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Sidestream cigarette smoke toxicity increases with aging and exposure duration

Suzaynn Schick, Stanton A Glantz

Objectives: To determine the effects of aging on the toxicity of sidestream tobacco smoke, the complex chemical mixture that enters the air from the lit end of burning cigarettes and constitutes the vast bulk of secondhand smoke.

Design: Statistical analysis of data from controlled experimental exposures of Sprague Dawley rats to fresh and aged (for more than 30 minutes) sidestream smoke for up to 90 days followed by histological sectioning of the respiratory epithelium. The data were obtained from a series of experiments conducted at Philip Morris’ formerly secret INBIFO (Institut für Biologische Forschung) laboratory in Germany.

Results: Using total particulate material as the measure of smoke exposure, aging sidestream cigarette smoke for at least 30 minutes increases its toxicity fourfold for 21 day exposures and doubles the toxicity for 90 day exposures, relative to fresh sidestream smoke.

Conclusions: These results help explain the relatively large biological effects of secondhand smoke compared to equivalent mass doses of mainstream smoke.

A bout one non-smoker dies from secondhand smoke exposure for every eight smokers who die from smoking, even though secondhand smoke doses (in terms of total mass inhaled) are substantially lower. In a previous analysis of unpublished sidestream cigarette smoke toxicity experiments done by Philip Morris, we showed that freshly-generated sidestream cigarette smoke is 3–4 times more toxic to laboratory animals than mainstream smoke (the smoke the smoker inhales). However, most secondhand smoke is not freshly generated. In typical indoor spaces secondhand smoke lingers for 1.5–2.0 hours. When sidestream smoke is released into the open air, it changes chemically and physically. A large percentage of sidestream smoke consists of oils and waxes that are emitted as small particles. These volatile and semi-volatile organic compounds evaporate as the smoke is diluted, forming gases and smaller particles. The vapours and small particles adsorb onto surfaces, then desorb over time, effectively increasing the exposure period.

Though these changes in secondhand smoke chemistry are known, there are few publications that compare the toxicity of freshly-generated and aged sidestream smoke. The tobacco industry has been concerned with these effects since the early 1980s. We identified research projects at several tobacco companies but limit our analysis to experiments done by Philip Morris at their formerly secret laboratory the Institut für Biologische Forschung (INBIFO) in Germany because of the consistency of methods and quality of data. Our analysis of these data show that the acute toxicity of sidestream smoke increases by a factor of 2–4 as it ages.

METHODS Tobacco industry documents
We found reports documenting Philip Morris’ in vivo experiments with sidestream cigarette smoke by searching the approximately 45 million pages of tobacco industry documents made public as a result of litigation against the tobacco companies. Between January and December of 2005, we searched the University of California, San Francisco (UCSF) Legacy Tobacco Documents Library (http://www.legacy.library.ucsf.edu), the UCSF British American Tobacco Documents Archive (http://bat.library.ucsf.edu/index.html), Tobacco Documents Online (http://tobaccodocuments.org), and Philip Morris documents (http://www.pmdocs.org), using standard strategies, starting with keywords “sidestream”, “aging”, and “lifetime exposure”. The initial searches yielded the identification numbers of projects and assays, which were then searched.

Sidestream inhalation studies at Philip Morris
Each of the experiments done at INBIFO had a unique identifying number, which we use. In 1989, after completing 35 biological assays of freshly-generated sidestream smoke, Philip Morris invented a sidestream smoke aging system. They piped hot sidestream smoke via a large cross-section duct into a 30 m³ room. Air in the room was circulated with a slowly rotating ceiling fan and a temperature of 26°C was maintained with two heat exchangers. Between 1984 and 1998 Philip Morris did five (3149, 3195, 3169, 3216, 3248) inhalation experiments at INBIFO to test the effects of aged sidestream smoke on rat respiratory epithelium (table 1). Experiments 3216 and 3248 were excluded from the analysis because data on individual animals were not available; study 3149 was excluded because it did not include complete assessment of histopathological damage to the vocal cords.

We included in our analyses experiments 3123, 3125 and 3127 (table 1) in which the rats were exposed to sidestream cigarette smoke that was piped directly from the smoking machine to the animals and was approximately 10 seconds old. We refer to this smoke as “fresh” sidestream. In experiments 3195 and 3169 the rats were exposed to sidestream cigarette smoke that had been held in a 30 m³ chamber with continuous air exchange rates of 0.75 or 2 air changes/hour before it was piped to the rats. We refer to these smokes as “aged” sidestream. (In their publications on aged...
sidestream smoke. \(^5\) Philip Morris referred to these smokes as "room aged sidestream" or RASS.1 Studies 3123, 3125, 3127 and 3169 used Kentucky Reference cigarette 2R1 and study 3195 used Kentucky Reference 1R4F. All five experiments included sham exposures where rats were placed in clean exposure chambers and exposed to clean, high exclusion particle arrestor (HEPA) filtered air as controls.

In experiment 3169 Philip Morris tested the effects of a 90 minute aging period with furnishings placed in the aging room (table 2). Adding the furnishings appears to have resulted in greater adsorption of TPM and nicotine onto surfaces in the aging chamber. In experiment 3195 they used a 30 minute aging and no furnishings. These differences are reflected in the ratios among CO and TPM and nicotine (table 3).

We combined data from experiments 3123, 3125 (21 day exposures) and 3195 (28 day exposures). Experiments 3127 and 3169 were 90 day exposures. The methods of exposure varied in the five experiments (Table 1). In whole-body exposure the animals were held in standard cages and the smoke was piped into the cages. In head-only exposure and nose-only exposure the animals were held in snug tubes, which were then mounted in holes in a smoke-filled duct so that only the head or nose of the animal projected into the smoke.

**Exposure calculations**

We normalised exposures either on the basis of concentration-hours of total particulate matter (TPM) measured at INBIFO as the mass of solids deposited on a glass fibre filter (Gelman #6004300) or on the basis of concentration-hours of carbon monoxide (CO) measured using non-dispersive infrared photometry.\(^{14}\) The glass fibre filter was rated by Gelman Company to retain 99.7% of particles \(\leq 300\) nm. Samples for all chemical determinations were taken from the breathing zone in the animal exposure chambers. Exposure times ranged from five hours a day, seven days a week to seven hours a day, seven days a week (table 1). To provide a common metric for exposure, we multiplied the TPM concentrations the animals were exposed to by the number of hours per day and number of days per week (TPM (mg/m\(^3\)) × hours/day × days/week) to obtain weekly exposure rates in TPM mg-h/m\(^3\)-week. CO ppm-h/week were calculated the same way.

**Histopathological scoring**

Fixation and sectioning protocols were consistent through the five experiments. The larynx was sectioned transversely, according to Lewis\(^24\). The trachea was sectioned longitudinally at the tracheal bifurcation. The nose was sectioned transversely according to Young\(^25\) to obtain tissue slices immediately posterior to the upper incisor teeth (nasal 1) and at the incisive papillae (nasal 2).

All tissue slices were embedded in Paraplast, cut at 5–6 \(\mu\)m, and stained with haematoxylin and eosin. In addition, some sections were stained with alcian blue/periodic acid Schiff's reagent to identify goblet cells. All slides were read by a veterinary pathologist at INBIFO.

To assess the effects of smoke inhalation, INBIFO scientists fixed and sectioned the upper respiratory tract tissues and examined them for pathological changes. Figure 1 shows the section locations, cell types and pathological changes that the INBIFO pathologists evaluated in at least one experiment; we based our analysis on those scored in all the INBIFO experiments we examined. All pathological changes were scored according to a subjective severity scale from 0 to 5: 0 = no visible lesion, 1 = slight lesion, 2 = slight to moderate lesion, 3 = moderate lesion, 4 = moderate to pronounced lesion, and 5 = pronounced lesion. The exact definitions of
slight, moderate, and pronounced lesions are not available, but the same veterinary pathologist oversaw all seven experiments so the criteria can be assumed to be consistent. We summed the histopathology scores from nasal section one through the trachea to create a total respiratory epithelium histopathology score for each animal. Thus, each animal had a total score from 0 (no lesions) to a maximum of 85 (17 locations × 5 (maximum score)). We excluded data from two obvious outliers: animal 007 in 3123, and animal 505 in 3169, and from any animal with scores missing for any section, cell type or pathology.

Statistical analysis
We tested the effects of TPM mg/m3-h-week or ppm/h-week, together with exposure duration and aging using a multiple regression implementation of an analysis of covariance on total respiratory epithelium histopathology score. We constructed this analysis by defining dummy variables using reference coding with the 21/28 day exposure to the fresh smoke condition as the reference condition: Aged = 0 if reference coding with the 21/28 day exposure to the fresh smoke was aged (30 to 90 minutes, as a continuous variable) and dummy variables for the presence of furnishings in the aging chamber, and exposure method. We also did a separate analysis including how long the smoke was aged, the presence of furnishings in the aging chamber, and exposure method. Calculations were done using SigmaStat version 3.1.1.

RESULTS
Using TPM mg-h/m3-week as the measure of smoke exposure and aging (versus fresh) smoke and exposure duration (21/28 y 90 days) as variables, demonstrates that aging sidestream cigarette smoke increases the slope of the respiratory histopathology dose–response relationship by a factor of 4.0 for 21 day exposures ([0.00386+0.01160]/0.00386) and by a factor of 2.1 for 90 day exposures ([0.00386+0.00751+0.0116+0.00129]/[0.00386+0.00751]) (table 4, fig 1). Increasing exposure duration from 21/28 days to 90 days increases the damage to the respiratory epithelium by a factor of 3.0 for fresh smoke ([0.00386+0.00751]/[0.00386]) and a factor of 1.6 for aged smoke ([0.00386+0.01160+0.00751]/[0.00386+0.01160]). The effects of aging the smoke and exposure duration on the slope are additive; the interaction term is not significant (table 4, fig 1).

Using CO ppm-h/week as the measure of smoke exposure demonstrates that aging sidestream cigarette smoke increases the slope of the respiratory histopathology dose–response curve by a factor of 3.8 for 21/28 day exposures, but decreases it by a factor of 0.68 for 90 day exposures (table 4, fig 2). Longer exposures increase the slope of the dose–response curve for damage to the respiratory epithelium by a factor of 2.8 for fresh smoke but for aged smoke the damage after 90 days exposure is 0.5 times that of the 21/28 day exposure. There is a significant interaction between aging and duration, with the effects being less than additive. We also tested the inclusion of exposure method (head only, nose only or whole body), the length of time the smoke was aged, and the presence of furnishings or carpet in the aging chamber in the model, allowing for effects on both the intercept and the slope in the full regression model. Including the exposure method produced a significant improvement in the fit, but the effect was small, with the R² increasing from only 0.874 to 0.881 for TPM and from 0.875 to 0.881 for CO. Including the length of time that the smoke was aged (30 to 90 minutes, as a continuous variable) and dummy variables for the presence of furnishings in the aging chamber did not significantly improve the fit. Because there are a limited number of experiments under each combination of conditions, these results need to be interpreted with caution.

### Table 2. Experimental variables in secondhand smoke generation

<table>
<thead>
<tr>
<th>INBIFO standard for fresh sidestream smoke (m³)</th>
<th>Mean smoke residence time, aging chamber (minutes)</th>
<th>Aging chamber surface area (m²)*</th>
<th>Aging chamber wall surface</th>
<th>Furnishings</th>
<th>Mean smoke residence time, exposure chamber (minutes)</th>
<th>Exposure chamber surface area (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3169</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&lt;1</td>
<td>0.06</td>
</tr>
<tr>
<td>3195</td>
<td>90</td>
<td>118</td>
<td>Painted wallpaper</td>
<td>Wool carpet, wool curtain, wooden bookshelf, books, magazines</td>
<td>&lt;1</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Includes surface area of heat exchanger.

INBIFO, Institut für Biologische Forschung.

### Table 3. Effects of aging on chemical composition of sidestream

<table>
<thead>
<tr>
<th></th>
<th>Fresh (3169)</th>
<th>Aged (3169)</th>
<th>Aged (3195)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPM (mg/m³)</td>
<td>1.5</td>
<td>8.7</td>
<td>1.2</td>
</tr>
<tr>
<td>CO ppm</td>
<td>5.5</td>
<td>27.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Nicotine (µg/m³)</td>
<td>410</td>
<td>2210</td>
<td>240</td>
</tr>
<tr>
<td>TPW/CO</td>
<td>0.28</td>
<td>0.31</td>
<td>0.098</td>
</tr>
<tr>
<td>TPW/nicotine</td>
<td>0.0037</td>
<td>0.0039</td>
<td>0.005</td>
</tr>
<tr>
<td>CO/nicotine</td>
<td>0.013</td>
<td>0.013</td>
<td>0.051</td>
</tr>
<tr>
<td>Particle mass median aerodynamic diameter (µm)</td>
<td>0.37</td>
<td>0.35</td>
<td>0.42</td>
</tr>
</tbody>
</table>

CO, carbon monoxide; TPM, total particulate matter.
they looked at a different measure of toxicity. Sonnenfeld and Wilson tested the toxicity of whole sidestream smoke on monolayer cultures of L-929 cells by measuring cell death. They found that toxicity decreased rapidly in the first 30 seconds of aging and predicted that the smoke would lose all toxicity to the cells after seven minutes aging. The differences in assessed toxicity between our analyses and their experiments may be because the INBIFO studies did not examine changes in toxicity between our analyses and their experiments may be because the INBIFO studies did not examine changes in toxicity.

DISCUSSION

We found only two previous publications on the effects of aging on sidestream toxicity. Sonnenfeld and Wilson tested the toxicity of whole sidestream smoke on monolayer cultures of L-929 cells by measuring cell death. They found that toxicity decreased rapidly in the first 30 seconds of aging and predicted that the smoke would lose all toxicity to the cells after seven minutes aging. The differences in assessed toxicity between our analyses and their experiments may be because the INBIFO studies did not examine changes in smoke toxicity during the first 30 seconds of aging or because they looked at a different measure of toxicity.

Philip Morris published the results of experiment 3169 in 1998. They compared the effects of aging on the toxicity of whole sidestream smoke on monolayer cultures of L-929 cells by measuring cell death. They found that toxicity decreased rapidly in the first 30 seconds of aging and predicted that the smoke would lose all toxicity to the cells after seven minutes aging. The differences in assessed toxicity between our analyses and their experiments may be because the INBIFO studies did not examine changes in toxicity between our analyses and their experiments may be because the INBIFO studies did not examine changes in toxicity.

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**Table 4** Effects of aging on sidestream smoke toxicity (linear regression results)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>SE</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPM</td>
<td>0.200</td>
<td>0.151</td>
<td>0.185</td>
</tr>
<tr>
<td>Exposure</td>
<td>0.00386</td>
<td>0.000238</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exposure × aged</td>
<td>0.01160</td>
<td>0.000566</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exposure × duration</td>
<td>0.00751</td>
<td>0.000975</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exposure × aged × duration</td>
<td>0.00129</td>
<td>0.00344</td>
<td>0.708</td>
</tr>
<tr>
<td>CO</td>
<td>0.0380</td>
<td>0.154</td>
<td>0.805</td>
</tr>
<tr>
<td>Exposure</td>
<td>0.00129</td>
<td>0.000783</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exposure × aged</td>
<td>0.000252</td>
<td>0.000207</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exposure × duration</td>
<td>0.000228</td>
<td>0.000293</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exposure × aged × duration</td>
<td>-0.00477</td>
<td>0.000424</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The regression equation is:

\[
	ext{Score} = \beta_0 + \beta_{\text{Exposure}} \times \text{Exposure} + \beta_{\text{Exposure}} \times \text{Aged} \times \text{Exposure} + \beta_{\text{Exposure}} \times \text{Duration} \times \text{Exposure} + \beta_{\text{Exposure}} \times \text{Aged} \times \text{Duration} \times \text{Exposure} + \beta_{\text{Exposure}} \times \text{Aged} \times \text{Duration} \times \text{Exposure} \times \text{Aged} \times \text{Duration}.
\]

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The experiments we analysed do not reveal the mechanism for the increased toxicity of aged sidestream smoke compared on the basis of equal exposures to TPM. Whatever the cause, the change in toxicity appears to happen in the first 30 minutes, because smoke aged 90 minutes was not significantly more toxic than smoke aged 30 minutes. It may be that because airborne TPM is lost to adsorption over time, equalising exposures on the basis of TPM increases the proportion of toxic gaseous components of sidestream smoke. Earlier Philip Morris experiments showed that the gas/vapour fraction of fresh sidestream smoke was more toxic to the respiratory epithelium than the particulate fraction. It is also possible that chemical reactions occur in secondhand smoke over time, producing compounds with higher toxicity.

The finding that aging increases toxicity 2.8-fold in 21/28-day exposures but decreases it by 0.5 for 90 day exposure when CO was used as the exposure metric (fig 2C,D) is remarkable because CO is a marker for the components of sidestream that are not lost to adsorption. We do not know why the relative toxicities of aged and fresh smokes were different for 21/28 and 90 day exposures when exposure was measured with CO. Regressions using data from experiment 3149, which used the Kentucky 2R1 cigarette, give similar results (data not shown), so potential differences between toxicity of Kentucky 2R1 and Kentucky 1R4F sidestream are not the cause.

Limitations
These experiments were conducted on Sprague Dawley rats, a well-established animal model. The criteria for the histopathological damage scoring system are not known. Because the same person supervised all of the scoring, we have assumed that the criteria were constant over time and consistent between studies. If the criteria were not constant and consistent, the pooled comparison we made may not be valid.

Conclusion
Philip Morris’ toxicological experiments using rats may help explain the epidemiological observation that approximately one non-smoker dies due to secondhand smoke exposure for every eight smokers who die of smoking. If aged sidestream smoke is approximately three times more toxic than fresh sidestream, and fresh sidestream smoke is approximately four times more toxic than mainstream smoke, then aged sidestream smoke is approximately 12 times more toxic than mainstream smoke. While the mass of smoke that non-smokers inhale is far lower than that which smokers inhale, the smoke itself appears to be substantially more toxic.

ACKNOWLEDGEMENTS
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What this paper adds
Secondhand smoke, the smoke that enters the air when people are smoking tobacco products, kills about one non-smoker for every eight smokers that active smoking kills. Fresh sidestream smoke (the smoke that comes from the lit end of the cigarette when it is smouldering) is 3–4 times as toxic to laboratory animals as the fresh mainstream smoke the smoker inhales. When sidestream smoke ages after it enters the air, it becomes 2–4 times more toxic to laboratory animals than fresh sidestream smoke. This result helps explain the relatively large biological effects of secondhand smoke compared to equivalent mass doses of mainstream smoke.
or preparation of the manuscript. Both authors were involved in all aspects of the research and serve as guarantors for the paper.

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Competing interests: All authors declare that the answer to the questions on your competing interest form (http://bmj.com/cgi/content/full/317/7154/291/DC1) are all No and therefore have nothing to declare.

Ethical approval: Not required.

REFERENCES


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