers. The results have been observed in studies conducted in eastern and western countries, in males and females, in cohort and case-control study designs, and with some adjustment for possible confounders.” (p. 7-28)

- OEHHA should formally indicate how it reached the determination of causality that warranted the inclusion of nasal sinus cancer in Table ES.1.

OEHHA Claim: “Active smoking is firmly established as a causal factor for cancers of the . . . nasal sinus cavity.” (p. 7-26)

Response:

- No references are provided for this contention. However, the 1982 and 1989 Surgeon General’s reports and the 1986 IARC Monograph (No. 38), cited elsewhere in the chapter by OEHHA, do not even mention nasal sinus cancer in their discussions of cancers purportedly associated with active smoking.

OEHHA Claim: “There are some data on the role of ETS for other cancer sites, including cancers of nasal sinus cavity . . .” (p. 7-26)

- The 1982 and 1989 Surgeon General’s Reports, cited by OEHHA, do not even mention nasal sinus cancer in their discussions of ETS.

OEHHA Claim: “Existing studies consistently show a significant positive association between exposure to ETS and nasal sinus cancer in nonsmokers.” (p. 7-28)

Response:

- **Lack of Statistical Significance:** Only one of the risk estimates cited by OEHHA in its discussion of the studies has a confidence interval that excludes 1.0. The single risk estimate that is “statistically significant” has an extremely wide confidence interval, based as it is on only nine cases.
- In Table 7.8 (p. 7-65), OEHHA lists the reported risk estimates from the three nasal sinus cancer studies. The table clearly shows that **only one of the reported risk estimates has a confidence interval that excludes 1.0.**
- None of the three spousal smoking risk estimates cited by OEHHA from the Hirayama study is statistically significant -- all the confidence intervals **include** 1.0. Thus, those risk estimates are consistent with neither an increase or a decrease in risk.
- Fukuda and Shibata do not even discuss the statistical significance of the individual risk estimates in their paper, but OEHHA has calculated confidence intervals. The great width (1.7 to 19.4) of the CI on the risk estimate for more than one smoker in the home illustrates the uncertainty associated with that risk estimate. When coupled with the fact that it is based on only nine cases, this makes any interpretation based on that number highly suspect.
- The authors of the Zheng, et al., study provide only limited information on the spousal smoking analysis. Moreover, the confidence intervals for both risk estimates presented

overlap 1.0 and are quite wide (as the authors themselves acknowledge), particularly the one for maxillary sinus cancers.

- **Sample Size:** OEHHA's conclusion of causality is based on three studies reporting data based on only 91 cases in total. Neither the number of studies nor the number of cases is sufficient support for such a conclusion.
- OEHHA even notes that the Hirayama (1983) study results are based on only 28 cases, but fails to point out that such a small sample size compromises the interpretation of the reported results. The low number of subjects results in very unstable risk estimates.
- Fukuda and Shibata (1990) acknowledge that their data set was of "relatively small size." However, OEHHA's discussion of this study obscures the fact that there are only 35 nonsmoking cases. OEHHA focuses on the risk estimates by exposure category and the purportedly statistically significant "trend," while failing to acknowledge the fragility of those risk estimates, based as they are on 15 and nine cases, respectively.
- While the Zheng, et al., study is fairly large (particularly given the rarity of this tumor), the risk estimates reported for spousal smoking are based on only 28 cases (those who were nonsmokers). Again, sample size is a distinct limitation of this study, a point which OEHHA fails to clearly state in their summary of the paper.

OEHHA Claim: “The results have been observed in studies . . . with some adjustment for potential confounders.” (p. 7-28)

Response:

- OEHHA states that the risk estimates from the Hirayama study included an adjustment for *husband's* age. Hirayama has adopted this approach consistently in his analyses of data from this study, when, as it has been noted elsewhere, “[i]t is absolutely routine in epidemiology to standardize for the age of the *subject*.” [emphasis added] (Lee, 1992) Hirayama’s odd approach would seem to raise questions about the overall validity of his study.
- Cancer of the paranasal sinuses is more common in Japan than in the United States. (DeVita, et al., 1993; for references to materials not cited by OEHHA, see **Attachment A**) This would imply that caution is warranted in applying the reported results of the Hirayama study (as well as the Fukuda and Shibata study) to the United States population. There may well be ethnic-specific and lifestyle risk factors that have not been considered, as have been reported for nasopharyngeal cancer. (Schottenfeld and Fraumeni, 1996)
- While Hirayama writes in the paper cited by OEHHA that “[n]o other risk factors studied significantly altered the risk of nasal sinus cancer in women,” he fails to provide the reader with information as to what those risk factors might be. (Hirayama, 1983) Possible risk factors for this cancer have been called “multifold,” and reportedly include exposure to

nickel; occupation in the furniture, textile, and shoe industries; occupational exposures to chromium, mustard gas, isopropyl alcohol, and radium; and possibly chronic sinusitis. (DeVita, et al., 1993)

OEHHA Claim: “The results have been observed in studies conducted in eastern and western countries, in males and females . . .” (p. 7-28)

Response:

- OEHHA’s statement misrepresents the available data, reported for only one sex in each of two countries.
- Two studies present data for nonsmoking *Japanese women* (Hirayama; Fukuda and Shibata) and one for nonsmoking *white American men* (Zheng, et al.). The Japanese studies do not have data on nonsmoking men, and the U.S. study does not have data on women.

Section 7.4.1. -- Cervical Cancer

OEHHA Claim: Cervical cancer is listed under “effects with suggestive evidence of a causal association with ETS exposure” in Table ES.1. (p. ES-2)

Response:

- Nowhere does OEHHA indicate how it reached a conclusion of “suggestive” evidence for “causality.” In fact, OEHHA’s statements in the text of the document do not even mention a causal relationship.
- The Table ES.1 conclusion is not justified or supported by the analysis presented in Chapter 7, which only states: “There is supportive evidence from epidemiological and biochemical studies implicating a role for ETS exposure in the etiology of cervical cancer in nonsmokers.” (p. 7-32) OEHHA presents no criteria for extrapolating from this position to a conclusion that there is “suggestive” evidence for “causality,” nor does it define what exactly constitutes “supportive” evidence or “suggestive” evidence.
- The reader of the OEHHA document is led to assume that the conclusion of “suggestive” evidence for “causality” is scientifically justified -- because it appears in the Executive Summary of the document -- when, in actuality, OEHHA provides *no* rationale at all for such a conclusion, and when the Executive Summary states a conclusion different from the conclusions stated in the text of Chapter 7.

- OEHHA should formally indicate how it reached the determination of “suggestive” evidence for causality that warranted the inclusion of cervical cancer in Table ES.1.
- OEHHA states in Chapter 7: “[H]uman papillomavirus [sic] . . . has been accepted as the sexually transmitted etiological factor in cervical cancer.” (p. 7-28) OEHHA thereby seemingly acknowledges the scientific consensus on the importance of the sexually-transmitted agent, human papillomavirus (HPV), in the etiology of cervical cancer. (See **Attachment A**: Winklestein, 1990; Bosch, et al., 1995; Munoz, et al., 1996; Bosch, et al., 1996) In fact, a consensus panel of the National Institutes of Health has concluded that cervical cancer is “causally related” to infection with HPV. (McNeil, 1996)
- Given the general acknowledgment of HPV as a cause of cervical cancer, it is curious that OEHHA would even evaluate ETS exposure in terms of “causality,” as indicated in Table ES.1.
- Moreover, a noted cancer textbook refers to active smoking as a “cofactor” for cervical cancer (DeVita, et al., 1993); given this position, it clearly seems very premature to conclude that there is suggestive evidence that ETS is *causally* associated with this disease, as OEHHA claims.

OEHHA Claim: “The relationship between ETS exposure and cervical cancer was investigated in one cohort and three case-control studies.” (p. 7-29)

Response:

- OEHHA’s review of the literature on ETS exposure and cervical cancer is incomplete. When additional studies are taken into account, however, no clear picture of “risk” emerges. What seems most likely, based upon the data from these and other studies, is that ETS exposure is a marker for a number of interrelated factors, and that the reportedly elevated risk estimates represent the residual effect of insufficient control for these variables.
- Munoz, et al. (1996), report cervical cancer risk estimates for husband’s smoking status that are not statistically significant when adjusted for a number of potential confounding factors. OEHHA did not reference this article.
- Bosch, et al. (1996), report statistically significant cervical cancer risk estimates for husband’s smoking, but conclude that the “two most likely reasons” for their reported results are misclassification of human papillomavirus exposure and insufficient adjustment. OEHHA did not reference this article.

OEHHA Claim: “Positive associations were observed in two of three case-control studies and a statistically nonsignificant positive association was observed in the only cohort study conducted.” (p. ES-7)

Response:

- **Misleading Presentation of Data:** Table 7.9 (p. 7-66) is incomplete, reporting data from only two of the available studies (Slattery, et al.; Coker, et al.). Nevertheless, Table 7.9 (p.7-66) clearly shows that *only one cited risk estimate from the two studies is statistically significant*. Chance has thus not been excluded as an explanation for the overwhelming majority of the reportedly increased risks cited by OEHHA in that table.
- The series of risk estimates OEHHA chose to include in Table 7.9 (p. 7-66) includes the largest risk estimate reported by Slattery, et al., for nonsmokers. The only statistically significant risk estimates for ETS exposure reported in Table 6 of the Slattery, et al., paper are for the highest exposure group for “all” reported ETS exposure or for home ETS exposure. This pattern would be consistent with confounding factors being clustered in that exposure group.
- **Sample Size:** It is also clear from Table 7.9 (p. 7-66) that the risk estimates for different ETS “exposure levels” are highly suspect, based as they are on very few cases.
- Moreover, the Slattery, et al., study, which seems to be key to OEHHA’s contentions, fails to even provide the numbers of cases and controls for the different levels. This incomplete reporting makes interpretation of the study’s reported results quite difficult.

OEHHA Claim: “There is supportive evidence from epidemiological . . . studies implicating a role for ETS exposure in the etiology of cervical cancer in nonsmokers.” (p. 7-32)

Response:

- Many questions remain about the ETS-cervical cancer epidemiologic studies cited by OEHHA, making any summary interpretation of their reported conclusions difficult. Many of the reported risk estimates are not statistically significant. The studies have generally (in some cases, completely) failed to adequately consider potential confounding factors. Given these limitations, OEHHA’s conclusion is unwarranted.

OEHHA Claim: “The current chapter extends or modifies the discussion of issues related to ETS exposure and cervical cancer (*e.g.*, on confounders in epidemiological studies . . .)” (p. iii)

Response:

- Compared to the cervical cancer section in the May 1994 External Review Draft, the current Chapter 7 does include some “modified” discussion of confounders, in that they are now briefly mentioned in the context of the data collected in the epidemiologic studies cited by OEHHA.

- However, OEHHA’s treatment of confounders can certainly be further “extended.” This is perhaps the most important issue in evaluating the cervical cancer studies, and OEHHA pays completely insufficient attention to it.
- ***Inadequate Treatment of Confounders in Individual Studies:*** The discussion in Chapter 7, plus statements from other reviews, clearly indicates shortcomings in the available literature’s treatment of confounding factors.
- It has been stated about the Hirayama study:

[T]here are a number of doubts about the results from this large prospective study, the most important of which appear to be incompleteness of follow-up and doubts about the validity of the statistical methods used. ***Failure to gather data on confounding variables relevant to specific cancers is also a problem.*** [emphasis added] (Lee, 1992)

OEHHA even acknowledges that Hirayama’s ETS data “were not adjusted for potential confounders including subjects’ or husband’s sexual activity” (p. 7-29). Given what is now known about the relationship between HPV and cervical cancer, this failure on Hirayama’s part means that this study can be treated as providing essentially no information relevant to the question of ETS exposure and cervical cancer risk.

- With respect to the Sandler, et al., study, Lee (1992) notes:

Apart from . . . the problems of multiple testing, it should be realized that the Sandler I study collected *no data* on sexual habits, which are strongly correlated both with risk of cervix cancer and with smoking habits. [emphasis added]

OEHHA acknowledges this as well: “[I]nformation typically obtained in studies of a specific cancer site (e.g., sexual activity in studies of cervical cancer) *was not collected.*” [emphasis added] (p. 7-29) As with the Hirayama study, the Sandler study can be given little weight in analyses of cervical cancer claims.

- Studies report that both personal smoking behavior and ETS exposure are correlated with a number of other lifestyle factors (related to sexual activity and alcohol and drug use) that appear to be markers for an increased risk of HPV infection. Disentangling the possible effects of these potential confounders is difficult, and the epidemiologic studies to date have not adequately addressed them.
- In a letter to the editor on the Slattery, et al., study, Zang, et al., note:

In this report, there clearly is undermatching of control patients with regard to important risk factors including sexual activity, religious background, and education. . . . Since the previously mentioned risk factors are correlated highly with one another as well as with active and passive smoking, the risk estimates relating smoking and cervical cancer may be subject to substantial bias and confounding. . . . The effect of adjustment on odds ratios is far greater than expected in case-control studies of this sort, as, for example, the decrease from 14.84 to 2.96 for passive smoke exposure. *In fact, the adjusted odds*

ratios are probably no more than the leftover effect of variables controlled imperfectly by logistic regression. [emphasis added]

- Zang, et al., also note:

The apparent association between cervical cancer and environmental smoke exposure may, in fact, be the result of increased exposure to papillomaviruses or herpes simplex virus 2 through contact with male sexual partners who smoke, since one might expect smokers to be generally more sexually active than nonsmokers, and case patients are more sexually active than control subjects.

- The author of a 1992 book on ETS chronic disease claims stated about the Slattery, et al., study:

[T]he major problem with interpreting this finding as cause and effect is the extreme difficulty of adequately adjusting for sexual habits. . . . Since the number of sexual partners is only an inaccurately measured surrogate of the true sexually related cause of cervix cancer . . . the adjustment will be incomplete and leave a residual confounding effect. ***It is entirely plausible that the whole of the adjusted relative risk could be explained by this.*** [emphasis added] (Lee, 1992)

- That potentially misleading results can come from studies with inadequate attention to confounding factors (e.g., Hirayama, as acknowledged by OEHHA; Slattery, as pointed out by other reviewers) seems clear when the Coker, et al. (1992), study is compared to those other studies. In that study, the authors included age, race, education, number of Pap smears, number of sexual partners, and history of genital warts as confounders in their analysis of ETS exposure and cervical cancer risk. As for their reported results, they note, “the

confidence intervals associated with each of these adjusted odds ratios were *wide and clearly not statistically significant.*” [emphasis added]

- ***Omission of Relevant Data:*** In the discussion of cervical cancer, OEHHA completely overlooked two articles presenting relevant data on the potential for confounding in the epidemiologic studies. Given OEHHA’s predilection for data from California elsewhere in the report, this omission seems particularly odd.
- Holly, et al. (1992), report data which “confirm that cigarette smoking is strongly associated with numerous life-style and behavioral factors, many of which have been linked directly or indirectly with cervical cancer.” These data were collected from women residing in the San Francisco Bay area.
- In another study from the same research group, Cress, et al. (1994), investigated whether women exposed to ETS differed from non-exposed women on a number of characteristics. This study was based on self-report of ETS exposure, just as are the epidemiologic studies of cervical cancer risk. Several statistically significant differences between “ETS-exposed” and non-exposed women were reported; they included marijuana use, beer consumption, and being divorced or separated. The authors concluded:

[W]omen nonsmokers exposed to passive smoke were different from those not exposed. Studies that examine the association between passive smoking and disease need to measure dietary, lifestyle,

sexual, and reproductive factors to adequately allow for these differences.

Attachment A to Comments on Chapter 7 – Carcinogenic Effects

Studies on Cancers Other than Lung Not Considered by OEHHA

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Attachment B to Comments on Chapter 7 – Carcinogenic Effects

Philip Morris Comment on 1996 External Review Draft

PHILIP MORRIS U.S.A.

COMMENTS ON:

**CARCINOGENIC EFFECTS OF EXPOSURE TO
ENVIRONMENTAL TOBACCO SMOKE**

EXCERPT: ETS AND LUNG CANCER

**California Environmental Protection Agency
Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment**

**External Review Draft
January 1996**

April 1, 1996

SECTION I: OVERVIEW AND INTRODUCTION

In the January 1996 external review draft, "Carcinogenic Effects of Exposure to Environmental Tobacco Smoke -- Excerpt: ETS and Lung Cancer" (hereinafter, the Excerpt), Cal/EPA contends that recently published epidemiologic studies support the conclusions reached in the U.S. EPA's 1993 Risk Assessment on environmental tobacco smoke (ETS). This contention is incorrect and is not supported by the data.

In this written comment, it will be well documented that the present draft of the Excerpt is a superficial treatment of the claimed association between ETS exposure and lung cancer risk. (Section II) Cal/EPA selectively cites and interprets studies and data in an attempt to support a conclusion that the recent epidemiologic data agree with the positions espoused in the U.S. EPA Risk Assessment on ETS.

Further, this comment will highlight and summarize a number of the unresolved criticisms of the U.S. EPA Risk Assessment on ETS, showing that the assumption that that document was a conclusive, thorough review of the literature is unfounded. (Section III) In particular, data on ETS chemistry, ETS exposure data, the limitations of questionnaire data, animal inhalation studies and genotoxicity data were not adequately addressed.

Finally, since Cal/EPA relies almost exclusively on the epidemiologic literature in reaching its conclusions, this comment will address a number of epidemiologic issues that were treated in insufficient detail in the Excerpt. (Section IV)

Given the shortcomings of the Excerpt, this comment concludes that Cal/EPA's stated position is not adequately supported. Specifically, when all of the relevant data are considered, those data do not support the claim that ETS exposure is associated with an increased risk of lung cancer in nonsmokers.

Based on the information contained herein and upon publicly available information, Cal/EPA should defer from taking any further action on this assessment of risks.

- To avoid "bad science," the Cal/EPA report should await the recommendations of the OEHHA Risk Assessment Advisory Committee. Cal/EPA Secretary James Strock has been quoted as saying that "scientifically sound risk assessments are the foundation upon which wise environmental decisions are made," which implies an obligation to assure that this report is sound science.

(i) The California Legislature has also recognized the significant impact of risk assessments. In 1993, the

Legislature enacted S.B. 1082 (Cal. Health & Safety Code sec. 57004), which required the director of Cal/EPA's Office of Environmental Health Hazard Assessment (OEHHA) to convene an advisory committee "to conduct a comprehensive review of the policies, methods, and guidelines to be followed by the state for the identification and assessment of chemical toxicity." The Advisory Committee was convened in 1994. It has held seven meetings; the next meeting will be held on April 10 and 11, 1996. At the same time that the work of the Advisory Committee is nearing completion, Cal/EPA is attempting to produce a formal risk assessment concerning ETS, complete with conclusions that may be calculated to command attention in the press but may be easily misinterpreted as to their true significance.

Given the universally recognized need for thorough and reasonable risk assessment guidelines,¹ it is difficult to see how Cal/EPA would now allow the release of any risk assessment without a determination that the assessment followed the recommendations of the Advisory

1. For example, note the current ongoing work of President Clinton's Commission on Risk Assessment and Risk Management (scheduled to issue a report on April 24, 1996) and the ongoing work of the U.S. Environmental Protection Agency to issue Revised Carcinogen Risk Assessment Guidelines.

Committee. Publishing an incomplete and unreviewed ETS assessment now has the potential of eclipsing the State's stated commitment to uniform, sound risk assessment practices.

- As developed more fully herein, Cal/EPA cannot take any action based on reliance upon U.S. EPA's Risk Assessment. Cal/EPA should not rely upon U.S. EPA's Risk Assessment on ETS, and in so doing, avoid the necessary and required original work by Cal/EPA. Under the California Health and Safety Code, 539660(c), Cal/EPA must evaluate the "quality of data" underlying any health evaluation. Cal/EPA cannot and should not pursue any report which relies upon EPA's Risk Assessment.
- Cal/EPA's ETS risk assessment (and specifically the lung cancer sub chapter) should not be pursued because Cal/EPA lacks the requisite authority to pursue such work. Cal/EPA's risk assessment on ETS was initiated prior to the enactment of AB 13, which imposed a state-wide ban on smoking in most places in California. See Labor Code sec. 6404.5(b). The restrictions of AB 13 encompass most nonresidential areas in the state, and even where smoking is restricted to designated areas, AB 13 requires ventilation equipment meeting specified standards to ensure that the air is not recirculated but exhausted outside. Even prior to AB 13, the Proposition 65

Scientific Advisory Panel listed ETS as a carcinogen. As a result, in those areas where limited smoking is allowed, Proposition 65 requires that warnings be posted to alert persons of their potential exposure to ETS. Given the existence of AB 13 and Proposition 65, and given the authority of the California Occupational Safety and Health Administration to generally regulate worker health and safety it is difficult to see any basis of authority for Cal/EPA to pursue a risk assessment concerning ETS.

- Cal/EPA should decline to take any further action on the ETS risk assessment given the extensive regulatory work already underway at the U.S. Occupational Safety and Health Administration (OSHA). Not only does Cal/EPA lack authority to pursue an ETS risk assessment, but if there were a current compelling need to review ETS issues, that need is being met by U.S. OSHA. Federal OSHA has completed the public comment phase of the lengthiest, most extensive regulatory hearing and review of potential ETS effects ever conducted. The agency held six months of public hearings, amassed more than 110,000 comments from interested parties and scientific experts, and collected extensive studies and data regarding the claimed health effects of exposure to ETS. Unlike the brief Cal/EPA workshops, the federal OSHA hearings were conducted as part of federal OSHA's regulatory responsibility for determining

- Reissue Chapter 7 of the Cal/EPA assessment in its entirety, as the new table of contents indicates some changes from the chapter on "other cancers" issued in May 1994.
- Substantially broaden the scope of its literature review to include all relevant materials. In particular, Cal/EPA should consider the public record compiled in response to U.S. OSHA's rulemaking on indoor air quality, which contains a vast amount of literature as well as perhaps the most recent and comprehensive analyses of ETS issues, many of which are cited herein.
- Given the unresolved criticisms of the U.S. EPA Risk Assessment on ETS, not rely on that document and its analyses, but obtain the relevant literature and re-analyze it. That is, Cal/EPA must "start from the beginning." In this regard, the agency should obtain and analyze the underlying data -- particularly the data from the Fontham, et al., study.
- Abandon the selective approach taken in the present draft of the Excerpt, and clearly define the criteria used in evaluating data and delineate the rationale for its approaches, in order to reduce the appearance of subjectivity.

- Revise and reissue in draft all other chapters in this assessment of ETS risks, to incorporate the suggestions inherent in the foregoing as to all chapters.
- Review the Congressional Research Service Report. Most of Cal/EPA's overall risk assessment does not have the benefit of the November 1995 Congressional Research Service (CRS) report on ETS which raised serious questions about the methodology of the U.S. EPA's ETS risk assessment. The CRS study was not addressed in Cal/EPA's most recently released draft excerpt.

SECTION II: CAL/EPA'S REVIEW IS SUBJECTIVE, SELECTIVE AND UNSCIENTIFIC

Cal/EPA's Excerpt clearly appears to be designed to support the conclusion that ETS exposure is associated with an increased lung cancer risk. The following discussion will illustrate a number of specific examples of subjectivity and selectivity in the Excerpt.

A. Cal/EPA Frequently Engages in Selective Reporting

Cal/EPA selectively references studies and reports data from studies in a manner that strengthens its positions. One example of this selectivity is found in the citation of the "first" studies to be published on spousal smoking and lung cancer. Cal/EPA cites only the Hirayama¹ and Trichopoulos, et al.,² studies, published in 1981. (p. 8) This statement is incomplete. Also in 1981, Garfinkel published data from a large cohort study conducted in the United States.³ Unlike the Hirayama and Trichopoulos studies, Garfinkel's data support **no increased lung cancer risk associated with ETS exposure**. The failure to include the Garfinkel (1981) study -- whether intentional or an oversight -- gives the appearance that it was omitted because it did not support the theme being developed by Cal/EPA.

Elsewhere, Cal/EPA claims that U.S. EPA's Risk Assessment on ETS is the "most recent" meta-analysis of the epidemiologic data on ETS exposure and lung cancer. (p. 8) This is inaccurate -- the results of several meta-analyses that fail to support U.S. EPA's conclusions have subsequently been published.⁴⁻¹⁰ Again, the omission of these references contributes to the impression that Cal/EPA is not presenting a thorough, complete representation of the scientific literature, and is instead focusing only on material that supports its conclusions.

Cal/EPA is also guilty of selectively reporting data from individual epidemiologic studies. For example, in the discussion of childhood ETS exposure, Cal/EPA references a reported risk for "high exposure" during childhood of 2.07 (95% CI 1.16-3.68) from the Janerich, et al., study. This is the **only** statistically significant association reported in that study.¹¹ Not only could a single statistically significant risk estimate have arisen by chance, given the number of analyses reported in that paper, but taking that result out of context misrepresents the Janerich, et al., study. Cal/EPA could just as well have highlighted the workplace risk estimate from the Janerich, et al., study, 0.91 (95% CI 0.80-1.04) in the Excerpt's workplace discussion -- but it did not.

Similarly, Cal/EPA selectively reports other data on workplace ETS exposures and lung cancer risk. Only two studies have reported workplace risk estimates that are statistically significant.^{12,13} The vast majority of workplace risk estimates are not statistically significant.^{11,14-25} While this is evident from the table of workplace results in the Excerpt, the text does not make this clear. Nor does the text indicate that recent meta-analyses of the workplace data report a summary risk estimate of approximately 1.0, consistent with no increase nor decrease in risk.^{4,5}

Cal/EPA also engages in "data-dredging" in discussing the workplace data. Namely, it creates a subcategory of three studies "in which the assessment of workplace exposure to ETS was complete," (p. 18) and claims that those studies are "generally supportive of an association between workplace ETS exposure and risk of lung cancer." (p. 18) However, Table 7.7 of the Excerpt shows that only one of the three risk estimates, that of Fontham, et al., is statistically significant. The workplace risk estimates for the other two studies cited to support Cal/EPA's proposition (Wu, 1985, and Wu-Williams, et al., 1990) are not statistically significant, and thus do not clearly support an association, contrary to the Cal/EPA's claim.

The review of workplace data by Cal/EPA appears to be an attempt by the agency to "make something out of nothing," because, as noted previously, meta-analyses of the workplace data consistently show no association.

Cal/EPA's discussion of the epidemiologic data on reported ETS exposures from sources other than the spouse is also flawed, because it relies in large part on only the reported results of the four most recent studies, rather than discussing the data presented in all the studies on this topic. As reported in a recent comprehensive review of the ETS-lung cancer literature, the data on lung cancer risks for non-spousal ETS exposures, taken as a whole, provide "little or no substantial evidence of an association."²⁶ Cal/EPA's approach to the data effectively ignores such analyses.

B. The Excerpt Contains Misleading Statements

There are a number of instances in the Excerpt where statements are framed in such a manner as to yield misleading impressions. Cal/EPA must draft its document so that its meaning is clear.

For instance, on p. 8, Cal/EPA states: "In order to gain a more accurate estimate of the association between ETS exposure

and lung cancer, a meta-analysis approach has been used to pool results of **comparable** studies." (emphasis added) This statement implies that the spousal smoking studies are "comparable," when it has been clearly illustrated that they are not.²⁶ Moreover, it implies that meta-analysis may be appropriately applied to these studies, which is not a universally accepted position.²⁷

On the same page, the Excerpt states: "Most of the individual studies found a small increased risk." (p. 8) Actually, of the 40 studies presently available,^{1-3,11-18,20-25,28-50} only eight report statistically significantly increased overall risk estimates for spousal smoking and lung cancer.^{1,2,13,18,25,35,41,42} The vast majority -- 80 percent -- of the spousal smoking studies report overall risk estimates that are not statistically significant. Without statistical significance, a reportedly increased risk is compatible with the null hypothesis of no association. Thus, Cal/EPA's reference to a "small increased risk" is misleading.

Another example of misleading reporting is found in Cal/EPA's treatment of the Fontham, et al., study. Cal/EPA states that cotinine measurements were obtained for 81 percent of self-responder cases. (p. 15) The choice of this percentage is misleading. Fontham, et al. (1994), state: "Urine samples were analyzed for 356 (53.5%) of 665 cases and 1064 (83.3%) of 1278 controls."¹³ Thus, cotinine samples were available for only about

half of all the cases in the study, according to the original report. Here, Cal/EPA's choice of the value to cite seems to be intended to portray the Fontham, et al., study's methods in the most favorable light.

In another section discussing the Fontham, et al., study, Cal/EPA states: "[T]he U.S. multicenter study corroborated the subjects' self-reported current nonsmoking status using the urinary cotinine level." (p. 21) While Cal/EPA acknowledges that cotinine measurements assess **only** current smoking, it accepts the misclassification rates of 0.6 percent in cases and 2.3 percent in controls reported by Fontham, et al., as accurate. The Excerpt does not mention that Fontham, et al., measured cotinine in hospitalized cases. The vast majority of hospitals severely restrict smoking (in fact, accreditation requires that smoking be banned); moreover, many lung cancer patients who happen to be smokers stop smoking after diagnosis. Thus, the cotinine measurements in this study do not even give a good indication of present smoking status, let alone previous long-term smoking status. The smoking status misclassification rates portrayed by Fontham, et al., as accurate are, in reality, **not** representative of the true situation. As reported by Lee and Forey, a review of the literature indicates that smoking status misclassification rates may range as high as 15 to 20 percent.⁵¹

C. Cal/EPA Fails to Provide Justification for Considering the Recent Epidemiologic Studies Separately from the U.S. EPA Risk Assessment

One of the most curious omissions in the Excerpt is its failure to discuss the outcome of a U.S. EPA-style meta-analysis in which data from the four epidemiologic studies published since 1991 are included. Cal/EPA provides no justification for this omission. Given Cal/EPA's acceptance (and even defense) of the U.S. EPA meta-analysis, the failure to consider a meta-analysis including all the studies can be interpreted as an attempt to exclude an analysis that would not support Cal/EPA's conclusions.

A review of the scientific literature reveals several post-EPA meta-analyses conducted by other researchers.^{4,7-10} These meta-analyses illustrate that, if the U.S. EPA's approach is adopted, inclusion of new studies lowers the overall summary risk estimate.

For instance, in a recent paper, LeVois and Layard report:⁴

Using the EPA's methods and assumptions, we have calculated a summary relative risk of 1.07 from a meta-analysis of 13 U.S. female spousal smoking studies, including these two recent studies [Brownson, et al., and Stockwell, et al.] This relative risk, with

95% confidence interval of 0.95-1.21, is not statistically significant.

The inclusion of two additional recent studies in the meta-analysis effectively reversed the conclusion of the U.S. EPA Risk Assessment on ETS. The summary relative risk is no longer statistically significant, and, therefore, does not support a conclusion of an association between spousal smoking and lung cancer.

Gross (1995) noted that when Kabat (1990, an initial report of the study discussed in the Excerpt⁵²), Stockwell, et al.,²² and Brownson, et al.,¹⁴ are included in a U.S. EPA-type meta-analysis, a summary risk estimate of 1.12 (95% CI 0.99-1.27) is calculated -- prior to adjustment for smoking status misclassification.⁷ Gross states: "Since any such adjustment lowers the relative risk, it implies the null hypothesis of no association cannot be rejected." In another 1995 paper, Gross provides the misclassification-adjusted summary risk estimate; even when the U.S. EPA's very low rate of 1 percent is used, the summary value is 1.07 (95% CI 0.95-1.21).⁸

Similarly, Gori (1994) observes that, while he does not endorse the U.S. EPA approach:⁹

Including the two latest studies [Brownson and Stockwell] explicitly excluded from the EPA's review and using the EPA's own [adjustment]

procedure, a realistic correction [for misclassification] of only 2.5% would nullify any excess risk estimates in the meta-analysis of the 13 available U.S. studies of spousal exposure.

Gori states that the published literature suggests smoking status misclassification rates between two and 10 percent (average four to five percent), compared to the 1.09 percent used by U.S. EPA.⁹

Sugita, et al., also reanalyzed the U.S. spousal smoking data, incorporating the Stockwell, et al., and Brownson, et al., studies, and calculated a summary risk estimate for the U.S. studies of 1.10 (95% CI 0.97-1.26).¹⁰ Based on this and other summary risk estimates calculated by Sugita, et al., they conclude:

From these odds ratios it has not been demonstrated scientifically whether or not ETS is a particular risk factor for lung cancer. The conclusion that the relationship between exposure to ETS and lung cancer is weak remains unchanged. (p. 180)

It seems clear that Cal/EPA facilitated its arguably predetermined conclusion by simply making observations about the "concordance" or "similarity" of the recent studies to U.S. EPA's conclusions, rather than by conducting its own statistical analysis. Cal/EPA should explain why the approach of comparing the individual post-1991 studies to the U.S. EPA summary risk estimate

was chosen instead of incorporating the new studies into a meta-analysis or other, more formal, comparison.

Cal/EPA should also provide further explanation for its position that the recent studies support the U.S. EPA's conclusion, given that the Congressional Research Service reached the following conclusion, based on a review of the same material:⁵³

The new studies, including the very large Brownson study, did not clarify the existence of a risk. Indeed, they complicated the interpretation of the evidence, since the two largest U.S. studies -- Fontham and Brownson - - found in one case a positive risk that was barely statistically significant and the other no risk at all. (p. 25)

D. Subjective Terms Are Used Throughout the Excerpt Without Definition

Throughout the Excerpt, Cal/EPA employs terms like "concordance" (p. 7), "very similar to" (p. 7), "closest to" (p. 7), "consistency" (p. 27), "similar" (p. 14), and "compatible" (p. 19) when discussing the results of two or more studies or analyses. Because these terms are not defined, their scientific meaning is unknown to readers of the Excerpt, who are therefore left with the impression that these terms are subjective descriptions without a rigorous scientific basis.

For example, Cal/EPA states: "Results from the largest population-based study, conducted in several metropolitan areas of the U.S. (Fontham et al., 1994) were **closest to** the pooled estimate of the U.S. EPA report." (p. 7) (emphasis added) The subjective phrase "closest to" has no scientific meaning. Used in this manner, it appears to be a transparent attempt to bolster the position that the newer studies support the conclusions of the U.S. EPA Risk Assessment, without conducting a statistical analysis.

E. The Excerpt Contains Unreferenced and Poorly Referenced Claims

In the Executive Summary of the Excerpt, Cal/EPA claims that there is "compelling biologic plausibility of an effect of ETS on lung cancer." (p. 7) No references are cited in support of this proposition. Thus, the public may take this statement at face value, when the statement is actually not supported by a review of the relevant scientific literature. Neither animal inhalation studies nor genotoxicity studies provide support for the claim, as discussed elsewhere in this submission (Section III).

Similarly, Cal/EPA provides only one reference for its claim that "there is no publication bias against statistically nonsignificant results on ETS in the peer-reviewed literature." (p. 10) The issue of publication bias cannot be summed up by a

single reference; Cal/EPA should review additional literature on this issue, including the literature on meta-analysis, where much of the debate on publication bias has developed.

Moreover, Cal/EPA does not address the issues of selective reporting and "data-dredging," another form of bias common in epidemiologic studies. For instance, the Fontham, et al., study reports more than 100 case-control comparisons.¹³ With this many comparisons, a number of statistically significant associations would arise by chance alone. This issue is not adequately addressed in the original studies, nor in the Excerpt.

F. Cal/EPA's Reanalysis of Kabat, et al. (1995), Is Not Justified

Cal/EPA's analysis of the 1995 Kabat, et al., case-control study¹⁷ certainly gives the appearance of a bias toward reporting results that support the position that the recent ETS epidemiologic studies support the conclusions of the 1993 U.S. EPA Risk Assessment on ETS. In fact, the approach taken by Cal/EPA has already been deemed "controversial" by observers.⁵⁴

The risk estimates based on the Kabat, et al., data, as originally reported in the publication, do not agree with Cal/EPA's position. As Kabat and colleagues stated: "[T]he pattern of odds

ratios [in this study] shows little indication of an association of environmental tobacco smoke with lung cancer in nonsmokers."¹⁷

After noting that "[t]here were no significant associations between spouses' smoking and risk of lung cancer in male or female subjects" in the original report of the Kabat, et al., study, Cal/EPA states: **"We calculated the OR for lung cancer in males and females combined to be 1.19 (95% CI=0.76-1.87) in association with spousal ETS exposure."** (p. 18) (emphasis added) Cal/EPA then summarizes its conclusions: "Although [Kabat et al.] . . . interpreted their findings (analyzing men and women separately) to be unresponsive of an association between ETS exposure and risk of lung cancer, the odds ratio we calculated from their results, though not statistically significant, was in fact very similar to the pooled estimate from the U.S. EPA report." (p. 19)

Because the author gives no statistical or scientific justification for combining the Kabat, et al., data for men and women, this approach appears to be an attempt to "make the data fit." The decision to combine the male and female data is even more curious when one considers that the U.S. EPA Risk Assessment based its conclusions on data for women only, completely ignoring the available data on males, which, taken as a whole, support no increase in lung cancer risk in men whose wives smoked.²⁶ Moreover,

the Excerpt implies that there is some importance in the reanalysis of the Kabat, et al., yielding a risk estimate of 1.19, the same as the U.S. EPA summary risk estimate, when this simple coincidence has little, if any, real meaning.

The interpretation by Kabat, et al., that their data do not support an association between ETS exposure and lung cancer risk is wholly appropriate. Kabat and colleagues report a number of risk estimates that do not achieve statistical significance. They also exercise caution in interpreting the few statistically significant risk estimates in their study. However, Cal/EPA does not simply accept this interpretation, apparently since it does not support Cal/EPA's position. Interestingly, Cal/EPA makes no comment about the unsupported interpretation by Brownson, et al., of the data in their case-control study.¹⁴

An examination of the reported results in the Brownson, et al., study shows that the overall risk estimate for spousal smoking is 1.0 (95% CI 0.8-1.2), clearly inconsistent with an increase in risk. The only statistically significant results in the Brownson paper were restricted to "qualitative" indices of exposure, where study subjects provided an "estimate [of] a perceived level" of exposure.¹⁴ Despite the vast majority of the data being consistent with no increase in risk, Brownson, et al., concluded:

[O]ur study and others conducted during the past decade suggest a small but consistent elevation in the risk of lung cancer in nonsmokers due to passive smoking.

According to a scientist who has reviewed and reanalyzed the Brownson publication and the raw data from the study, "influences directed to the identification and reporting of an association between ETS [exposure] and lung cancer may have affected the reporting of the results" from this study and others.⁵⁵

G. Conclusion

As the lung cancer Excerpt is written, it appears to be an attempt by Cal/EPA to make recent data agree with the conclusions of the U.S. EPA Risk Assessment on ETS. The selectivity and subjectivity of the Cal/EPA discussion lead to conclusions which simply are not supported by the data and objective, thorough scientific analyses.

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SECTION III: CAL/EPA RELIES INAPPROPRIATELY ON THE U.S. EPA RISK ASSESSMENT ON ETS, WITHOUT SUFFICIENT ATTENTION TO THE DEMONSTRATED FLAWS IN THAT DOCUMENT

Cal/EPA relies extensively on the 1993 U.S. EPA Risk Assessment on ETS, which purportedly reviewed the literature on ETS and lung cancer through 1991.¹ Cal/EPA has apparently accepted the U.S. EPA Risk Assessment as conclusive. While mention is made of certain criticisms of the U.S. EPA document (e.g., pp. 9-10), Cal/EPA simply lists those criticisms, without providing sufficient rebuttal. Cal/EPA sums up its discussion of the U.S. EPA Risk Assessment by stating: "[T]he U.S. EPA's report is based on the total weight of evidence, and not on an individual study." (p. 10) The following discussion will show that (i) the criticisms leveled against the U.S. EPA report are much more severe than the superficial treatment by Cal/EPA would imply, and (ii) U.S. EPA omitted a large amount of relevant data and information in reaching its conclusions about ETS, so that the "weight of the evidence" claim is misleading.

A. Criticisms of the U.S. EPA Risk Assessment Have Never Been Adequately Addressed by the Agency

Criticisms have been raised about the U.S. EPA's Risk Assessment since the initial release of a draft for public review in 1990. At that time, independent scientists and interested parties raised concerns about many important issues; over 100

submissions were made to the U.S. EPA public comment docket. The numerous criticisms of the 1990 draft were summarized in a document prepared for the Agency, which stated:²

The predominant theme in comments related to lung cancer is that the classification of ETS as a Group A carcinogen, causally related to increased risk of lung cancer, is unwarranted. . . . The claim that it is biologically plausible that ETS is a lung carcinogen is contentious and unjustified in the Report. (pp. 3-4)

It is not possible to summarize comprehensively in just a few pages the remarks from the thousands of pages of comments submitted. (p. 4)

The criticisms -- on toxicological, chemistry, exposure, epidemiologic and statistical issues -- raised with respect to the 1990 draft of the U.S. EPA Risk Assessment were not adequately addressed in the revised draft that was issued in 1992. At that time, comments were again submitted by a number of independent scientists and other interested parties. In addition to reiterating many of the criticisms initially raised, and confirming that those criticisms were not adequately addressed in the new draft, the comments also raised new issues related to the second review draft.

Following the release of the final document in January 1993, essentially identical to the heavily criticized 1992 draft,

a number of articles critical of the Risk Assessment have appeared in the scientific press.³⁻⁹ For instance, Huber and colleagues (1993) wrote:³

[T]he EPA's risk assessment is built on the manipulation of data, ignores critical chemical analyses and key epidemiological data, violates time-honored statistical principles, fails to control adequately for important confounding influences (factors other than the one studied that may affect a result or conclusion) that provide alternative explanations for its conclusions, and violates its own guidelines for assessing and establishing risk to a potential environmental toxin. It lacks credible quality control and adequate external unbiased peer review. In short, in its report on ETS, the EPA did not comply with accepted principles of toxicology, chemistry, and epidemiology, nor with its own guidelines for undertaking cancer risk assessment. In fact, the conclusions drawn by the EPA are not even supported by the EPA's own statements. (p. 45) (emphasis added)

In a 1994 commentary on the U.S. EPA Risk Assessment, Gori stated:⁴

[T]his figure [3,000 deaths] is . . . the **result of an unprecedented exercise in data manipulation.** Among other unjustifiable gambits, this EPA report stands out for its unorthodox insistence on one-tailed statistics and 90% confidence intervals, for arbitrary and unproven adjustment procedures, and for its selective use of epidemiologic evidence. For instance, without explanation the report has summarily excluded workplace data and the latest epidemiologic studies, which together void the report's conclusions when added to the body of evidence. (emphasis added)

Furthermore, in another discussion published in 1995, Gori observed:⁵

EPA claims to have used a weight of evidence approach. In reality, ETS has been characterized only indirectly by an analogy to active smoking that EPA itself discounts. Negative epidemiologic reports were summarily ignored. Statistical standards were relaxed. Competing and well-known risk factors were disregarded. Documented misclassification biases were discounted. **By its own admission, EPA made very selective use of available studies, emphasizing only those that support its preconceived conclusions.** (pp. 20-21) (emphasis added)

In a technical analysis, Gross (1995) illustrated clearly that the U.S. EPA methodology for calculating deaths purportedly attributable to ETS exposure "is **far from an exact or even approximate science.** . . . [M]inor to moderate changes in the model parameters lead to large changes in the estimated number of [deaths]."⁶ (p. 411) (emphasis added) Further, he stated that "EPA has not given very much thought to the uncertainty of its estimates," and noted that the epidemiologic data on ETS and lung cancer are consistent with a risk estimate of 1.0, which, in U.S. EPA's model, would yield an estimate of zero "attributable" deaths.

More recently, the Congressional Research Service (CRS) has issued two reports that raise substantial questions about the

U.S. EPA Risk Assessment.^{10,11} In 1994, one of the authors of the 1994 CRS review, Jane Gravelle, described the conclusions of that document as follows:¹²

[O]ur evaluation was that **the statistical evidence does not appear to support a conclusion that there are substantial health effects of passive smoking.** (emphasis added)

A compilation of materials criticizing or questioning the U.S. EPA Risk Assessment is submitted as Appendix I to this comment.

B. Data on ETS Chemistry Were Omitted from the U.S. EPA Risk Assessment

The U.S. EPA Risk Assessment failed to discuss or even to reference a significant amount of relevant literature on the physical and chemical properties of ETS.

A number of scientists have concluded that ETS is not the same as either mainstream or sidestream smoke.¹³⁻¹⁷ ETS is an aged and diluted mixture of sidestream and exhaled mainstream smoke; ETS is a dynamic, ever-changing mixture that undergoes chemical transformations and physical changes as it ages and is diluted in the air.¹³⁻¹⁵ As one researcher in tobacco smoke chemistry has observed, "there are profound physical and quantitative chemical

differences" among the three kinds of smoke (mainstream smoke, sidestream smoke and ETS).¹³

Studies also indicate that constituents in ETS are **hundreds to thousands** of times more dilute than either sidestream or mainstream smoke.^{16,17} Concentrations of ETS constituents in real-life situations are often below the limits of detection and measurement for even the most sensitive air monitors. Often, the contributions of ETS constituents to the ambient air are indistinguishable from background levels of the same constituents generated by other sources.¹⁶

The strategy of comparing mainstream and sidestream smoke employed by U.S. EPA in the Risk Assessment (e.g., Chapter 3) ignores the profound effect of dilution in the ambient air upon tobacco smoke constituents. As two tobacco smoke chemists report:¹⁴

The important question is not the ratio of sidestream/mainstream but rather what is the concentration of the constituent in the indoor environment and how does it compare to levels from sources other than ETS. Studies based solely on observations of fresh sidestream, or highly and unrealistically concentrated ETS should take into account the possible differences between these smokes and ETS found in real life situations.

Similarly, the 1986 Report of the Surgeon General notes: "SS [sidestream smoke] characteristics, as measured in chambers, do not

represent those of ETS, as inhaled by the nonsmoker under non-experimental conditions."¹⁸

C. Data on Measurements of ETS Exposure Were Omitted from the U.S. EPA Risk Assessment

Cal/EPA focuses heavily on the epidemiologic literature in the Excerpt. In failing to mention the literature on issues of ETS exposure, Cal/EPA seems to be implying that the U.S. EPA Risk Assessment provides sufficient discussion of these issues, which is not the case.

The initial public review draft for the U.S. EPA Risk Assessment (1990) did not even contain an exposure assessment. Comments submitted to the public docket for the 1990 draft risk assessment observed that U.S. EPA had failed to provide an exposure assessment that considered data from the numerous published studies on actual levels of ETS constituents in the air of public places and workplaces. In apparent response to that criticism, the revised 1992 draft of the U.S. EPA Risk Assessment contained a chapter entitled "Estimation of Environmental Tobacco Smoke Exposure," but the chapter's authors failed to consider at least 35 pertinent exposure studies on ETS constituent levels in public places.

Furthermore, during its review of the chapter in July of 1992, the U.S. EPA Science Advisory Board rejected the chapter and returned it to the author for rewriting. Nevertheless, without either an exposure assessment or recourse to any of the data pertaining to it, the Science Advisory Board endorsed U.S. EPA's estimate of exposure and risk for the entire U.S. population. A revised chapter on ETS exposure occurs in the final U.S. EPA Risk Assessment; however, the studies and data therein are not integrated into the U.S. EPA Risk Assessment.

Only a small number of actual ETS exposure studies available in the published literature are even discussed in the U.S. EPA Risk Assessment. Many of the studies of exposure to ETS constituents under realistic conditions in public places, workplaces and homes omitted from the U.S. EPA Risk Assessment report minimal exposures to ETS; these reported exposures do not support the conclusions of the U.S. EPA Risk Assessment.^{17,19-62}

For instance, studies on ambient measures of nicotine in offices and restaurants report average levels of approximately 2.0 and 3.5 ug/m³ nicotine, respectively. These exposures are equivalent to 1/400 to 1/200 of the nicotine found in a single cigarette. Averages for nonsmoking areas in workplaces with smoking restrictions are even lower, averaging less than 1 ug/m³ nicotine, or about 1/1,000 of the nicotine in a single cigarette.

This means that the typical nonsmoking worker would have to spend from 200 to more than 1,000 hours in an office, restaurant or public place in order to be exposed to the nicotine equivalent of a single cigarette.^{35,55-58}

The cigarette equivalent approach has been criticized because different results would be obtained if different reference compounds are chosen. These criticisms are based simply on calculating the sidestream/mainstream ratios for the different reference compounds. Such a calculation would be accurate if there were no chemical and physical changes which occur when sidestream smoke is transformed into ETS. This, as has already been clearly pointed out earlier in this submission, is not the case. As a consequence, it would not be at all correct to state that a typical nonsmoking worker who spends 200 to 1,000 hours in an office, restaurant or public place is exposed to the **smoke** equivalent of one cigarette, since ETS is not at all chemically or physically identical to the smoke to which a smoker is exposed.

With regard to the calculation of cigarette equivalents based on other reference compounds, it is important to note that the vast majority of ETS constituents are found, generally in much greater amounts, in indoor environments in the absence of ETS. Researchers report that there is little difference in ambient levels of carbon monoxide or nitrogen oxides in smoking and

nonsmoking areas of workplaces and public places and in homes with or without smokers.^{16,27,30,31,49,59,60} Similarly, levels of volatile organic compounds such as formaldehyde and benzene in the presence of smoking are often indistinguishable from levels reported in nonsmoking areas.^{16,32,35,50-52} Studies that have examined ETS constituent levels of nitrosamines also report minimal contributions to overall ambient air levels in homes, offices and public places.^{48,61,62}

D. Limitations of Exposure Estimates Based on Questionnaires Were Not Discussed by U.S. EPA

The "exposure" estimates in epidemiologic studies on ETS are wholly independent of a large body of data on ambient exposure measurements to various constituents of ETS, which, taken as a whole, suggests that ETS exposures are minimal. The epidemiologic studies rely on questionnaire data, the accuracy of which depends on an individual's ability to recall past events, such as how much a husband smoked in the past 20 to 30 years.⁶³⁻⁶⁵ The two bodies of data, epidemiologic/health effects, on the one hand, and exposure measurements on the other, have never been integrated into one comprehensive study.

In a 1994 article critical of the U.S. EPA Risk Assessment, Gori wrote:

A major limitation of epidemiologic studies on ETS has been the unreliable estimates of dose, which compound the uncertainties of personal or proxy recall of the intensity, frequency, and duration of exposures over individual lifetimes. Even the simple dichotomous classification of exposed and non-exposed subjects presents recognized uncertainties, such as those deriving from the self-classification of some smokers as non-smokers. (p. 327)

U.S. EPA relied heavily on the epidemiologic data in its Risk Assessment on ETS, without adequately exploring the limitations of questionnaire data.

1. "Spousal Smoking" Is Not Equivalent to ETS Exposure

A fundamental problem with Cal/EPA's Excerpt and with U.S. EPA's Risk Assessment is the **uncritical** acceptance of the risks reported in spousal smoking studies as **true** risks due to **exposure to ETS**. The central question is whether or not "ETS exposure" is the same thing as "spousal smoking"; that is, does living with a smoker imply exposure to ETS?

Even though U.S. EPA concedes that spousal smoking status is a "crude measure" of ETS exposure and one that is "prone to exposure misclassification,"⁶⁶ the Agency nevertheless equates spousal smoking status with household exposures to ETS.⁶⁷ However, the extent to which the spousal smoking index is an accurate,

reliable and valid quantitative indicator of ETS exposure was not assessed by U.S. EPA in its Risk Assessment. The quality of the underlying exposure data from the spousal smoking studies is thus of critical importance for epidemiologic studies on ETS and chronic disease, because "the accuracy of any statistical analysis is limited by the accuracy of the information upon which it is based."⁶⁸

The spousal smoking index is not an accurate marker for exposure to ETS. That "marriage to a smoker" is **not** equivalent to home exposures to ETS has been demonstrated in two studies.^{69,70} Friedman, et al., polled nearly 38,000 nonsmokers and ex-smokers in California about ETS exposure. Nearly 35 percent of respondents classified by their spouse's smoking status reported no exposure overall to ETS; 47 percent of women married to smokers reported zero hours of exposure at home.⁶⁹ The researchers concluded that "using the spouse's smoking status to classify persons resulted, as far as can be discovered with our relatively crude questionnaire, in a considerable amount of [exposure] misclassification."⁶⁹

A 1994 published study conducted in the U.K. reports a similar conclusion.⁷⁰ The researchers compared subjective estimates of ETS exposure with objective measurements of airborne ETS markers via personal monitors (breathing zone measurements). They reported that 45 percent of subjects with a smoking partner assessed their

exposure as "none or low." Similarly, 30 to 40 percent of individuals married to smokers were actually exposed to less than the average ETS levels measured among subjects with a nonsmoking partner. The researchers conclude: "Clearly, spousal smoking status would not be a reliable means to assess the ETS exposure of individuals or small groups of subjects."⁷⁰

Thus, "marriage to a smoker" may -- or may not -- imply exposure to ETS; it cannot serve as an accurate, quantitative surrogate for actual exposure measurements.

2. Attempts to "Refine" the Spousal Smoking Index Do Not Yield Better Data

In attempting to "refine" the spousal smoking index, a number of spousal smoking studies have employed exposure estimates beyond the simple index of "marriage to a smoker."^{65,71} Questions regarding ETS exposures have included those related to the **intensity** of exposure (e.g., the number of smokers in the home or the number of cigarettes smoked per day or per year), or the **duration** of exposure (e.g., hours of exposure, years of marriage to a smoker or years of smoke exposure).⁷¹ Estimates of the intensity or duration of spousal smoking exposure are derived by questionnaire or interview of the study subject (the case) or next of kin.⁷²

There is no standardized or validated questionnaire available for use in epidemiologic studies on ETS, and no single questionnaire was used in the published studies on spousal smoking.⁷³ As a consequence, definitions of various ETS exposure indices have differed considerably from study to study with respect to source, intensity and duration.

3. Questionnaire Responses Are Neither Reliable Nor Accurate

The questionnaire, by its very nature, can provide only a crude, qualitative estimate of exposure. Questionnaire data do not, and cannot, provide information on **concentration** (e.g., actual levels of airborne ETS constituents) or **frequency** of ETS exposure. As the U.S. National Academy of Sciences observed in 1991: "Exposures [to ETS] occur at a wide range of concentrations for highly variable periods and in numerous indoor environments. Unlike active smoking, exposure to ETS cannot now be easily assessed. . . ." ⁷³ The questionnaire is thus only an indirect means of assessing exposure. This, according to a NIOSH witness at the U.S. OSHA Public Hearing, is one of "the principal weaknesses in the epidemiologic evidence to date." ⁷⁴

Where exposure is defined as "concentration over time" or "intensity, frequency and duration," the questionnaire's inherent shortcomings are obvious. Thus, Coultas, et al., note:⁷⁵

Questionnaires on exposure to environmental tobacco smoke generally assess the strength of the source, e.g., the number of smokers, the number of cigarettes consumed and the duration of exposure. The concentration of environmental tobacco smoke, however, depends not only on the source strength, but on room size, mixing, adsorption of smoke components, and the rate of exchange of indoor with outdoor air. Personal exposure also varies with the nonsmoker's proximity to the smoker. Questionnaires cannot comprehensively and accurately assess each of these factors.

Estimates of "intensity" and "duration" of exposure also depend upon respondent memories of literally decades of potential exposure scenarios. Complete recall, of course, is impossible, and even partial recall may be faulty. For example, studies indicate that while spouses (or children) may generally agree in reporting a partner's (or parent's) smoking status (i.e., whether or not a spouse smoked), agreement regarding the amount smoked or duration of smoking is often very poor.^{63,65,76-79}

Pron, et al., examined the reliability of self-reported ETS exposure histories by interviewing and re-interviewing study subjects.⁶⁵ Consistency of responses about exposure between initial interview and re-interview was poor, and correlations between responses were low, especially for questions related to intensity and duration of exposure. The authors conclude:

[T]his is the first study to assess the reliability of information reported on passive smoke exposures in personal interviews. Test-

retest estimates of reliability suggest that misclassification of such exposure may be extensive.

In "An Assessment of the Validity of Questionnaire Responses Provided by a Surviving Spouse,"⁷⁶ Lerchen and Samet report that wives generally agree with the smoking status classification given by their spouse, but that 44 percent of the spouses could not provide a detailed smoking history of the spouse. The authors observed that wives tended to report that their spouse smoked 20 cigarettes per day, even when the husbands reported that they smoked substantially more or less. The researchers remarked that "the validity of such surrogate information, when available, is uncertain." Kolonel, et al., reported similar results in 1977.⁸⁰

Sandler and Shore investigated the degree of agreement on parental smoking status from interviews of parents and their (adult) children.⁷⁷ These authors reported reasonable agreement in responses about the smoking status of the parent, but agreement on exposure level estimates was extremely poor. The authors conclude that childhood ETS exposure information obtained via questionnaire from adults "cannot readily be used to estimate levels of exposure" to ETS, and that "data on levels of exposure were not of high enough quality to allow for detailed evaluation of dose-response."⁷⁷

Similar results were reported in a study by Coultas, et al., in 1989.⁶³

Brownson, et al., in a 1993 study, assessed ETS exposure histories through interviews and re-interviews of cases and controls in a spousal smoking study on lung cancer.⁷⁹ Agreement for spousal smoking status after re-interview was 84 percent, indicating that 16 percent of cases and controls actually reversed responses regarding the smoking status of their spouse. The agreement rate for exposure histories in adulthood among cases was only 49 percent. Overall, the reliability of responses (assessed by agreement in responses over time) about quantities of exposure was extremely poor, and lower for cases than for controls.⁷⁹

Despite representations to the contrary,^{77,79} studies indicate that the personal interview may not even guarantee a reliable answer concerning the smoking status of one's spouse, much less a reliable estimate of ETS exposure duration or intensity. Of course, the **reliability** of questionnaire data is determined only by agreement of respondent answers concerning **estimates** of exposure. The **accuracy** of the estimate itself is not addressed. No standard exists for validating a history of exposure to ETS, or for assessing the accuracy of exposure estimates.

4. Questionnaire Responses Are Influenced by Perceptions

Reconstruction of a life history of ETS exposure is highly influenced by recall accuracy -- imagine recalling, with **any** degree of accuracy, the duration, frequency and degree of personal ETS exposure over the previous week, year or decade, or during a marriage, during adulthood, etc. Studies suggest that even **recent perceptions** of ETS exposure may be wholly inadequate indicators of **actual** ETS exposures.^{64, 70, 81-83}

O'Connor, et al., compared self-reported exposures to ETS with ambient levels of nicotine collected by personal monitoring devices.⁸¹ Thirty-six percent of women who reported ETS exposure by questionnaire were misclassified as "exposed" to ETS according to objective measures of exposure.

Coultas, et al., in a study of 10 homes, found that questionnaire responses about ETS exposures "were poor predictors of concentrations of respirable particles and nicotine."⁶⁴ Similarly, a workplace study conducted by Schenker, et al., reported no association between respirable nicotine concentrations and self-reported exposures to ETS.⁸² The authors conclude that "self-reported exposure to ETS is an inaccurate measure of passive smoking in the occupational setting."

Two recent, large-scale studies in the U.S. and the U.K. compared perceptions of individual exposures to ETS (by self-report) with actual levels of ETS constituents collected in the subjects' breathing zones by personal monitors in agreement with OSHA practice.^{70,83} In the U.S. study, greater than 50 percent of all self-reports of ETS exposures occurred at work. Actual exposure measurements, however, indicated that average exposure levels were approximately four to six times greater in venues **outside** the workplace.⁸³ In the U.K. study, individuals subjectively ranked relative contributions of ETS as leisure > work > home > travel.⁷⁰ Measured exposure levels, however, indicated a ranking of: home > leisure > work > travel. The authors suggest that the discrepancy between subjective rankings and objective measurements of exposure may be due to the subject's inaccurate estimate of the time spent in each venue. Other researchers have studied variations in subjective perceptions of exposure and have reported that perceptions will vary depending on whether or not the exposure source (i.e., the smoker) can be seen.⁸⁴

5. Cotinine Measurements Do Not Validate Questionnaire Responses

Nicotine, because it is characteristic of tobacco smoke in the air, has been used extensively as an ambient air exposure marker for ETS.^{35,70,83,85} Likewise, cotinine, one of the substances converted from nicotine by the body, has been used as a biomarker

(internal dose marker) for nicotine (and ETS) exposures.⁸⁶⁻⁹⁰ Several researchers, including Riboli, et al., of IARC, have used measurements of cotinine in an attempt to validate self-reports of ETS exposures.^{87,90-92}

The advantage of body fluid measurements of cotinine over nicotine is that cotinine has a longer half-life (17 hours vs. 3 hours). Despite this longer half-life, however, cotinine-derived estimates are of little value in determining past (greater than one week) exposures to nicotine. As Reasor has written:⁹³

At present, however, there is no reliable way, through the use of biological markers, to assess long-term exposure to ETS.

Cotinine has even more fundamental problems when used as a biomarker for exposure to ETS.^{81,88,93-97} For example, an individual will metabolize and clear cotinine from his/her system at different rates at different times (intra-individual variation), and clearance rates vary considerably from individual to individual (inter-individual variation).^{93,95,98} Common foods also contribute trace levels of nicotine (which are thus converted to cotinine), thereby confounding inferences about ambient nicotine (ETS) exposures.⁹⁹ Moreover, body fluid levels of cotinine do not correlate well with ambient levels of nicotine (or any other constituent of ETS),^{64,70,81,93,96} and saliva, plasma and urine levels of cotinine are also poorly correlated.^{64,95}

Large intra- and inter-subject variabilities in the conversion, metabolism and clearance of cotinine call into question the validity of its use in surveys that employ single-point measurements (of cotinine) to represent ETS dose.¹⁰⁰ Dr. Paul Nelson of R.J. Reynolds recently observed: "It is likely that inter-individual variations in nicotine and cotinine metabolism or excretion would far outweigh the small incremental increase in cotinine concentration following exposure to typical levels of ETS nicotine. In other words, the variation between people is larger than the variation due to normal exposures."¹⁰¹

Virtually every cotinine measurement survey relied upon by U.S. EPA for ETS exposure estimates was restricted to single-point measurements of cotinine.^{86,87,91,92} However, an individual's cotinine level at a single point in time will be determined by the timing of the specimen collection, and by the individual's own rates of uptake, metabolism and clearance. Thus, Idle observes:⁹⁵

Single point cotinine concentrations can give no more than a clue of past exposure to pyridine alkaloids [such as nicotine] of unknown amounts, at an unspecified time, by an unknown route of entry and from unknown origins.

Phillips, et al., reported extremely poor correlations between salivary cotinine values and 24-hour respirable particle and nicotine exposure measurements.⁷⁰ The data from the Phillips,

et al., study indicate that some subjects who were exposed to high levels of particles and nicotine had **no** detectable cotinine levels, while some subjects exposed to low levels of ETS, as determined by personal monitor, exhibited high levels of cotinine.

O'Connor, et al., reported levels of urinary cotinine that "did not differ" among ETS exposed and non-exposed.⁸¹ Ogden, et al., reported "virtually identical median levels of salivary cotinine" for all subjects, even though nicotine exposures varied nearly three-fold between exposed and non-exposed individuals.⁹⁶

Cotinine measurements likewise do not correlate well with self-reports of ETS exposures.^{70,75,80,87,102,103} Wagenknecht, et al., found that 58 percent of 575 study participants who reported ETS exposures of 42 hours or more had **no** detectable serum cotinine levels; of the 186 individuals who reported no known exposures, 23 percent had a detectable cotinine level.¹⁰² Delfino, et al., examined salivary cotinine levels and compared them with questionnaire-derived responses about ETS exposures.¹⁰³ No correlations were reported.

In 1994, Emmons, et al., reported that nearly half of those individuals who recalled exposure to ETS at work had nondetectable cotinine concentrations, as did 29 percent of those who reported exposures at home.⁸⁷ Coultas, et al., found that both

urinary and salivary cotinine levels of workers, measured post-workshift, "varied widely with self-reported exposures."⁷⁵ Similar results were reported by Heller, et al.,⁹⁴ and Suadicani and co-workers.¹⁰⁴ In the latter study, investigators reported that individuals who were classified (by self-report) as "often exposed," "occasionally exposed" and "rarely/never exposed" exhibited similar average serum cotinine levels of 25, 22, and 24 ng/ml, respectively.¹⁰⁴ Tunstall-Pedoe and coworkers recently reported poor correlations between self-reported ETS exposure and serum cotinine level.⁸⁹ They concluded that "their poor correlation with each other . . . undermine[s] the validity of the two measures of passive smoking."

Despite acknowledged shortcomings in the use of cotinine as a quantitative biological marker for ETS, some researchers nevertheless have used cotinine measurements in an effort to validate self-reports of ETS exposure.^{86,91} Riboli, et al., for example, reported that mean urinary cotinine levels among their study population showed a linear increase with self-reports of ETS exposure, and that cotinine levels were indicative of reports concerning the duration of exposure and the number of cigarettes smoked in the presence of the subject.⁹¹

Close examination of the Riboli, et al., study data reveals, however, that 20 percent of the study population had

nondetectable cotinine levels, and that a predicted increase of 5 ng/ml urinary cotinine could be calculated from self-reported exposures ranging anywhere from six to 83 cigarettes per day.⁹¹

Riboli, et al., do not indicate the existence of any overlap in cotinine measures among the groups examined.⁹¹ Other researchers, however, have reported wide variations of cotinine measures within specific levels of reported exposure.^{63,70,87} For example, Phillips, et al., reported "considerable variation in the direct measurements [of cotinine] corresponding to the higher grades of subjective [ETS] assessment."⁷⁰

Even proponents of the use of cotinine to validate self-reports of ETS exposures realize the limitations of the method.⁶³ Cummings, et al., provide the following caveats to their study:⁶³

Cotinine was chosen as a biological marker of ETS exposure because it is specific to tobacco smoke. However, cotinine levels in body fluids may not only reflect environmental exposure to tobacco smoke, but also factors that influence uptake and metabolism of nicotine.

And:

The relatively modest correlation between reported ETS exposure and urinary cotinine indicates that other factors such as differing metabolic rates and body size may have a confounding effect on the relationship between cotinine levels and questionnaire measures of ETS exposure. In view of this finding, we

would recommend against using cotinine levels as a strictly quantitative indicator of ETS.

6. A Potential Bias in ETS Questionnaire Data

Self- (and surrogate) reports of exposure to ETS are neither accurate nor reliable when compared to a standard of personal measures of exposure to airborne ETS constituents. Questionnaires are limited by accuracy of recall and the individual's ability to provide comprehensive, quantitative estimates of exposure over time. Other problems beset the questionnaires used in studies on ETS. As Tager notes:¹⁰⁵

Among the most significant limitations of existing questionnaire data [on ETS] are the facts that many of the questions were not designed specifically to investigate involuntary exposure, or the questionnaires have been incomplete in their probing of the circumstances of exposure (e.g. intensity, duration, specific location, etc.). These limitations have made it difficult to provide even semi-quantitative exposure estimates over time.

The accuracy of an exposure estimate is of obvious importance because of its profound effect on risk estimates in epidemiologic studies.¹⁰⁶ Garfinkel, et al., in a spousal smoking study published in 1985, reported different risk estimates ranging from 0.46 to 3.57 depending upon who responded to questions about exposure. If a nonsmoking wife or smoking spouse responded to the

exposure questions, the estimated risk from spousal smoking was below unity, i.e., no increased risk. If a son or daughter responded, the estimated risk rose to over 3.00. Responses were also influenced by changing the wording of the exposure-related questions.¹⁰⁶

An entire range of potential biases is introduced through the improper design and administration of specific questionnaires. One particular bias, however, is directly applicable to the ETS exposure issue. It is one that arises, in part, from the way in which ETS exposure questions are phrased and presented -- called "recall bias."^{72,107-109} Recall bias in relation to ETS is a differential bias, in that cases and controls are likely to be affected differently.⁷²

Given the tremendous publicity generated, for example, by the U.S. EPA's classification of ETS as a "known human" carcinogen, it is without question that a nonsmoking lung cancer case could be influenced by such publicity in the effort to account for his/her disease. If ETS is mentioned or prompted in any way by the questionnaire or interviewer, the likelihood increases that ETS will be selected by the respondent as "the correct answer."¹⁰⁷ A control, or someone without lung cancer, is not likely to respond in that way. Tunstall-Pedoe, et al., for example, found that self-reports of exposure exhibited strong associations with symptoms,

while the relationships were "weak or absent" for cotinine levels.⁸⁹ The authors suggest that individuals with respiratory conditions may exaggerate ETS exposures, thereby creating a (recall) bias in self-reported exposure estimates.

The influence upon the respondent by the way a question is phrased or asked may be extensive. It reflects, in Wynder's words, a "wish bias," a "tendency on the part of the subject or the investigator to reach a desired result."¹⁰⁷ Wynder writes:

Research workers, like everyone else, often develop an affection for their own hypotheses and may prefer to see them supported rather than refuted. This may lead to incomplete review of the literature in which papers failing to support the hypothesis may be ignored or more subtly, may be rejected because they are considered to be of worse quality than papers that support it. Sometimes hypotheses are based on a single piece of evidence The wishes of the investigator may also enter into the collection of the data. Greater care and thoroughness may be given to collection of the data from the cases than from the controls.

The foregoing analysis demonstrates that ETS exposure and the surrogate "spousal smoking history" are not the same thing. A spouse living with a smoker may or may not be exposed at home, and he or she may or may not be exposed elsewhere. If an individual is truly exposed to ETS, the questionnaire response provides no information on the concentration or frequency of exposure. Thus,

if meaningful at all, the surrogate "spousal smoking" may be more of a measure of the many lifestyle factors surrounding marriage to a smoker than it is of ETS exposure; indeed, a history of spousal smoking may have a number of implications for disease that have nothing whatsoever to do with exposure to ETS.¹¹⁰

E. Relevant Animal Inhalation Studies Were Essentially Overlooked in the U.S. EPA Risk Assessment

U.S. EPA's risk assessment overlooked the vast majority of relevant animal data,¹ citing only a few studies. This omission severely undermines the claim that the U.S. EPA's conclusions are based on the "weight of the evidence." As the following discussion will show, if Cal/EPA were to review all the relevant animal data, it would be clear that those data provide no support for a claim that ETS exposure is associated with lung cancer.

The data in animal inhalation studies reported to date provide no support for a claim that there is "biological plausibility" for the position that ETS exposure is causally related to lung cancer. These data are not referenced in the Excerpt.

The "carcinogenic agents" supposedly identified in tobacco smoke (e.g., the "list" of suspected carcinogens referred to in the U.S. EPA Risk Assessment) either are not suspected **pulmonary** carcinogens or have not been unequivocally demonstrated as tumorigenic to human tissue or to the lung tissue of experimental animals.^{13,111,112}

In conjunction with U.S. OSHA's lengthy rulemaking process (which is still underway), several reviewers have examined and summarized the available animal inhalation studies relevant to U.S. OSHA's claim that animal inhalation studies provided supporting data for an ETS-lung cancer relationship. These reviewers unanimously conclude that the animal studies do not support the claimed carcinogenicity of ETS.¹¹³⁻¹¹⁷

One reviewer, Christopher R.E. Coggins, Ph.D., concluded that animal studies using close surrogates for ETS "show no meaningful toxicological changes, even at massive exaggerations of real-world ETS concentrations."¹¹³ Similarly, Gordon Newell, Ph.D., told U.S. OSHA that studies using a number of animal species have "failed to support the hypothesis that fresh tobacco smoke causes lung cancer" in those species.¹¹⁴

Moreover, Gio B. Gori, Sc.D., noted in his submission to U.S. OSHA's rulemaking record:¹¹⁵

Experimental data offer no plausible argument to classify ETS as a human risk . . . The arbitrariness of a priori assumptions of ETS-related human risks is further underscored by equivocal and uninterpretable epidemiologic reports.

Overall, tobacco smoke inhalation studies have not produced an increased incidence of lung tumors in experimental animals compared to controls; the relevance of other routes of exposure (e.g., skin painting) is questionable.^{116,117} The vast majority of the available data from animal inhalation studies using surrogates for ETS deal with subchronic exposures, which are of minimal relevance to the question of the claimed "carcinogenicity" of ETS. Nevertheless, none of the subchronic studies report data supporting any permanent changes following subchronic exposure of animals to sidestream smoke at levels exceeding those encountered in "real-life" situations. Brief summaries of the subchronic studies follow.

In two 1987 papers, Haley, et al., present preliminary reports on an American Health Foundation study, in which hamsters were exposed to mainstream or sidestream smoke 7 days/week for 18 months.^{118,119} Apparently, however, no final report has been published. In those reports, the authors note that smoke-exposed animals were living **longer** than were sham or cage control animals. No additional information was presented.

von Meyerinck and colleagues (1989) describe a study in which rats and hamsters of both sexes were exposed to sidestream smoke at a concentration of 4 mg/m³ TPM and 25 to 30 ppm carbon monoxide for 10 hours/day, five days/week for 90 days.¹²⁰ These authors note about their exposure system: "The levels in the exposure chamber were at least 1 and in some instances 2 orders of magnitude higher than reported for smoke-polluted rooms under real-life conditions." (Elsewhere, the authors described these conditions as "unrealistically high."¹²¹) One hundred animals of each species were exposed, 115 of each species were sham controls, and 100 of each species were room controls. The authors reported minor, completely reversible histopathological changes in the nasal cavity in rats only, and no alterations in any other part of the respiratory tract.

Male rats and male hamsters were nose-only exposed to fresh sidestream smoke (FSS) for seven hours/day, seven days/week for 90 days, in a study reported in 1994 by Teredesai and Pruehs.¹²² One group of 20 animals was exposed to FSS with a total particulate matter (TPM) concentration of 2 ug/L, one to FSS with TPM of 6 ug/L, and one served as a sham exposure group. Histopathological changes described as "slight" were reported in the nose and larynx of exposed rats, "mainly in the high FSS concentration group." These changes were reversible following cessation of exposure. The

authors noted that "[n]o smoke-exposure-related histopathological changes were observed in trachea and lungs."

In the subchronic study of Coggins, et al.,¹²³ aged and diluted sidestream smoke was used as a surrogate for ETS. This substance may be a more appropriate approximation of ETS than are other forms of tobacco smoke. Effects (hyperplasia and inflammation) were reported only in animals exposed to particle concentrations some 100 times higher than typical real-world concentrations. Coggins, et al.,¹²⁴ also report the same minor, completely reversible histopathological changes. The changes did not progress over longer periods of exposure, and once again occurred **only** at particle concentrations some 100-fold higher than real-world levels.

In a 14-day inhalation study, one would not expect lung tumors to develop. Thus, the relevance of the work of Coggins, et al., to the discussion of cancer is limited. Nevertheless, Coggins, et al. show only minor, reversible cellular changes following intense exposure to a surrogate for ETS.¹²⁵

Only one animal inhalation study to date has used exposures to a surrogate for ETS of a duration sufficient for it to be considered a chronic study.¹²⁶ Witschi, et al., recently reported the results of a study, specifically designed to produce

lung tumors in the strain of mouse used, which failed to show any significant difference between unexposed animals and animals exposed to a surrogate for ETS. The strain used lives less than one year and is known to develop lung tumors within four to six months after the beginning of exposure to various chemicals.

In the Witschi, et al., study, these mice were exposed (whole-body) to concentrations of aged sidestream smoke well in excess of measured "real-world" levels. (While not equivalent to ETS, sidestream smoke has been used as a surrogate in animal studies.) Despite the demonstrated sensitivity of this strain of mouse, the authors reported the following: "The number of animals bearing lung tumors was the same in smoke-exposed as in filtered air-exposed animals as was the average number of tumors per lung." These data are compatible with the conclusion that sidestream smoke, under the test conditions, is not a lung carcinogen in this strain.

In a recent review of the relevant literature, Rodgman cautions:¹³

Classifying a substance as tumorigenic or 'carcinogenic' can be misleading. Often, these terms are overinterpreted. One must be aware of the precise meaning and limitations of the terms tumorigenicity and carcinogenicity when applied to specific

compounds and must exercise considerable care in the use of these and related terms.

* * *

Many of these 43 MS and/or tobacco components [claimed to be carcinogens] should be excluded from the list on the basis of published data on their tumorigenicity (or lack of it) in laboratory animals at levels determined in MS, their lack of tumorigenicity in most instances on inhalation, and the equivocal evidence of their tumorigenicity in humans at levels in MS.

In this major review, Rodgman also writes:

[I]nhalation studies from 1936 to date involving lifetime exposure of laboratory animals to whole cigarette MS have consistently failed to induce squamous cell carcinoma . . .

The failure to produce in MS-exposed laboratory animals the tumor type reported to be associated with smoking in humans is important not only with regards to the biological properties of MS itself but also with respect to that of diluted MS delivered to the caged animals. . . .

If, as Stewart and Herrold (1962) noted, these smoke-inhalation experiments more closely resembled passive smoke (or ETS) exposure than human exposure during actual smoking, then substantial evidence is available to demonstrate that exposure to 'passive smoke' (or ETS), more concentrated than that encountered in the human situation, is ineffective in induction of the tumor type supposedly associated with cigarette smoking in humans . . .

The above discussion illustrates that the available animal inhalation data provide no support for the claimed "biological plausibility" that ETS exposure is associated with an increased lung cancer risk. The unreferenced statement made in the Excerpt is not representative of the actual state of the scientific literature, and as such, results in a misleading impression being conveyed to readers of the Excerpt. Cal/EPA should review the relevant animal inhalation studies and the critiques/discussions already in the U.S. OSHA rulemaking record as it revises this Excerpt.

F. Data on Genotoxicity and Related Endpoints Were Essentially Overlooked in the U.S. EPA Risk Assessment

The U.S. EPA Risk Assessment fails to reference a number of actual studies comparing levels of mutagens and other genotoxic markers in the body fluids of exposed and non-exposed nonsmokers.¹²⁷⁻
¹³⁶ The results of those studies suggest no statistically significant increases in mutagenic activity in the body fluids of nonsmokers exposed to realistic levels of ETS compared with nonsmokers who are not exposed.

When considering data on genotoxicity, it is important to put the genetic changes reported in such studies into context. All forms of life are constantly exposed to physical and chemical

agents in the environment (e.g., radiation) and to endogenous (internal) agents with the ability to cause changes in DNA. According to Bruce Ames, developer of the Ames assay for mutagenicity, human exposure to potentially mutagenic or carcinogenic substances is much greater than generally appreciated, i.e., the environment can be thought of as "filled with potential carcinogens."¹³⁷ DNA has been called an "unstable" molecule, and it has been noted that **endogenous** DNA damage may occur at the rate of 100,000 base pairs **per cell, per day.**^{137,138} Thus, DNA is **not** completely stable; changes are regularly occurring, but for the most part, do not result in heritable effects on the organism. As toxicologist Christopher R.E. Coggins of R.J. Reynolds stated in testimony before U.S. OSHA, "Toxicologically, I'm not sure that we really know what mutagenesis really means because of . . . DNA repair."¹³⁹

Therefore, conclusions about genotoxicity obtained from in vitro systems, while certainly providing some information about the substance being tested, must nevertheless be put into the proper biological context. The magnitude of a genotoxic response in the whole organism may be substantially different than that observed in a bioassay.^{140,141} As Ames and Gold noted:¹⁴⁰

[H]umans have numerous inducible defense systems against mutagenic carcinogens, such as DNA repair, antioxidant defenses, glutathione

transferases, and so forth . . . [L]ow doses of carcinogens appear to be both much more common and less hazardous than is generally thought.

The difficulty of extrapolating from in vitro genotoxicity to in vivo carcinogenicity is illustrated by data presented in the recent animal inhalation study of Witschi, et al., which used sidestream smoke.¹²⁶ Other studies suggest that tobacco smoke condensates may be mutagenic when tested using in vitro systems; however, there are no such studies using ETS condensates. In their recently published chronic inhalation study, Witschi and colleagues reported no differences in the total number of animals with tumors or in the average number of tumors per lung in the smoke-exposed animals when compared to filtered air-exposed control animals -- consistent with sidestream smoke **not** being carcinogenic under the test conditions -- even though they did report positive results for molecular biomarkers. Thus, these data suggest that the relationship of mutagenicity to "carcinogenicity" is not clear-cut.

In another study, Nikula, et al. (1995), investigated the inhalation carcinogenicity of two substances that were essentially equivalent, except that one contained mutagens and the other did not.¹⁴² If mutagenic properties were relevant to carcinogenicity, it would be expected that the substance with mutagens would have

produced a stronger carcinogenic response. However, as the authors noted, the two substances yielded "very similar" responses in the test system. Thus, these data, too, suggest that positive genotoxicity results cannot necessarily be correlated with carcinogenicity.

In its Proposed Rule, U.S. OSHA discussed a number of studies in which cigarette smoke or cigarette smoke condensate was tested in the Ames Salmonella typhimurium assay, and an increased mutation rate was reported. OSHA's inclusion of studies dealing with mainstream and sidestream smoke revealed the misconception pervading the Proposed Rule that ETS, mainstream, and sidestream smoke are equivalent.

Moreover, OSHA omitted at least one relevant study from this discussion in the Proposed Rule. In 1991, Bombick, et al., reported on a cellular smoke exposure technique using rat liver cells and the Ames Salmonella assay.¹³⁵ After a three-hour exposure using ETS at a concentration of 1.5 mg total particulate matter/m³, the authors report:

Using the neutral red cytotoxicity and Ames mutagenesis assays there were no differences observed in the ETS-exposed cells and their respective room air controls, indicating that ETS was **biologically inactive as tested.**
(emphasis added)

Studies have reported that various constituents and extracts of ETS collected from indoor air are capable of inducing mutations in the Ames assay.¹⁴³⁻¹⁴⁹ However, the significance of such reported findings has not been established. Virtually all air samples, whether in the presence or absence of smoking, can be shown to be mutagenic in various bioassays. Indeed, many substances, including foods and other "natural" materials, have been shown to exhibit mutagenic and/or carcinogenic properties.¹⁴⁸

Of relevance, Sonnenfeld and Wilson report that sidestream smoke exhibits reduced activity as it ages and becomes diluted, that is, as it becomes ETS.¹⁴⁹ These authors report on an experiment in which cultured mouse fibroblast-like cells were exposed to mainstream or sidestream smoke of various ages. In this report, cytotoxicity (cell mortality) is used as a measurement of DNA damage sufficient to cause cell death. The authors write:

Aging of SS smoke resulted in a rapid decline in the mortality generated by the smoke. As calculated from the linear regression curve, an increase in age of SS smoke of 30 [seconds] after generation would have resulted in a **total** loss of cytotoxic effects. (emphasis added)

Another area of relevant research comprises those studies that have compared the mutagenicity of body fluids of nonsmokers exposed to ETS and nonsmokers not exposed to ETS. Several of these

studies report no significant difference in mutagenic activity.^{129,130,134,136,150,151}

For instance, in a study by a team of German researchers, ten nonsmokers were exposed to ETS, generated by human smokers, for eight hours under two exposure conditions.¹³⁸ The two experiments were characterized by CO levels of 10 ppm and 25 ppm, respectively; according to the authors, both exposure regimes represent higher exposures than "real-life" situations. Elsewhere, they describe Experiment 2 as "far from being realistic,"¹³⁴ and bearing "no relation to a real-life situation."¹³⁶ In addition, the authors controlled for the effect of mutagens from the diet by keeping their subjects on a diet low in polycyclic aromatic hydrocarbons. Urine samples from both smokers and nonsmokers were tested in the Ames Salmonella assay. The authors report:¹³⁰

All urine extracts of ETS exposed non-smokers were found to be negative in the mutagenicity test when applying the [criterion] of Ames (doubling of spontaneous mutation rate).

Thus, even at exposure levels higher than would be expected on average, **no** increase in mutagenicity could be measured. These data do not support claims that ETS exposure is associated with an increase in mutagenic activity; moreover, because the samples come from exposed humans, the influence of physiological processes following exposure is indirectly taken into account.

Citing the high variability of measures of urinary mutagenicity and questions about the relevance of increased urinary mutagenicity to cancer risk, the authors write:¹³⁶

The data suggest that nonsmokers in real-life situations take up very low doses of ETS constituents, and detoxification of the genotoxic substances inhaled is effective.

And:¹³⁷

Whether ETS exposure can lead to an elevated urinary mutagenicity is a matter of controversy. In most investigations no significant increase has been observed. . . .

. . . The results of our investigations, as well as those of other authors, suggest that urinary mutagenicity, which would be a potential marker for ETS particle exposure, remains unchanged after ETS exposure.

The few studies reporting statistically significant increases in urinary mutagenicity among individuals exposed to ETS do not employ realistic levels of exposure to ETS, nor do they control adequately for the presence of mutagens in the diet of the study subjects.¹⁵²⁻¹⁵⁴ For instance, in the Bos, et al., study, the exposure condition consisted of the smoking of 157 cigarettes over six hours in a room with "poor ventilation."¹⁵² The relevance of such an exposure to "real-life" conditions is certainly

questionable. With respect to diet, Bartsch, et al., acknowledge, concerning their study, that¹⁵⁴

Urinary mutagenicity is influenced also by dietary habits; although we collected information on diet, the dimension of the study (particularly as far as passive smokers are concerned) does not allow adequate statistical treatment of this potential confounding factor.

Other related studies have examined levels of various DNA changes in nonsmokers exposed to ETS.^{131,138,151,155-158} Based on the data presented in these studies, nonsmokers exposed to ETS do not appear to exhibit increased DNA adduct formation, nor do studies report increased levels of chromosomal changes in cells of nonsmokers exposed to ETS. Discussion of these studies follows.

Collman, et al., collected data from 16 nonsmokers, 15 "passive smokers" (currently living with one or more smokers), and 13 current smokers, all women.¹⁵⁶ Sister-chromatid exchange (SCE) frequencies in lymphocytes (a type of white blood cell) were compared with and without coincubation with a chemical that enhanced the frequency of SCEs. Based on both assays, the authors report that "the frequency of SCEs in persons passively exposed to smoke was not higher than in nonsmokers."

In a report by Husgafvel-Pursiainen, peripheral blood lymphocytes were examined for SCE frequency, a sensitive test.¹⁵⁷ This test uses cells **from the exposed individual**, rather than another organism, and also considers repair mechanisms, thus being a better representation of actual events at the cellular level. Study groups consisted of 12 smoking waiters and waitresses, 20 nonsmoking waiters and waitresses who were occupationally exposed to ETS, and 14 nonexposed office workers. The author reports that "[t]he mean SCE level in exposed non-smokers did not differ from that observed in the non-exposed group." Although no ETS measurements from the restaurants were reported, the author characterizes them as "heavily polluted," and the exposure as "long-term." This study, which reports data from persons exposed in a "real-life" situation, does not support claims of the genotoxicity of ETS.

Chromosomal aberrations (CAs) and SCEs were examined in peripheral blood lymphocytes from nine smoking waiters, 16 nonsmoking waiters exposed to ETS at work, and seven reportedly nonexposed nonsmokers by Sorsa, et al.¹⁵⁵ The authors report that "[n]o significant differences were seen between the groups or subgroups in the 2 parameters." Thus, no "genotoxic" effects could be detected in persons exposed to ETS at "real-world" occupational exposures.

Holz, et al., report that DNA adduct levels were compared in monocytes (a type of white blood cell) of smokers and "heavily exposed passive smokers," who had been exposed in a chamber.¹³¹ DNA adducts above background were reported in smokers; they disappeared in less than 40 hours. The authors report **no** above-background adduct levels in study subjects exposed to ETS.

In a study by Gorgels, et al., 50 self-reported ETS-exposed men ("passive smokers"; average 72.8 hours exposure per week) were compared with 56 self-reported low ETS-exposed men (average 5.1 hours per week).¹⁵⁸ SCEs in cultured lymphocytes were examined; the authors report that "[n]o difference was observed between low exposed non-smokers and the passive smokers." They conclude:

Our results are in accordance with previous smaller studies in less homogeneous populations of non-smokers. These studies also failed to demonstrate even a tendency for an association between passive smoking and SCE levels.

Five male smokers, five male nonsmokers, and five male nonexposed nonsmokers were compared in Holz and colleagues' 1993 paper.¹³⁸ The endpoint examined was DNA single-strand breaks (SSBs), "considered to be an important parameter of genotoxic stress," in lymphocytes. The authors write:

All probands revealed measurable and varying SSB levels. Since DNA is an unstable molecule and estimated endogenous damage exceeds 100000 affected base pairs per cell per day, we assume that SSB base levels reflect unrepaired lesions. Active smoking caused an increase in SSBs in peripheral blood lymphocytes. This effect could not be found in passive smokers.

ETS exposure in this study consisted of five smokers each smoking 24 cigarettes in eight hours in an exposure chamber. This study provides no support for claimed genotoxic effects of ETS, even at a high exposure level.

This review of data from studies in which genotoxicity was assessed in persons actually exposed to ETS thus provides little, if any, support for the contention that ETS is genotoxic at levels encountered in workplaces and other indoor environments. As Doolittle stated in his submission to U.S. OSHA:¹⁵⁹

The hypothesis that ETS causes lung cancer is not supported by any of the available genotoxicity data. There is no evidence that ETS at or near ambient levels of exposure produces genotoxicity. The available published evidence comes to the opposite conclusion, namely ETS is not genotoxic.

G. Conclusion

In conclusion, the U.S. EPA Risk Assessment on ETS is not the definitive document portrayed by Cal/EPA. A large number of

unresolved questions and uncertainties remain. Moreover, more recent data on exposure, animal inhalation studies and genotoxicity do not conclusively support U.S. EPA's conclusions, contrary to the statements made by Cal/EPA.

A large number of the submissions and articles critical of U.S. EPA's risk assessment are now part of the U.S. OSHA rulemaking record. U.S. OSHA also relied heavily on the U.S. EPA Risk Assessment, and commenters have called upon OSHA to critically evaluate its conclusions.

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**SECTION IV: CAL/EPA'S TREATMENT OF THE EPIDEMIOLOGIC DATA
OVERLOOKS A NUMBER OF IMPORTANT ISSUES**

The following discussion will address a number of problems with Cal/EPA's treatment of the epidemiologic literature on ETS exposures and lung cancer in the Excerpt. A selection of relevant references is submitted as Appendix II.

A. Cal/EPA Fails to Acknowledge the Magnitude of the Limitations of Weak Association Epidemiology

Members of the scientific community have criticized the epidemiologic studies on ETS exposure and lung cancer for failing to consider certain factors, namely bias and confounding, that could affect the validity of the studies' risk estimates. The impact of such factors is particularly important in studies that, like these studies, report risk estimates that are "weak."^{1,2} A weak association is represented by a risk estimate of less than 2.0 or perhaps even less than 3.0.³⁻⁶ As Wynder notes:³

[E]pidemiology has problems when the associations are of a low order of magnitude. In such instances, findings in the literature are, in general, inconsistent. . . .

When risks are small, and especially when effects occur many years after their causes, detecting them, estimating their magnitude, and assessing their importance for the community in light of other relevant factors pose problems of study design, data collection, analysis, and interpretation which can be exceedingly difficult. (p. 139)

Specifically, Gori (1995) comments about the ETS epidemiology:⁷

The weak data on ETS are compatible with either a slight increase or a decrease of risk, but are impotent to certify either conclusion on a scientific basis. In the unlikely hypothesis that ETS were a risk, it would have to be small beyond detection because of the **inevitable and excessive noise to signal ratios of its epidemiology.** (p. 20) (emphasis added)

1. **Cal/EPA's Discussion of the Potential Impact of Confounders in the Spousal Smoking Studies Is Incomplete**

In the Excerpt, Cal/EPA essentially dismisses the possibility that confounding factors could contribute to the increased lung cancer risk reported in some of the spousal smoking studies. (p. 22) Cal/EPA cites only a few studies other than some of the spousal smoking studies themselves, in a review that is clearly incomplete and selective.

The use of spousal smoking status as a proxy for ETS exposure introduces substantial uncertainties into estimates of risk. Spousal smoking (marriage to a smoker) does not measure ETS exposure alone, but rather encompasses numerous variables that may be related to lung cancer risk.⁸⁻¹² These other variables, or

confounding factors, are factors associated with the exposure being studied (here, spousal smoking status) **and** with an increased risk of the outcome under consideration (in these studies, lung cancer). Confounders may seriously affect estimations of risk purportedly due to ETS exposure.

As noted in a recent review:¹³

Because the relative risks or odds ratios for human diseases reported to be associated with ETS exposure are typically no larger than the risks for confounding lifestyle factors, epidemiological studies of the association between ETS exposure and chronic disease should be designed to maximize data quality and statistical power.

The ETS and chronic disease epidemiology studies conducted to date have not adequately controlled for all of the known confounding variables.

Few of the spousal smoking studies upon which Cal/EPA relies have taken even some of the many potential confounding factors into account. Therefore, the possible impact of confounders on risk estimates attributed to ETS is of paramount importance. Since actual ETS exposures are not measured in these studies, where reported spousal smoking status is used as a surrogate for ETS exposure, the risk estimates claimed for ETS are instead risk estimates for "marriage to a smoker." A number of

potential lung cancer risk factors are associated with household smoking status.^{14,15}

One of the most important potential confounders of the claimed ETS-lung cancer relationship is dietary differences between "smoking" and "nonsmoking" households. [A "smoking" household is one in which at least one person smokes; a "nonsmoking" household contains no smokers.] In epidemiologic studies, when a nonsmoking case or control reports marriage to a smoker, this would correspond to living in a smoking household.

Diet appears to be a true confounder of the spousal smoking-lung cancer relationship.^{16,17} Diet satisfies the first necessary condition for being a confounder by being associated with lung cancer risk, as has been shown in a number of studies.^{18,19} Second, dietary differences are associated with household smoking status; that is, the diets of nonsmokers living with smokers differ from the diets of nonsmokers living with nonsmokers.

Data show that smokers' diets are generally different from nonsmokers' diets.²⁰⁻²⁸ In particular, the data, already suggestive of differences in diet according to household smoking status, have been dramatically strengthened by the addition of several recent studies.^{8,29-32} Taken as a whole, the data strongly support an association between household smoking status and diet.

Since diet is associated with lung cancer risk, it could well be a confounder in the spousal smoking studies which have failed to adequately account for diet in their analyses. The magnitude of the risks reported for some dietary exposures suggests that if only a few cases in a study had such exposures, it could have a significant impact on the risk estimates attributed to ETS exposure.

The following are brief comments on some of the recent literature that illustrates the correlation between diet and other lifestyle factors and household smoking status.

- A 1992 British study examined the consumption of fried foods, fats, fruits, vegetables, and sweets in smokers, nonsmokers, and exsmokers.²² The authors reported that nonsmokers who live in smoking households "have a diet more like smokers," and that "diet could be an important confound in epidemiological studies of ETS." The authors also noted:

Our analysis showed that non-smokers in smoking households ate fried food more often, more chips [french fries], less fruit in winter, more butter and less margarine high in polyunsaturates than non-smokers in non-smoking households. As we have pointed out, these habits are thought to increase the probability of cancer.

These results suggest that it is wise to show caution when interpreting the disease patterns

of non-smokers in smoking households. Studies to date have failed to take into account the effect that differences in dietary and lifestyle behaviour between 'smoking' households and 'non-smoking' households may have on the incidence of cancer or heart disease.

In a 1993 paper, the same authors reported that, in addition to having higher intakes of saturated fats, never smokers living in smoking households consumed fats more often, drank more alcohol, and ate fewer root vegetables and cereal than did never smokers living in nonsmoking households.²³

- Thornton, et al. (1994), examined 33 lifestyle factors in a survey of 9,003 British adults.⁸ They report:

[L]ike current smokers, passive smokers tended to be less educated; of lower social class; work in 'risky' occupations; drink more alcohol; do nothing to keep healthy; take longer before their first meal of the day; eat more fried foods and bread; eat less cereal, fruits, salads and low fat/polyunsaturated spread; drink more tea (but not more coffee); use more sugar in tea and coffee; not cut down on fatty foods; and be more neurotic and extrovert.

Thornton, et al., summarize their study as follows: "It has not perhaps been documented clearly before that smokers and non-smokers differ in so many lifestyle characteristics and that these are **nearly always in the direction of predicting a**

higher risk of disease." (emphasis added) The authors also point out the importance of considering these factors, particularly when investigating weak associations in epidemiology, such as those claimed for ETS.

- Matanoski, et al. (1995), report on analyses of data from the NHANES I study, comparing nutritional and behavioral characteristics between nonsmoking women whose husbands smoked and nonsmoking women whose husbands were nonsmokers.²⁹ Nonsmoking women with smoking spouses were statistically significantly more likely to have an urban residence, to consume beef and the skin on poultry (both suggestive of increased fat intake), to drink alcohol, and to consume less of certain vitamins and other nutrients than did nonsmoking women married to nonsmokers. The authors summarize the importance of their findings as follows:

[E]xposure to household tobacco smoke may not represent just a single exposure but a complex of factors, many of which, such as low vitamin intake and high alcohol intake, have been shown to influence the risk of cancer.

While the Matanoski, et al., study is referenced in the Cal/EPA Excerpt, the treatment is superficial and does not give adequate attention to the importance of this study's findings.

- Emmons and colleagues (1995) also compared the diets of persons living with smokers and living with nonsmokers.³⁰ Their data further support the differences between smoking and nonsmoking households: persons living with smokers had less healthy diets overall, consuming more fat and less fiber, fewer fruits and vegetables, and fewer micronutrients than did nonsmokers who lived with nonsmokers. All the reported differences were statistically significant.

The importance of the potential impact of dietary confounding on reported estimates of lung cancer risk from the spousal smoking studies was described by Layard in a submission to U.S. OSHA's rulemaking record.¹⁰

Dietary confounding alone could easily be large enough to explain the summary U.S. spousal smoking-lung cancer relative risk of 1.09 from [Layard's] meta-analysis of female studies.

Other data suggest the importance of numerous factors associated with cancer risk that may affect the reported results of the spousal smoking studies. Few, if any, of these risk factors have been considered in the epidemiologic studies on lung cancer, nor has U.S. EPA or Cal/EPA convincingly shown that these other confounders or independent risk factors for lung cancer may be discounted. These potential confounders or risk factors would not

have to apply to all the spousal smoking studies; if they influenced only a few of the studies, U.S. EPA's and Cal/EPA's position would be seriously affected.

For instance, recent publications have reported the following lung cancer risk factors: body weight,³³ history of radiation therapy,³⁴ occupation,³⁵ previous lung disease³⁶ and family history of lung cancer.³⁷ Moreover, in some cases, the risks reported for these other factors are far in excess of the risk estimates suggested for spousal smoking.³⁴

As pointed out in the Report of the Independent Working Group to the Health Care Committee of the Australian National Health & Medical Research Council (NH&MRC), while it is difficult for epidemiologic methods to detect very low relative risks, it is conceivable that factors having low relative risks could still impact the outcome of a study.³⁸ The Report notes:

[T]hree factors, each having a relative risk of 1.1, if present together would induce (assuming no interaction positively or negatively between them) an observed relative risk of 1.33. It is impossible that confounding risk factors of this magnitude would be detected in the studies we have examined. Hence although these risk factors would not be detected, they could, in aggregate, produce a relative risk at least as large as that observed for exposure to ETS.

Similarly, a 1992 review also addressed the possibility that confounding factors may have a combined effect on estimations of lung cancer risk:²⁶

In the absence of calculations of lung cancer risk when multiple factors apply, one can only speculate on the combined effect on an individual who, for example, might have a family history of lung cancer (RR = 2-4), lived in an urban area (RR = 1.2-2.8), worked in an occupation associated with elevated lung cancer risk (RR = 2 or more), was among the physically less active groups of the population (RR = 2) and, if a female, had the risk associated with a short menstrual cycle (RR = 2.2).

Until epidemiologic studies fully account for the possible impact of confounders and independent risk factors on estimates of lung cancer risk for spousal smoking, those risk estimates must be viewed with caution and carefully interpreted. **There is no indication in the Excerpt that Cal/EPA has done this.** As Gio Batta Gori, Sc.D., writes in a comment in the U.S. OSHA public record:¹¹

[A]ttributions of epidemiologic risk to ETS cannot be rationally sustained unless confounders and biases have been convincingly controlled, and adjustments have been objectively justified.

2. Cal/EPA Has Inappropriately Dismissed the Potential Impact of Several Sources of Bias in the Spousal Smoking Studies

When interpreting the results of epidemiologic studies, the potential effects of biases must be considered. Bias refers to factors in the design, conduct, analysis, or interpretation of an epidemiologic study that erroneously lead to the appearance of a stronger or weaker association than in fact exists. Bias (and confounding) becomes particularly important when dealing with weak associations, i.e., risk estimates of 3.0 or less.^{1,3-6,8}

One important type of bias particular to the spousal smoking studies is smoking status misclassification bias.^{10,39} Smoking status misclassification occurs when smokers erroneously report themselves as nonsmokers in response to study questionnaires. None of the spousal smoking studies to date has been able to discount smoking status misclassification as a potential source of bias.⁴⁰

It has been implied that the Fontham, et al., study⁴¹ is superior to the other spousal smoking studies because it had accounted for smoking status misclassification. This is an incorrect portrayal. While Fontham and colleagues did the best they could to exclude **current** active smokers from among cases and controls, based on cotinine measurements, cotinine does not allow

the determination of **past** smoking status.¹² Moreover, Fontham, et al., measured cotinine in hospitalized cases. The vast majority of hospitals severely restrict smoking (in fact, accreditation requires that smoking be banned); moreover, many lung cancer patients who happen to be smokers stop smoking after diagnosis. Thus, the cotinine measurements in this study did not even give a good indication of present smoking status, let alone previous long-term smoking status. The smoking status misclassification rates portrayed by Fontham, et al., as accurate are, in reality, **not** representative of the true situation.⁴²

Smoking status misclassification could have a dramatic impact on the reported risk estimates in the Fontham, et al., study, and in other spousal smoking studies. For instance, a recent Congressional Research Service (CRS) Report on ETS calculated that, in the Fontham, et al.,⁴¹ and Brownson, et al.,⁴³ studies, smoking status misclassification rates of **less than 10 percent** alone would account for **all** the reported elevation in risk at the highest exposure levels, and that misclassification rates of **less than three percent** would mean that those risk estimates would not achieve statistical significance at the 95 percent level.⁴⁴ Such misclassification rates are certainly possible, according to a recent review of the literature on this subject.⁴²

In addition to smoking status misclassification, another potentially important source of bias in the epidemiologic studies on ETS is misclassification of disease status, i.e., diagnosis of lung cancer. In many of the spousal smoking studies, disease diagnosis is haphazard and incomplete. Even in some of the better-designed studies, the possibility that tumors appearing in the lung may have metastasized from other sites remains likely. None of the studies confirmed lung tumor diagnosis via autopsy.

In a recent study, Kaye, et al., reported that in "emotionally charged situations," misclassification of disease diagnosis could inflate cancer risk estimates by some 30 percent.⁴⁵ Self-reports of cancer were compared with medical diagnoses of cancer for two groups of people. One group lived in a community with a hazardous waste treatment facility (test population); the other (control population) did not. The risk estimate for malignant tumors for the test population **decreased by 31 percent** when the more precise medical diagnoses were used instead of self-reports of having had cancer. The authors conclude:

This study demonstrates the importance of verifying reported cases of disease, even a disease as well defined as cancer, in emotionally charged situations such as living in communities surrounding hazardous waste sites. If reported cases of cancer had not been verified, it would have incorrectly

appeared that community A had almost twice the rate of cancer as community B and that an association existed between living in community A and having cancer.

Given the increasing public attention paid to ETS, and the claims that ETS exposure is causally associated with lung cancer, ETS could be considered an "emotionally charged" issue. While this might not affect the earlier studies on ETS, it is certainly of potential importance for studies conducted in the last few years.

B. A Number of Unresolved Questions Exist Concerning the Fontham, et al., Study, Rendering Its Interpretation Less Clear than Cal/EPA Portrays

Cal/EPA offers essentially no critical comment on the Fontham, et al., (1994) spousal smoking study.⁴¹ In fact, Cal/EPA appears to favor this study, commenting that its reported results are "closest to" those reported by the U.S. EPA in its Risk Assessment on ETS. (p. 7, 19) Moreover, Cal/EPA claims that this study "successfully addressed" the many weaknesses inherent in the spousal smoking study design. (p. 27)

Despite the contentions to the contrary in the Cal/EPA Excerpt, the Fontham, et al., (1994) study is still subject to the same limitations as the other spousal smoking studies. A number of criticisms of the Fontham study have been submitted to the public record at U.S. OSHA.^{12,46,47} For instance, Sears and Steichen listed

nine major categories of problems with the Fontham, et al., study, which they characterized as "significant design and execution flaws."¹² These included:

[T]he study population has no male representation and is not even representative of the nonsmoking U.S. female population, under-representing rural subjects and massively over-representing minorities (especially Asians);

[T]he percentage of adenocarcinoma is unusually high, possibly a reflection of abnormal demographics in the study population;

[T]he phenomenon that urinary cotinine analyses fail to detect active smoking cases only, suggests that misclassification is more prevalent among the lung cancer cases than among the controls, leading to an inflated relative risk point estimate;

[T]he failure to promulgate the use of colon-cancer controls to account for recall bias results in over-estimation of risk;

[T]he use of frequency-only matching within age categories, combined with the high sensitivity of cancer incidence to age differences, likely introduces a bias resulting in inflated estimates of risk;

[T]he categorization of individuals by broad race groupings fails too account for important lifestyle differences, especially among the large Asian subset of this study;

[T]he inability of the standard linear logistic regression approach to fully account for strongly-coupled confounding variables . . . results in inaccurate estimation of risk;

[T]he non-independence of the spousal-, workplace- and social-exposure study subpopulations forces Fontham's workplace

relative risk estimate to include potential contributive effects (including confounding) from both spousal and social exposure.

[T]he absence of dose from the 'risk equation' necessitates reliance upon the recall of exposures that may have taken place decades earlier, often by a surrogate respondent.

Moreover, recent publications have raised additional questions about the Fontham, et al., study.^{36,48,49} Perhaps the most serious questions about the study are raised in a post-hearing brief submitted to the U.S. OSHA record that points out what appears to be a significant misinterpretation.⁴⁹ In that submission, William Butler focuses on the data in the Fontham, et al., study concerning women who reported both childhood and adult ETS exposure. Fontham, et al., originally presented risk estimates for adult ETS exposure regardless of childhood ETS exposure status.

Butler divided the study subjects into four categories: (i) neither childhood nor adult ETS exposure (the reference group); (ii) childhood but not adult exposure; (iii) adult but not childhood exposure; and (iv) both childhood and adult exposure. He calculated a statistically significantly **negative** lung cancer risk for women with childhood but not adult exposure (OR = 0.35, 95% CI 0.12-0.99), which, he proposes, is a result of some bias in study design or data collection. Butler states that "Fontham et al.'s

failure to mention this fact makes their analysis incomplete and their interpretation misleading."

Fontham, et al., included cases and controls from the group with the decreased risk in the comparison group for their analysis of adult lung cancer risk. According to Butler, the underlying bias became a "source of artificially inflated statistical estimates that incorrectly indicate a positive association between adult ETS exposure and lung cancer."

According to Butler, Fontham, et al., appeared to recognize that there was an "interaction" between childhood and adult exposure in the 1994 paper, but did not mention the bias identified in his reanalysis. He states that adjustment for the bias would be "expected to reduce" the risk estimates for spousal, household, workplace, and social ETS exposures. Butler then notes that Fontham, et al., have not provided sufficient data in their publications for these adjustments to be made, and suggests that the raw data from the study would be needed and should be released.

A number of other issues have been raised concerning this study. For instance, as noted earlier, despite the Fontham, et al., study's use of cotinine to assess current tobacco use, the authors themselves acknowledge that misclassification of ever smokers as **lifetime** never smokers is "problematic" because there is

"no biomarker of lifetime tobacco use."⁴¹ Moreover, only slightly more than half (54%) of cases had cotinine determinations, so that even **recent** active smoking was not excluded for nearly half of the cases.⁴¹

While the Cal/EPA Excerpt repeatedly stresses that the Fontham, et al., study is a **multicenter** case-control study, if the characteristics of the study population are examined, it is seen that the vast majority -- 81 percent and 86 percent, respectively -- of cases and controls come from two areas in California (Los Angeles and the San Francisco Bay area). Given this breakdown, the "multicenter" label is fairly misleading. Moreover, the authors provide no breakdown of the data by study center, and it is not possible to ascertain whether the reported risks were consistent across the centers. Heterogeneity in the data among study sites would argue against combining the data as was done by Fontham, et al.

Although Fontham, et al., state that "dietary cholesterol" was considered as a potential confounder, they do not provide sufficient information in the study publication describing this factor. Apparently, Fontham, et al., did not consider dietary saturated fat intake, recently reported by Alavanja, et al., to be associated with relative risks as high as 6.0 to 11.0.²¹ The risk estimate was highest for nonsmoking women with **adenocarcinoma**; over

75% of the cases in the Fontham, et al., study were adenocarcinomas. Alcohol consumption, another potential confounder, was also not mentioned. Recent studies have reported that smokers, and the persons living in their households, are likely to consume more fatty foods and more alcohol.^{8,29}

The "adjustment" of the reported risk estimates is difficult to interpret, as the adjustment takes into account both study design variables (e.g., subject age) and potential confounders. For instance, the workplace risk estimate in the final report of the Fontham, et al., study was 1.12 before "adjustment" for several factors.⁴¹ The "crude" risk estimate was not statistically significant. After adjustment, however, Fontham, et al., reported a statistically significant workplace risk estimate of 1.39. As was pointed out in submissions to the U.S. OSHA rulemaking record, the direction of the change was opposite what would be expected, and of a magnitude greater than many of the other adjustments in the paper.^{12,46} Fontham, et al., do not provide adequate discussion of this unexpected outcome of adjustment, e.g., they do not explain which factor(s) had the most impact.

The above discussion illustrates that there are many unresolved issues concerning the Fontham, et al., study. For the Cal/EPA Excerpt to accurately reflect the nature of the ETS

epidemiology, it should review the criticisms that have been raised concerning the Fontham, et al., study, and address them.

C. The Argument that a Number of the Spousal Smoking Studies Demonstrate a "Dose-Response" Relationship Is Scientifically Flawed and Cannot Be Used to Support Cal/EPA's Conclusions

In the Excerpt, Cal/EPA refers to reportedly increased risks in the "high exposure" subgroups from some of the spousal smoking studies and to claimed positive trends in risk estimates. (e.g., p. 19, p. 26) By so doing, Cal/EPA invokes the argument that these studies exhibit a "dose-response."

The "dose-response" argument is based on the claim that positive results of statistical tests for trend on epidemiologic data show that lung cancer risk increases with increasing reported exposure; that is, that those tests demonstrate that a "dose-response" has occurred. Those who adopt this argument further suggest that positive tests for trend satisfy the criterion for dose-response used in evaluating a causal relationship in epidemiology. As will be shown below, however, such claims about "dose-response" based on the epidemiologic studies do not withstand critical scientific scrutiny.

1. The ETS Epidemiologic Studies Contain No Actual Exposure Data; The Purported "Dose" Levels Cannot Be Assumed to Represent Dose at All

The ETS epidemiologic studies do not measure actual exposures to ETS in the home or workplace. Rather, the studies rely on imprecise estimates based on individual recall. Other recent studies clearly illustrate that questionnaire responses are **not** accurate representations of actual ETS exposures, and are subject to bias.⁵⁰⁻⁵² As Wynder and Hoffmann⁵³ note:

[I]n all ETS-lung cancer studies in 'never-smokers,' assessment of their lifetime exposure remains problematic as long as reliable biomarkers of uptake are lacking.

Perhaps the greatest uncertainty about the epidemiological data is due to the unreliable information obtained by questioning volunteers in regard to their smoking habits.

H. Daniel Roth, Ph.D., noted in his submission to U.S. OSHA: "The ETS exposure data in the overwhelming majority of studies are far too weak for drawing epidemiological conclusions."⁵⁴ If the data are weak in terms of ETS exposure as a whole, they are likely even more limited when dealing with reported "specifics," such as levels of perceived exposure (including the number of cigarettes smoked per day or the duration of a smoking history).

An examination of the ETS-lung cancer epidemiologic studies reveals that they use a variety of definitions to "quantify" spousal smoking. For instance, some use number of cigarettes smoked per day without consideration of duration, while others use duration regardless of amount smoked. In addition, the studies do not use the same intervals to categorize amount smoked, e.g., the "highest exposure" in one study may be >20 cigarettes per day, while in another study, it might be >40 cigarettes per day. Thus, the highest exposure category in the first would be an intermediate category in the second. As noted in the 1995 CRS Report:⁴⁴

One implication of the potential disparity between the different types of exposure measurements is that combining risk [estimates from] several studies at the highest exposure levels probably yields misleading results. (p. 31)

The authors of the CRS Report note elsewhere that U.S. EPA calculated just such an overall risk estimate for the highest exposure levels in the spousal smoking studies.

Given the limitations inherent in exposure estimates based on personal recall and recall by surrogate respondents, claims that the "exposure level" data in the ETS epidemiologic studies can be used to illustrate dose-response are based on a weak and inadequate foundation. Uncertainty exists with respect to both

the actual exposures encountered by study participants and the degree to which study subjects may be misclassified according to exposure. These limitations alone suggest that it would be prudent to exercise great caution in making interpretations about "dose-response" from these data.

2. Claims that the Spousal Smoking Studies Exhibit Dose-Response Relationships Are Most Commonly Based on the Results of Statistical Tests for Trend; Such Tests Are Clearly Not Tests for Dose-Response and Should Not Be Interpreted as Such

Epidemiologists frequently imply that the results of a statistical test for trend (e.g., the Mantel extension test) provide evidence of a dose-response relationship. According to Maclure and Greenland, this overstates the evidence for dose-response, particularly if "dose-response" is considered to be a monotonic relationship, that is, one in which risk increases with each increment of exposure.⁵⁵ In fact, Maclure and Greenland point out:

Tests for overall trend, such as the Mantel extension test, are **widely but erroneously** believed to be tests of the hypothesis that a monotonic dose-response relation exists -- that is, a relation in which risk continues to increase with each increment of exposure. (emphasis added)

Statistical assessments of "trend" are **not** equivalent to developing a dose-response relationship.³⁸

Maclure and Greenland point out several major statistical problems with applying the Mantel extension test to questions of dose-response.⁵⁵ First, they demonstrate mathematically that when there are few subjects in one of the exposure categories, the Mantel extension test is essentially algebraically identical to a test for overall association (the Mantel-Haenszel test). They explain the interpretation error that can arise from this misconception as follows:

Numerous articles can be found in which authors conclude that the extension test for trend in risk over a trichotomous exposure, **when there are few subjects in one of the exposure categories**, is telling something extra. In fact, it is little more than a restatement of the results of the Mantel-Haenszel test of the collapsed dichotomous table. (emphasis added)

That is, mathematical considerations dictate that, if the subdivisions ("exposure categories") include one or more with few subjects, the Mantel extension test will have a positive result in a study reporting an increased risk when only two categories are considered. This is frequently interpreted as evidence of dose-response, when it is essentially only an artifact of the mathematics involved. The issue is relevant because a number of

the spousal smoking studies have at least one category with less than 20 cases. Peter Lee has noted that dose-response relationships are frequently reported by those studies that report an association in the first place, an observation that would follow from the mathematical considerations described above.²⁰

Moreover, Maclure and Greenland also note another limitation of the Mantel extension test: that it "**assumes** a particular dose-response model as part of its justification."⁵⁵ (authors' emphasis) They continue:

[The Mantel extension test] is not a test of the appropriateness of that model: instead, it is a test for magnitude of trend given that the shape of the dose-response relation implied by the linear-logistic model is appropriate.

Similarly, J. Lee, et al., note that a number of possible dose-response models could be applied to the ETS-lung cancer data.³⁸ Noting the inconsistencies among the various studies in terms of the data reported, they point out that the analyst's choice of model to apply to the data can affect the conclusions:

Any conclusions about dose response relationships should not simply be a reflection about the type of dose response model chosen by the analyst. If a linear model is fitted to data which truly have a threshold then it is highly likely that the

straight line will be judged to provide an adequate fit. **However this does not prove that the dose response is linear and any interpretations based on extrapolation could be quite erroneous.** (emphasis added)

Another problem with the Mantel extension test is that it does not take incremental increases in risk at **each** exposure category into account.⁵⁵ The nature of the test is such that a single increase in risk at **one** exposure category could be sufficient to produce a statistically significant test for trend. Specifically, Maclure and Greenland state:

The heart[™] of the dose-response hypothesis is found in the words . . . 'continuously increasing risk.' Not only does the first small dose of exposure influence risk, but additional doses further increase risk relative to the effect of the previous dose. All dose increments are hypothesized to have effects, not just one of them. **Because the extension test can yield a small p value if only one dose increment had an effect, it does not test the hypothesis of interest.**⁵⁵ (emphasis added)

Another limitation in using a test for trend was noted by J. Lee, et al., who pointed out that inclusion of the unexposed, or reference, group in the test for trend can result in a statistically significant outcome, even though the only difference is between nonexposed and exposed.³⁸ That is, the different exposure levels (the point of a dose-response analysis) may

actually not be statistically significantly different from one another.

[A]ssessment of this [dose-response] relationship should be independent of the assessment of a possible overall association, and hence . . . the **unexposed** group should be excluded from the analysis. The rationale for this exclusion is that RRs greater than unity for exposures greater than zero may not differ from each other and yet the test for trend will be significant. In this case a trend may not be present in the RR for exposures above zero. (authors' emphasis)

Thus, inclusion of the unexposed group in a test for trend can lead to an artifactual conclusion of a statistically significant trend. Moreover, because there is no error term associated with the control value (i.e., the unexposed group), the control value will have an infinite statistical impact on the reported relationship.

In a 1995 paper, Tweedie and Mengersen evaluate "dose-response," taking into account "the possible confounding effect that inclusion of the unexposed group may have."⁵⁶ In agreement with J. Lee, et al., and with Maclure and Greenland, they note that "an observed dose-response relationship may be in fact simply evidence of overall association but not of increasing (or decreasing) risk with increasing dose."

Tweedie and Mengersen illustrate this using hypothetical data,⁵⁶ where all the risk estimates are equal at different dose levels, an exercise also carried out by Maclure and Greenland.⁵⁵ Tweedie and Mengersen also use actual data from one of the spousal smoking studies, where "we see a raised relative risk but no discernible increase of effect with increasing dose," to illustrate the same point. A "raised relative risk," as discussed later in this section, may well be due to the effects of bias and confounding; because of the nature of the test for trend, the spuriously elevated risk can lead to an improper claim of "dose-response."

Similarly, the authors of the CRS Report comment that, of the spousal smoking studies they reviewed,⁴⁴

All of the trend analyses include zero exposure. If the trend was linear down to zero exposure, then including that level in the trend analysis would yield the same results as when excluded. If there was a threshold effect, then a trend test which included the zero exposure level might show a trend even if an analysis which included only exposures above zero did not show such a trend. **In other words, a sharp rise at some exposure level above zero could incorrectly be interpreted as a dose response trend over all exposure levels.** (emphasis added) (p. 29)

So, if an elevated risk is reported (overall or for one exposure category), and if the nonexposed group is included in a

test for trend, the result of the test will be statistically significant, even though risk does not increase with each increment of "dose." Thus, the use of a test for trend does not reflect dose-response, but simply the fact that, mathematically, the test is being "driven" by some reportedly elevated risk.

3. Claims of Dose-Response in the ETS Lung Cancer Studies Reflect an Increased Risk at the Highest Exposure Levels; Such Risk Estimates Do Not Reflect ETS Exposure, But Rather the Influence of Other Factors Associated with Spousal Smoking

The preceding discussion illustrates that a single elevated risk estimate can drive a so-called dose-response assessment, when a test for trend is inappropriately used to reach a conclusion about dose-response. This explanation could certainly account for the "positive" trends reported in the ETS epidemiology. As Paul Switzer, Ph.D., a statistician at Stanford University, notes:⁵⁷

[W]ithout the highest spousal exposure group there would be very few individual studies with statistically significant effects or significant dose-response relationships.

Why, then, are the risk estimates elevated at the highest reported spousal exposure levels? The most likely explanation relates to the fact that the elevated risk estimates are the result

of unexplained bias and confounding. For instance, Springall writes in his submission to U.S. OSHA that data exist suggesting that spousal concordance (the tendency for smokers to marry smokers) increases with the amount smoked and therefore, "an enhanced tendency to a false dose-response exists in the presence of [smoking status] misclassification."¹⁴ As smoking status misclassification has been adequately excluded in **none** of the spousal smoking studies, it is certainly possible that misclassification may contribute to the claimed dose-response relationships reported.²⁰ As noted earlier, only very small rates of smoking status misclassification could account for the risk estimates reported at high exposures by Fontham, et al.,⁴¹ and Brownson, et al.,⁴³ demonstrating the clear potential for this form of bias to have an effect in this context.

Another source of bias, exposure misclassification, may also contribute to the reportedly higher risks at higher exposure levels. As the authors of the CRS Report note:⁴⁴

The more specific the question about exposure, the more precise the measure, **but the less accurate the recall**. That is, there is likely to be a very small error rate in reporting marriage to a smoker, but there could be a significant error in reporting actual amounts of exposure, such as numbers of cigarettes smoked by a spouse, particularly in the past. (emphasis added)

Recall bias is also likely to produce an artificial dose-response trend in the spousal smoking data. Layard points out that cases may be motivated to try to explain their disease, which could lead to more complete recall or exaggeration of high exposure levels by cases than by controls.⁵⁸ This differential would produce apparently elevated risk estimates at high exposures. Layard also notes that, under this scenario, one would expect to see a reduction in risk (i.e., a risk estimate below 1.0) at low reported exposures, just as is seen when the data from the U.S. case-control studies on women are combined in a meta-analysis.

As noted earlier, the spousal smoking studies are not measuring ETS exposures, but rather are essentially addressing risks associated with "marriage to a smoker." Spousal smoking status carries with it a number of associated lung cancer risk factors that may be associated with amount smoked.²⁰

As Layard writes:¹⁰

[M]any potential confounders of reported spousal smoking-lung cancer associations, as well as smoking-status misclassification bias, are correlated with spousal smoking in a dose-dependent fashion, and such correlations could account for apparent dose-response trends.

That is, if the effects of confounding factors and biases were indeed highest in the highest exposure group, the risk estimates for those groups would be expected to be larger. Thornton, et al., report data from their survey of risk factors in a British population that support such a relationship.⁸ The highest prevalence of many of the risk factors (dietary and behavioral) they investigated was found in smokers of 20 or more cigarettes per day. This study also showed that persons exposed to ETS had higher prevalences of risk factors than did non-exposed persons. Thus, these data are strongly suggestive of a relationship such as that described by Layard.¹⁰ As most studies of risk factor clustering have not focused on associations with amount smoked, this area requires further research. This explanation for the increased risks reported at higher spousal exposures nevertheless remains a viable one.

4. A More Rigorous Analysis of the Data Does Not Support the Dose-Response Claims that Have Been Made

In general, claims about dose-response are tenuously based on qualitative reviews of data from the spousal smoking studies. Moreover, they accept the equivalence between a positive test for trend and dose-response, which, as described herein, is inappropriate and misleading. The weakness of such claims is clearly demonstrated when Tweedie and Mengersen's more rigorous

analysis of the "dose-response" data, published in the peer-reviewed literature, is considered.⁵⁶

Tweedie and Mengersen's 1995 paper compares several techniques for assessing dose-response in epidemiologic studies. It includes both reanalyses of the dose-response data from the individual studies and a meta-analytic assessment. The authors comment that they "provide a more rigorous approach than the purely qualitative assessments which have often been used in the literature."⁵⁶

As an initial step, Tweedie and Mengersen recalculate tests for dose-response for all the spousal smoking studies reporting sufficient data, using three alternate methods. The first (the Armitage method) simply tests whether the reported risks at different categories are significantly different. The authors note that its "major benefit" is that no assumption about the pattern of dose-response is made. (Since the shape of the dose-response, if any, is unknown, using models that impose a shape will influence the outcomes of the test.) The other two methods fit models, one exponential and one linear, to the data, i.e., each entails a different assumption about the nature of the dose-response. For each of the three methods, the authors perform one test **including** the unexposed group, and one test **excluding** the unexposed group.

Tweedie and Mengersen report that when the unexposed group is **excluded**, for all three tests, only one study fits one of the models; **no other studies show a statistically significant "dose-response."** Conversely, depending on the model, when the unexposed group is included, anywhere from two to all of the studies are statistically significant (at least at the 10 percent level). Thus, these analyses illustrate the sensitivity of conclusions about dose-response to the method of analysis.

In addition to the individual-studies analysis, Tweedie and Mengersen analyze the data from the case-control studies using meta-analytic techniques. Again, three models are used, and the results differ by model and, particularly, by whether the unexposed group is included.

In conclusion, Tweedie and Mengersen note that "a simple assessment of point estimates -- without consideration of their accuracy as expressed by associated confidence intervals and without a more rigorous method of synthesising the results from individual studies -- can be quite misleading."

Overall, Tweedie and Mengersen conclude:⁵⁶

From the meta-analysis of studies of lung cancer and exposure to ETS in non-smoking females given here, our conclusion is that,

despite a significant observed relative risk associated with overall exposure, there is little increase in relative risk with increasing dose. One explanation that accounts for this somewhat unexpected situation, of an overall observed increased relative risk but little indication of positive dose response, is that some bias may be inflating all of the observed risks. (emphasis added)

5. Conclusion: Claims About a "Dose-Response" Relationship Derived from the ETS Epidemiologic Studies Are Unfounded and Should Not Be Relied Upon by Cal/EPA

The foregoing discussion clearly illustrates that the claims that data in some of the epidemiologic studies on spousal smoking and lung cancer support a dose-response relationship are based on a misinterpretation of the use and meaning of statistical tests for trends. Positive trend results can be explained due to spuriously elevated risk estimates arising from uncontrolled confounding and biases. Moreover, the results of more appropriate, more rigorous statistical analyses reveal inconsistencies among and within studies in terms of "dose-response." Because so many questions remain, "dose-response" claims should not be used by Cal/EPA to support its analysis of ETS.

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Attachment C to Comments on Chapter 7 – Carcinogenic Effects

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Attachment D to Comments on Chapter 7 – Carcinogenic Effects

New Literature Since OEHHA's January 1996 External Review Draft on Lung Cancer

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