

## RESEARCH PAPER

## Philip Morris toxicological experiments with fresh sidestream smoke: more toxic than mainstream smoke

S Schick, S Glantz

Tobacco Control 2005;14:396-404. doi: 10.1136/tc.2005.011288

See end of article for authors' affiliations

Correspondence to:  
Suzaynn Schick, PhD,  
University of California,  
San Francisco, Box 1390,  
San Francisco, California,  
94143-1390, USA;  
suzaynn.schick@ucsf.edu

Received 26 January 2005  
Accepted 20 June 2005

**Background:** Exposure to secondhand smoke causes lung cancer; however, there are little data in the open literature on the in vivo toxicology of fresh sidestream cigarette smoke to guide the debate about smoke-free workplaces and public places.

**Objective:** To investigate the unpublished in vivo research on sidestream cigarette smoke done by Philip Morris Tobacco Company during the 1980s at its Institut für Biologische Forschung (INBIFO).

**Methods:** Analysis of internal tobacco industry documents now available at the University of California San Francisco Legacy Tobacco Documents Library and other websites.

**Results:** Inhaled fresh sidestream cigarette smoke is approximately four times more toxic per gram total particulate matter (TPM) than mainstream cigarette smoke. Sidestream condensate is approximately three times more toxic per gram and two to six times more tumourigenic per gram than mainstream condensate by dermal application. The gas/vapour phase of sidestream smoke is responsible for most of the sensory irritation and respiratory tract epithelium damage. Fresh sidestream smoke inhibits normal weight gain in developing animals. In a 21 day exposure, fresh sidestream smoke can cause damage to the respiratory epithelium at concentrations of 2 µg/l TPM. Damage to the respiratory epithelium increases with longer exposures. The toxicity of whole sidestream smoke is higher than the sum of the toxicities of its major constituents.

**Conclusion:** Fresh sidestream smoke at concentrations commonly encountered indoors is well above a 2 µg/m<sup>3</sup> reference concentration (the level at which acute effects are unlikely to occur), calculated from the results of the INBIFO studies, that defines acute toxicity to humans. Smoke-free public places and workplaces are the only practical way to protect the public health from the toxins in sidestream smoke.

Secondhand tobacco smoke is a complex mixture containing known carcinogens, teratogens, and toxins that causes 53 000 deaths per year in the USA, about one non-smoker for every eight smokers.<sup>1</sup> There is a broad scientific consensus that secondhand smoke (SHS) causes lung cancer,<sup>1-3</sup> beginning with the 1986 publication of the US Surgeon General's report *The health effects of involuntary smoking*.<sup>2</sup> Despite this consensus, the tobacco industry has vigorously challenged the link between SHS and lung cancer,<sup>4-8</sup> including funding of research published in 2003 challenging the evidence linking SHS and lung cancer.<sup>8,9</sup> However, while it publicly challenged the link between SHS and lung cancer, Philip Morris Co privately performed extensive in vivo toxicological testing of sidestream smoke at its secret Institut für Biologische Forschung (INBIFO) in Germany.<sup>10</sup> (Sidestream smoke is the smoke that rises from the tip of the burning cigarette between puffs and constitutes approximately 85% of SHS.<sup>2</sup>) This paper reviews and summarises this large body of high quality research.

## METHODS

We located the INBIFO research among approximately 40 million pages of tobacco industry documents made public as a result of litigation against the tobacco companies. Between June 2004 and December 2004, we searched the websites of the University of California San Francisco (UCSF) Legacy Tobacco Documents Library (<http://www.legacy.library.ucsf.edu>), Philip Morris (<http://www.pmdocs.com>), Tobacco Documents Online ([www.tobaccodocuments.org](http://www.tobaccodocuments.org)), and the Centers for Disease Control (<http://www.cdc.gov/tobacco/industrydocs>) using standard strategies,<sup>11</sup> starting with keywords "sidestream" and "INBIFO", including misspellings and truncations. The initial searches yielded names of

projects, research institutions, researchers and assays, which were then searched.

Between 1981 and 1989, INBIFO did at least 115 studies of sidestream smoke. Of these 115 studies, 67 were basic research studies and 48 were simple product testing studies. Of the 67 basic research studies, 22 tested freshly generated sidestream smoke and 11 tested sidestream smoke condensate or "tar" of University of Kentucky standard reference cigarette 2R1.<sup>12</sup> Of these 33 studies, 28 were completed and all or part of their results are available in the tobacco documents. These studies form the basis of this paper. The remaining 34 basic research studies include tests of the effects of aging on sidestream toxicity, comparisons of sidestream and diesel engine smoke toxicity, and methods development studies. They will be treated in a future paper. Each INBIFO study had a unique identifying number, which we use to identify the studies.

## RESULTS

## 21 day inhalation studies comparing mainstream and sidestream smoke

The first study of sidestream smoke at INBIFO, study 3047,<sup>13-16</sup> was a 21 day inhalation study started in November 1981. Male Sprague Dawley rats were exposed to equal concentrations (based on total particulate matter (TPM) per litre) of mainstream cigarette smoke, sidestream smoke from a cigarette that was puffed according to the Federal Trade

**Abbreviations:** CalEPA, California Environmental Protection Agency; DMBA, 7,12 dimethylbenz(a)anthracene; FTC, Federal Trade Commission; INBIFO, Institut für Biologische Forschung; LOAEL, lowest observed adverse effect level; NOEL, no observable effect level; SHS, secondhand smoke; TPM, total particulate matter

Commission (FTC) protocol,<sup>17</sup> and sidestream smoke from a free-burning cigarette.<sup>14</sup> Because fresh sidestream cigarette smoke particulates have a mass-median aerodynamic diameter of 0.23  $\mu\text{M}$ ,<sup>18</sup> modern particulate measurements of  $\text{PM}_{2.5}$  and  $\text{PM}_{3.5}$  are equivalent to the TPM measurements used by INBIFO.

The concentration of smoke was 170  $\mu\text{g/l}$  TPM (170 000  $\mu\text{g}/\text{m}^3$ ), approximately one third of the maximum tolerated dose determined by INBIFO's earlier inhalation studies on mainstream smoke.<sup>19, 20</sup> Twenty rats in each exposure group were exposed to the smoke seven hours a day for 21 days. The method of exposure was "head only", meaning that the rats were placed head first in snug fitting plastic tubes with a screened head portion that projected into a duct through which the smoky air flowed. Controls for the experiment consisted of rats kept in their normal cages (cage controls) and rats that were placed in exposure tubes but were given fresh air to breathe (sham exposure).

None of the control rats died, one rat from the mainstream exposure group died, 11 rats from the puffed sidestream group died or were killed in a moribund state, and 12 rats from the free burning sidestream group died or were killed in a moribund state<sup>14</sup> (table 1). At the end of the daily exposures, the mean body temperature was 37°C for sham exposed and cage control rats, 36°C for mainstream exposed rats, and 32°C for the sidestream exposed rats<sup>14</sup> (table 1). The increase in mortality indicates that the sidestream smoke was more acutely toxic and the drop in body temperature suggests shock and depressed metabolic function in the sidestream exposed rats.<sup>21</sup>

The respiratory frequency of sham and cage control groups was 107 breaths/min, mainstream exposed rats 91 breaths/min, and sidestream exposed rats 86 breaths/min.<sup>14</sup> The sham and the cage control rats gained weight during the 21 day exposure to approximately 150% of their initial body weight; the mainstream exposed group reached 130% of their initial body weight; the sidestream exposed groups lost weight, dropping to 80% of their initial body weight<sup>14</sup> (table 1). The decreased body weights were associated with decreases in food consumption and suggest shock and cachexia.<sup>21</sup>

After 21 days exposure, 6% of the mainstream exposed rats showed slight atrophy to the olfactory epithelium, and 13% showed slight basal cell hyperplasia of the ciliated epithelium in the nasal cavity. All the sidestream exposed rats showed pronounced atrophy or ulceration of the olfactory epithelium and both hyperplasia and squamous cell metaplasia of the ciliated epithelium in the nasal cavity, with cornification in some cases (table 1). The investigators concluded: "sidestream exposure induced more frequent and more severe epithelial lesions in the olfactory and ciliated epithelium of the nasal cavity than the mainstream. If one extrapolates from the experience of previous mainstream inhalation studies, the mainstream total particulate material concentration of this study would have to be increased by a factor of three to produce similar strong reactions than seen with sidestream exposure in this study."<sup>14</sup>

A total of seven 21 day inhalation studies comparing mainstream and sidestream smoke were performed between 1982 and 1985 (3057,<sup>25-27</sup> 3061,<sup>20, 22-24</sup> 3069,<sup>25-29</sup> 3081,<sup>30-33</sup> 3108,<sup>38</sup> 3113<sup>39, 40</sup>). Four of these studies (3057, 3061, 3069, 3081) used concentrations of smoke that were sufficient to kill 10% or more of the rats (table 1). Weight loss and rectal temperature below 33.5°C correlated with mortality. Averaged over the four studies, sidestream smoke caused similar rates of mortality at one third the concentration (140  $\mu\text{g/l}$  TPM) of mainstream smoke (469  $\mu\text{g/l}$ ).

The technicians who handled the animals also noted other, more subtle, signs of the comparative toxicity of sidestream cigarette smoke. Study 3061 compared a 380  $\mu\text{g/l}$  TPM

mainstream dose to a 90  $\mu\text{g/l}$  sidestream dose. The animal handlers noted: "all smoke treated rats showed general signs of exhaustion after the end of the daily exposure. In contrary to all the other rats which recovered until the next morning, the rats of the highest sidestream group continued to show shaggy fur and slightly decreased rectal temperature."<sup>24</sup> Considering these factors together, the authors of integrating reports for studies 3069 and 3081 stated: "The mainstream and high dose sidestream exposed groups reacted approximately similarly, although the TPM concentration in the high dose sidestream-exposed group was approximately a factor of four lower than in the mainstream-exposed one."<sup>25, 41</sup>

The results of the histopathological examination of the respiratory epithelia support the idea that sidestream smoke is four times more toxic than mainstream smoke per gram. Compared on the basis of TPM, the concentration of sidestream smoke sufficient to cause necrosis of the epithelium lining the nasal cavity was 23% that of mainstream (table 1). The concentration of sidestream smoke sufficient to cause atrophy of the olfactory epithelium was one tenth that of mainstream and the concentration of sidestream sufficient to cause squamous metaplasia of the nasal epithelium was one third (table 1).

In March 1982, INBIFO researchers compared mainstream and sidestream smoke condensates (collected in an impaction trap) in a bacterial mutagenesis test (3067<sup>42</sup>). They used the plate incorporation assay with S9 microsomes and two strains of *Salmonella typhimurium*, TA 98 (which detects frameshift mutations), and TA 100 (which detects DNA base pair substitution). Strain TA 98 showed no difference, but strain TA 100 showed 30% higher activity with sidestream condensate. These results suggest that sidestream condensate and its metabolic breakdown products induced more base pair substitutions per gram than mainstream condensate.

### Tumorigenesis

In 1982, sidestream smoke condensate was included in an 80 week skin painting tumorigenesis experiment on mice (3068<sup>43-45</sup>). Half of the mice received a single pretreatment with 7,12 dimethylbenz(α)anthracene (DMBA), half did not. Chemical compounds like DMBA, which are sufficient to cause tumours by themselves, are called tumour initiators. Chemical compounds that do not cause tumours on their own, but which increase the incidence or multiplicity of tumours when applied after a tumour initiator, are called tumour promoters. The doses of cigarette smoke condensate applied were 60, 90, or 120 mg per mouse per week.

Signs of intoxication (frantic activity, prone position, panting, closed eyes) were more pronounced among the sidestream treated mice and persisted for longer. Only sidestream condensate caused dosage dependent mortality<sup>45</sup> (table 2). Among the mice that were not treated with DMBA, the incidence of tumours was 3.4–5 times higher in the mice treated with sidestream condensate than the mice treated with mainstream condensate (table 2), suggesting that sidestream condensate is a far more potent tumour initiator than mainstream condensate. Taking the time to tumour development, tumour incidence, and tumour multiplicity into account, the INBIFO researchers concluded that sidestream condensate, without DMBA treatment, was 2–6 times more tumourigenic than mainstream condensate and that sidestream condensate, preceded by DMBA treatment, was 2–3 times more tumourigenic than mainstream condensate.<sup>45</sup>

### Acute toxicity

The first acute toxicity study (3071<sup>46, 47</sup>) compared single applications of mainstream and sidestream cigarette smoke condensate, ranging from 100–2115 mg/kg body weight, to mice.<sup>48</sup> The mice were studied for two weeks after the

**Table 1** 21 day smoke inhalation studies on rats: high concentrations

Project number	Smoke type µg/l TPM†	Mortality	% initial body weight	Rectal temperature °C‡	Respiratory frequency‡	Nasal reserve cell hyperplasia	Nasal squamous metaplasia	Nasal epithelial cornification	Atrophy olfactory epithelium	Necrosis nasal epithelium	Trachea reserve cell hyperplasia
3047 <sup>14</sup>	Sham	0	150%	37	107	-	-	-	-	-	-
	MS 170	1/20 (5%)	36	32	91	+	-	±	±	-	-
	SS 160	23/40 (57%)	80%	36.7	86	+	-	+	+	+	-
3061 <sup>20, 22-24</sup>	Sham	0	137%	29.6	113	-	-	-	-	-	-
	MS 371	17/20 (85%)	97%	29.6	98	+	+	+	+	+	+
	MS 181	0	117%	34.2	111	-	-	-	-	-	-
	MS 86	0	127%	36.8	123	-	-	-	-	-	-
	SS 89	0	114%	33.7	89	+	+	+	+	+	+
3069 <sup>25-29</sup>	SS 89 1/2*	0	126%	34.9	95	+	+	+	+	+	+
	Sham	0	140%	37	107	-	-	-	-	-	-
	MS 510	16/20 (80%)	78%	28.3	95	-	-	-	-	-	-
	SS 119	2/24 (8%)	89%	32.7	74	-	-	-	-	-	-
	SS 68	0	114%	34.7	82	-	-	-	-	-	-
3081 <sup>30-33</sup>	Sham	0	130%	36.3	137	-	-	-	-	-	-
	MS 526	14/24 (58%)	82%	31.1	96	-	-	-	-	-	-
	MS 261	0	116%	35.9	110	-	-	-	-	-	-
	SS 133	11/24 (46%)	86%	33.2	79	-	-	-	-	-	-
	SS 59	0	116%	36.0	97	-	-	-	-	-	-
3113 <sup>34</sup>	Sham	0	142%			-	-	-	-	-	-
	MS 60	0	134%			+	+	+	+	-	+
	SS 60	0	124%			+	+	+	+	-	+

-, not observed; +, observed; ±, observed in 10% or fewer of subjects; empty cell: no data.

\* 1/2: indicates half day exposure (210 minutes instead of 420 minutes).

† µg/l: 1000 µg/m<sup>3</sup>

‡ Body temperature and respiratory frequency were measured at the end of the exposure.

MS, mainstream smoke; SS, sidestream smoke.

**Table 2** Skin tumourigenicity of mainstream and sidestream whole smoke condensate<sup>43–45</sup>

Dose (µg/week)	DMBA	Mortality at 80 weeks		Tumour probability at 80 weeks	
		MS	SS	MS	SS
0	–	33		0	
0	+	19		0.003	
60	–	29	41	0.12	0.55
90	–	29	55	0.21	0.71
120	–	30	61	0.13	0.65
60	+	27	63	0.38	0.65
90	+	30	74	0.48	0.82
120	+	35	86	0.49	0.86

DMBA, 7,12 dimethylbenz(a)anthracene; MS, mainstream smoke; SS, sidestream smoke.

condensate application. The LD<sub>50</sub> concentration (concentration sufficient to cause death in 50% of the animals) for sidestream condensate was 608 mg/kg. The animals painted with mainstream condensate did not reach 50% mortality during the study, but the extrapolated LD<sub>50</sub> was 2370 mg/kg. The sidestream LD<sub>50</sub> for dermal exposure was about one third the mainstream LD<sub>50</sub>.

Study 3099,<sup>47</sup> a toxicity test on human lung tissue culture cells, also demonstrated the difference in toxicity between mainstream and sidestream smoke. The cells were seeded in confluent monolayers, allowed to attach overnight, and exposed to fresh smoke.<sup>49</sup> The smoke was pumped into the sealed airspace above the lung cells and culture medium in varying concentrations and retained overnight, then the cells were detached from the flasks and tested for viability the next morning. The LD<sub>50</sub> for sidestream smoke was one third that of mainstream smoke.<sup>49</sup>

In 1984, research on sidestream smoke began to turn from acute toxicological effects and tumourigenesis to testing its effects on specific cell types and cellular systems in an effort to determine which chemical components made sidestream smoke uniquely irritating, and to find a no observable effect level (NOEL) for inhalation of sidestream smoke.

### White blood cells

Three studies tested the effect of smoke inhalation on the white blood cell populations within the lung (3108,<sup>38</sup> 3113,<sup>39</sup> 40 5015<sup>50</sup>). Inhalation of mainstream and sidestream smoke did not have as notably different effects on these cells as it had on the respiratory epithelium. Both kinds of smoke increased the number of polymorphonuclear leucocytes and pulmonary alveolar macrophages recovered from the lungs,<sup>38</sup> but decreased number of total leucocytes recovered from the blood.<sup>39</sup> Lymphocytes recovered from the lungs of mainstream exposed animals had slightly higher rates of mitogen induced proliferation than those from sidestream exposed animals.<sup>50</sup> Lymphocytes recovered from sidestream exposed animals were less viable than those from mainstream exposed animals.<sup>38–50</sup> The findings of reduced lymphocyte viability suggest that the immune capacity of the animals exposed to sidestream smoke might be reduced relative to that of the animals exposed to mainstream smoke.<sup>51</sup>

### Chemical basis for enhanced sidestream toxicity

The first study to examine the chemical basis for the difference between mainstream and sidestream smoke compared sidestream smoke and ammonia vapour in a one day inhalation experiment with rats (3104<sup>52–54</sup>). Sidestream smoke contains between 15–300 times more ammonia than mainstream smoke. The introduction to report 3104 states: “ammonia was expected to be one of the main irritative complements in sidestream smoke.”<sup>52</sup> Five concentrations of

sidestream smoke were tested, ranging from 13–253 µg/l TPM. The five ammonia vapour concentrations tested ranged from 51 µg/l (about the ammonia in 253 µg/l TPM sidestream) to 414 µg/l (about 10× the ammonia in 253 µg/l TPM sidestream).

Twelve of the 20 rats exposed to the highest sidestream concentration died. None of the rats in the ammonia groups died.<sup>52</sup> Averaged over the entire seven hours, the respiratory rate of the rats in the highest dosage sidestream group was less than half that of sham controls. The respiratory rate of the rats in the highest dosage ammonia group equalled the sham controls.<sup>52</sup> At the higher sidestream concentrations, the body temperature of the rats dropped steadily over the seven hours exposure, from 37°C down to 30°C, but the temperature of the ammonia groups did not change. INBIFO scientists concluded that sidestream smoke is 10 times more irritating than the ammonia vapour it contains.<sup>52</sup>

Study 5061<sup>55</sup> was a follow up to study 3104. It tested the effects of five chemicals found in high concentrations in sidestream smoke—formaldehyde, acetaldehyde, acrolein, ammonia, and isoprene—in a three day exposure that measured the amount of carbon dioxide exhaled by the rats.<sup>55</sup> Changes in the amount of exhaled carbon dioxide indicate changes in both respiratory rate and tidal volume. Decreased respiratory rate and shallow breaths are responses to sensory irritation.<sup>56</sup> The study found that the combination of all five compounds, at the concentrations at which they are present in sidestream smoke, was only 10–20% as irritating as whole sidestream smoke.<sup>55</sup>

The next study (3124<sup>57–61</sup>) to examine the chemical basis for the toxicity of sidestream smoke compared the effects of whole sidestream smoke, the particulate phase of sidestream smoke, the gas and vapour phase of sidestream smoke, and a recombined sidestream made of the particulate phase and the gas and vapour phase. Rats were exposed to the different smokes for seven hours a day for three days. Respiration, symptoms of irritation, body temperature, and body weight were monitored, then the rats were killed and their respiratory tracts examined.

Whole sidestream smoke was the most irritating, followed by the gas/vapour phase, the recombined smoke phase, and finally the particulate phase.<sup>58</sup> The gas/vapour phase had much stronger effects on the epithelium lining the respiratory tract than the particulate phase.<sup>60</sup> The incidence of hyperplasia and metaplasia was higher among the animals exposed to the gas/vapour phase than among the animals exposed to the particulate phase.<sup>60</sup> The only site within the respiratory tract where the particulate phase did more damage than the gas/vapour phase was the anterior larynx,<sup>60</sup> possibly because the anterior larynx is a major site of impact for inhaled particles in the rat.<sup>62</sup>

Experiment 5062<sup>63</sup> compared the effects on the upper respiratory epithelium of whole sidestream smoke, sidestream gas/vapour phase and a mixture of formaldehyde, acrolein, and ammonia at twice the concentration at which these compounds are present in the whole sidestream smoke. The mixture caused approximately 35% of the damage caused by whole sidestream smoke.<sup>63</sup>

Experiment 3126<sup>64–66</sup> compared sidestream smoke from 2R1 cigarettes to sidestream from a non-tobacco cigarette, sidestream from the non-tobacco cigarette with added nicotine, and aerosols of pure nicotine, formaldehyde, acetaldehyde, acrolein, and ammonia. The concentrations of the aerosols of single chemicals were scaled so that the highest concentration would be approximately twice as high as the concentration of that component in 2R1 smoke.

The end points assayed included weight gain, carbon dioxide (CO<sub>2</sub>) exhalation, and histopathology of the respiratory tract. 2R1 smoke decreased weight gain the most,

**Table 3** 21 day smoke inhalation studies: low concentrations

Project number	Exposure type	Smoke type µg/l PM	Nasal reserve cell hyperplasia	Nasal squamous metaplasia	Upper vocal cord hyperplasia	Larynx ventral depression thick	Trachea reserve cell hyperplasia
3114 <sup>67, 68</sup>	Head only	Sham	—	—	+	—	±
		SS 17	+	+	+	+	+
		SS 17 ½*	+	+	+	—	+
		SS 5	+	—	+	—	+
		SS 5 ½*	+	—	+	—	+
		SS 2	—	—	—	—	+
		SS 2 ½*	—	—	—	—	—
3125 <sup>69</sup>	Nose only	Sham	—	—	—	—	—
		SS 6	—	—	+	+	+
		SS 2	—	—	—	+	—
3123 <sup>34, 71-73</sup>	Whole body	Sham	—	—	—	—	—
		SS 19	+	—	—	—	±
		SS 60	+	+	—	+	±

—, not observed; +, observed; ±, observed in 10% or fewer of subjects; empty cell: no data.  
\*½: indicates half day exposure (210 minutes instead of 420 minutes).

followed by the non-tobacco cigarette with added nicotine, the non-tobacco cigarette, then pure nicotine and acrolein.<sup>66</sup> 2R1 smoke and nicotine, at their highest concentrations, decreased body temperature by 5°C and 3.5°C, respectively.<sup>66</sup> The medium and highest concentrations of 2R1 smoke decreased CO<sub>2</sub> exhalation by approximately 50%.<sup>66</sup> The highest concentration of pure nicotine decreased CO<sub>2</sub> exhalation by approximately 20%.<sup>66</sup> Acrolein aerosol had 20–25% of the effect of 2R1 smoke on the histopathology of the respiratory epithelium.<sup>64</sup> In the larynx, the highest acrolein aerosol concentration caused almost as much thickening of the epithelium as the 2R1 smoke.<sup>64</sup> Formaldehyde, acetaldehyde, and ammonia had no effects. Taken together, these experiments show that although acrolein and nicotine can account for some of the effects of sidestream smoke, the whole smoke is more toxic than the sum of the effects of its major components.

### No observable effect levels

A series of experiments began in 1985 that was designed to compare different methods of exposure and find NOEL for exposure to short term (21–90 day) exposures of fresh sidestream smoke using concentrations from 2–20 µg/l TPM and three different methods of smoke exposure. The method used in study 3114<sup>67, 68</sup> was head only, the standard method used in all of the previous experiments. Studies 3125<sup>69</sup> and 3127<sup>70</sup> used a nose only exposure. This approach required a more restrictive exposure chamber and resulted in greater stress upon the animals. The sham exposed rats, confined in nose only exposure tubes and given fresh air to breathe, weighed 20% less than the cage control rats. Study 3123<sup>34, 71-73</sup> used a whole body exposure. In the whole body exposure system, the animals were exposed to smoke while they were in their cages, moving freely in groups of two, instead of being confined solo in an exposure tube.

**Table 4** 21 day versus 90 day inhalation studies

Project number	TPM µg/l	Nasal reserve cell hyperplasia	Total vocal cord hyperplasia	Total vocal cord metaplasia
3125 <sup>69</sup> (21 day)	Sham	—	—	—
	SS 2	—	—	—
	SS 6	—	+	+
3127 <sup>70</sup> (90 day)	Sham	—	—	—
	SS 2	+	+	—
	SS 6	+	+	+

Study 3114<sup>67, 68</sup> tested sidestream smoke at 3, 5, and 17 µg/l TPM. The rats were divided into two groups, one receiving the smoke for 3.5 hours/day and the other for 7 hours/day. They also tested the effects of a 14 day recovery period after the smoke exposure. Based on the results, the INBIFO scientists proposed different NOELs for each site within the upper respiratory tract: 2 µg/l for reserve cell hyperplasia of the epithelium in the front of the nose, 17 µg/l for cornification of the epithelium in the front of the nose and atrophy of the olfactory epithelium, and between 2–17 µg/l for hyperplasia of the epithelium of the vocal cords depending on the exact site within the vocal cords (table 3). (These levels are not true NOELs, since some effects were still observed.) All of the observed changes to the respiratory epithelium caused by 21 days of inhalation were reversible after 14 days recovery.

Study 3123<sup>34, 71-73</sup> measured the effects of higher concentrations of sidestream smoke (20 and 60 µg/l TPM) using a whole body exposure system. The technicians responsible for maintaining the smoke concentration at the specified levels sampled the smoke just before it was released into the cages and from within the cages and noticed that: “The ‘real’ concentrations in the cages were lower than those determined at the inlet by the factor 0.8–0.9 for TPM, 0.6 to 0.8 for nicotine, 0.3 for ammonia, and 0.4 to 0.5 for formaldehyde. With the other components the factor was nearly one.”<sup>71</sup> The lower concentrations of some smoke components were reflected in less severe damage to the respiratory tract.<sup>72</sup> Despite the low smoke concentrations and non-restrictive exposure chambers, the smoke exposed rats still showed decreases in weight gain of approximately 15%<sup>71</sup> (table 3).

Studies 3125<sup>69</sup> (21 days) and 3127<sup>70</sup> (90 days) tested 2 µg/l and 6 µg/l TPM using nose only exposure. In the 21 day exposure neither concentration caused hyperplasia in the nasal epithelium and the 6 µg/l concentration caused hyperplasia and slight metaplasia in the vocal cords (table 3) as well as a decrease in weight gain of 12% for the low concentration and 24% for the high concentration group.<sup>74</sup> In the 90 day study both dosages caused hyperplasia in the nasal epithelium and the vocal cords<sup>70</sup> (table 4), but there was no effect on weight gain. At 6 µg/l TPM for 90 days there was significant metaplasia at the vocal cords. The damage to the respiratory epithelium at both concentrations was more severe and more prevalent after 90 days than after 21 days<sup>70</sup> (table 4), which demonstrates that longer exposures to sidestream smoke cause more severe damage to the respiratory tract.

### DISCUSSION

Based on its chemical composition, it has been inferred that sidestream smoke should be more toxic than mainstream

smoke.<sup>2</sup> There are several published bacterial mutagenesis studies indicating that sidestream particulate matter and condensates are more mutagenic than mainstream particulate matter and condensates.<sup>75-77</sup> One experiment indicated that sidestream condensate is more tumourigenic than mainstream condensate,<sup>78</sup> and another that, when injected, sidestream condensate induces more cell proliferation in the bone marrow.<sup>79</sup> Exposure to sidestream smoke results in higher concentrations of carboxyhaemoglobin, nicotine, and cotinine in blood of research animals than exposure to equal quantities of mainstream smoke.<sup>80</sup> However, there are no inhalation experiments in the open literature comparing the effects of mainstream smoke to sidestream smoke or sidestream smoke constituents that are comparable to the work Philip Morris did at INBIFO from 1981 to 1989.

The other strengths of the INBIFO research are its consistency and breadth. The studies discussed in this paper all utilised the same cigarettes, the same smoking machines, and, for a given method, the same exposure equipment and techniques. Each study is also complemented by detailed chemical analyses of the smoke.

Comparison of the INBIFO mouse skin tumourigenesis study 3068, conducted between 1982 and 1984, and the study published by Mohtashampur *et al*<sup>78</sup> in 1990 illustrates the greater depth of the INBIFO work. Mohtashampur *et al* measured the benzopyrene content of the smoke condensates they used in their experiment, whereas INBIFO study 3068 measured the pH, the concentration of nicotine, catechol, six different nitrosamines, and 14 different polycyclic aromatic hydrocarbons, and did gas chromatography of the volatile compounds.<sup>43</sup> Mohtashampur *et al* recorded the lifespan of each mouse, number of tumours, and did histopathological examinations of the treated skin areas from all mice. Study 3068 assessed an 80 point checklist of symptoms of intoxication twice daily, measured body weight and temperature, noted all visible skin irritations and lesions, recorded the size and the date of onset of each lesion, and did histopathological examinations on the treated skin areas and matching untreated skin areas from all mice.<sup>43-45</sup> Mohtashampur *et al* classified skin lesions into three types (skin tumours, mammary tumours, and precancerous skin lesions); INBIFO classified skin lesions into 25 types. The fourfold difference Mohtashampur *et al* found between sidestream and mainstream in the number of animals with tumours is comparable to the two to sixfold difference from INBIFO study 3068.

INBIFO's 1986 finding<sup>58</sup> that the gas/vapour phase of sidestream smoke is responsible for the majority of its toxicity to the respiratory epithelium and its sensory irritation preceded similar conclusions in the open literature by more than a decade. In 1997, Witschi *et al*<sup>81</sup> demonstrated that inhalation of sidestream gas/vapour phase is just as carcinogenic as whole sidestream smoke in the A/J mouse and, in 2002, Melkonian *et al*<sup>82</sup> demonstrated that sidestream gas/vapour phase has most of the inhibitory effects on angiogenesis in the chick chorioallantoic membrane of whole sidestream smoke. Likewise, studies 3104, 5061, and 5062 support the risk assessment of Fowles *et al*<sup>83</sup> which showed that the observed cancer incidence attributable to smoking is five times higher than calculated estimates for cancer risk based on the cancer potency factors of the major compounds present in mainstream tobacco smoke.

The NOEL studies with fresh sidestream done in the 1980s are the unpublished prologue to the studies that Philip Morris published beginning in 1994.<sup>84-88</sup> The first of these published studies<sup>84</sup> reported slight hyperplasia of the squamous epithelium at the arytenoid projections and the lower medial surface of the vocal cords in fewer than 20% of the animals. These results are similar to the unpublished study

### What this paper adds

Second hand smoke (SHS) contains higher concentrations of some toxins than mainstream smoke, but there is little information in the open scientific literature on the relative toxicity of a mainstream and fresh sidestream cigarette smoke.

Philip Morris did an extensive series of studies of the toxicity of sidestream cigarette smoke (sidestream smoke comprises approximately 85% of SHS) at the laboratory it owned in Germany during the 1980s. Their studies of freshly generated sidestream smoke show that it is approximately four times more toxic per gram total particulate matter (TPM) than mainstream smoke by inhalation. In 21 day exposures, fresh sidestream smoke can cause damage to the epithelium lining the respiratory tract at doses as low as 2 µg/l TPM and this damage increases with longer exposures. Sidestream tar also causes two to six times more tumours per gram, when painted on the skin of mice. None of the studies comparing sidestream and mainstream smoke were ever published.

3127<sup>70</sup> by the same authors, which examined many more end points and found effects in the majority of the animals. (This example is similar to a Philip Morris funded study of SHS in airliners that was edited to remove or downplay results that did not support the industry's argument that smoke-free policies were not needed on airlines.<sup>89</sup>) Another published study reported that the effects of inhaled sidestream cigarette smoke on rats do not increase in severity with longer exposure.<sup>86</sup> The unpublished research from INBIFO contradicts this claim. Studies 3125<sup>69</sup> and 3127<sup>70</sup> show that the damage to the respiratory epithelium does increase in severity with longer exposures and that sidestream exposed rats gain less weight than sham exposed rats, even at 2 µg/l (3123,<sup>34</sup> 71-73 3125<sup>69</sup>).

It is possible to use the data from Philip Morris' research to estimate an acute reference exposure level for fresh sidestream smoke using the guidelines set by the California Environmental Protection Agency (CalEPA). CalEPA defines an acute reference exposure level as "the concentration level at or below which no adverse health effects are anticipated for a specified exposure duration".<sup>90</sup> INBIFO found acute effects at the lowest doses tested (tables 3 and 4), 2 µg/l (2000 µg/m<sup>3</sup>) TPM. Considering this concentration as an estimate of the lowest observed adverse effect level (LOAEL), the CalEPA procedure applies uncertainty factors of 10 to allow for interspecies variation, 10 for variation within susceptibility by humans, and 10 to account for the fact that the experiments only identified a LOAEL, not a NOAEL. Applying this 1000 total uncertainty factor to 2000 µg/m<sup>3</sup>, we compute an acute reference exposure level of 2 µg/m<sup>3</sup> TPM, below which one would not expect acute effects in humans. Mean particulate concentrations in public places where smoking occurs range from 27 µg/m<sup>3</sup> to 686 µg/m<sup>3</sup><sup>91</sup> and peak levels can be substantially higher. This level of pollution is orders of magnitude above the 2 µg/m<sup>3</sup> acute reference exposure computed based on the results found at INBIFO. To reduce the concentration of smoke in public areas to this level through ventilation would require increases in current ventilation rates by factors of 14 (27/2) to 343 (686/2). Because these increases are impractical, the data from INBIFO support smoke-free public spaces as the only practical way to protect non-smokers' health from the toxins in sidestream smoke.

### Limitations

The cigarette used in these studies, the University of Kentucky 2R1 standard reference cigarette, is a high tar, unfiltered cigarette designed to model the cigarettes popular

in the 1950s. The advantages of the 2R1 cigarette are that it does not change over time or from market to market. There is evidence that the sidestream smoke from the filtered "light" cigarettes that now constitute the majority of market<sup>92</sup> is significantly more toxic, per gram and per cigarette, than that from "full-flavor" cigarettes similar to the 2R1.<sup>77</sup> Thus, the results of the INBIFO work in the 1980s may underestimate the toxicity of modern sidestream smoke.

## Conclusions

The results of the research done at INBIFO on fresh sidestream smoke and condensates from the University of Kentucky 2R1 cigarette show that sidestream condensate is 2–6 times more tumourigenic per gram than mainstream condensate.<sup>43</sup> By inhalation, whole fresh sidestream smoke is 2–6 times more toxic per gram TPM than mainstream smoke, depending on the end point. The gas/vapour phase of sidestream smoke is responsible for the majority of the sensory irritation and damage to the respiratory tract epithelium. Fresh sidestream smoke can cause damage to the respiratory epithelium at low levels, and damage to the respiratory epithelium increases with longer exposures. The number, variety, and results of the fundamental toxicological experiments done by Philip Morris at INBIFO are without parallel in the open scientific literature. These studies were neither published nor revealed to the government in rule making hearings by the US Occupational Safety and Health Administration or in risk assessments by the US EPA and CalEPA. The unpublished research on sidestream smoke condensates and freshly generated cigarette sidestream smoke that Philip Morris did supports the institution of smoke-free policies in workplaces and public places as the only practical way to protect the public from the known risks of lung cancer and other diseases associated with cigarette sidestream smoke.

## ACKNOWLEDGEMENTS

The authors would like to thank Katharine Hammond, PhD, and Melanie Marty, PhD, for their comments on the manuscript.

## Authors' affiliations

**S Schick, S Glantz**, Center for Tobacco Control Research and Education and Division of Cardiology, University of California San Francisco, San Francisco, California, USA

This research is supported by the California Tobacco-Related Disease Research Program (12FT-0144) and the National Cancer Institute (CA 87472). The funding agencies had no role in the conduct of this study or the preparation of the manuscript.

Competing interests statement: Dr Glantz has received honoraria for lecturing on the effects of secondhand smoke and advocated for smoke-free policies. Dr Schick has nothing to disclose.

## REFERENCES

- 1 **California Environmental Protection Agency**. Health effects of exposure to environmental tobacco smoke: the report of the California Environmental Protection Agency. *Smoking and tobacco control monograph number 10*. Bethesda: US Department of Health and Human Services, National Institutes of Health, National Cancer Institute; 1999 August.
- 2 **US Department of Health and Human Services**. *The health consequences of involuntary smoking. A report of the Surgeon General, 1986*. Rockville, Maryland: Public Health Service, Centers for Disease Control, 1986. (DHHS Publication No (CDC) 87-8398.)
- 3 **US Environmental Health Protection Agency**. *Respiratory health effects of passive smoking: the lung cancer and other disorders*. Washington DC: US Environmental Protection Agency; 1992 December 1992, Report No.: EPA/600/6-90/006F.
- 4 **Bero LA, Glantz SA, Rennie D**. Publication bias and public health policy on environmental tobacco smoke. *JAMA* 1994;**272**:133–6.
- 5 **Barnes DE, Bero LA**. Scientific quality of original research articles on environmental tobacco smoke. *Tobacco Control* 1997;**6**:19–26.
- 6 **Barnes DE, Bero LA**. Why review articles on the health effects of passive smoking reach different conclusions. *JAMA* 1998;**279**:1566–70.
- 7 **Kennedy GE, Bero LA**. Print media coverage of research on passive smoking. *Tobacco Control* 1999;**8**:254–60.
- 8 **Bero LA**. Tobacco industry manipulation of research. *Public Health Reports* 2005;**120**:200–8.
- 9 **Enstrom JE, Kabat GC**. Environmental tobacco smoke and tobacco related mortality in a prospective study of Californians, 1960–98. *BMJ* 2003;**326**:1057.
- 10 **Diethelm P, Rielle J, McKee M**. The whole truth and nothing but the truth? The research that Philip Morris did not want you to see. *Lancet* 2004;**36**:4998–5006.
- 11 **Malone R, Balbach E**. Tobacco industry documents: treasure trove or quagmire? *Tobacco Control* 2000;**9**:334–8.
- 12 **Davis D**. The reference and research cigarette series. 1984. Bates No. HK1180005/0020. <http://legacy.library.ucsf.edu/tid/1818100>.
- 13 **Romer E, Schnell P**. INBIFO. Subreport IB A 0500/3047 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette type 2R1 on rats. 1981. Bates No. 2029190417/0508. <http://legacy.library.ucsf.edu/tid/xol81f00>.
- 14 **Gugel H, Reininghaus W, Romer E**. INBIFO. Integrating report A 0500/3047 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette type 2R1 on rats. 29 Jul 1982. Bates No. 2029190329/0354. <http://legacy.library.ucsf.edu/tid/qzg83e00>.
- 15 **Romer E, Schnell P, Speck M, et al**. INBIFO. Subreport AC 0500/3047 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette 2R1 on rats. 1981. Bates No. 2029190355/0412. <http://legacy.library.ucsf.edu/tid/rzg83e00>.
- 16 **Mull R, Romer E, Rylander R, et al**. INBIFO. Subreport BC A 0500/3047 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette 2R1 on rats. 1981. Bates No. 2501371057/1098. <http://legacy.library.ucsf.edu/tid/til32e00>.
- 17 **Peeler CL**. *Cigarette testing and the Federal Trade Commission: a historical overview*. Washington DC, 1996. Report No. NIH publication number 96–4023.
- 18 **Klepeis NE**. Using computer simulation to explore multi-compartment effects and mitigation strategies for residential exposure to secondhand tobacco smoke. Berkeley California: University of California Berkeley, 2004.
- 19 **INBIFO**. Report A 0500/1540 56 d - smoke inhalation study on rats with 2R1 and net cigarettes and added nitric oxide part 3: biological data volume I. 7 Sep 1978. Bates No. 1002937200/7616. <http://legacy.library.ucsf.edu/tid/leu53e00>.
- 20 **Mies M, Romer E, Schnell P, et al**. INBIFO. Subreport Py 0500/3061 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette type 2R1 on rats. Aug 1982. Bates No. 2501103727/3973. <http://legacy.library.ucsf.edu/tid/fnk32e00>.
- 21 **Jones TC, Hunt R**. *Veterinary pathology*, 5th ed. Philadelphia: Lea and Febiger, 1983.
- 22 **Romer E, Speck M, Vandongen C**. INBIFO. Subreport analytical chemistry A 0500 / 3061 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette type 2R1 on rats. 26 Nov 1982. Bates No. 2029190730/0809. <http://legacy.library.ucsf.edu/tid/eah83e00>.
- 23 **Becker D, Mull R, Romer E, et al**. INBIFO. Subreport biological chemistry 0500 / 3061 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette type 2R1 on rats. 24 Nov 1982. Bates No. 2029190652/0729. <http://legacy.library.ucsf.edu/tid/dah83e00>.
- 24 **Romer E, Schnell P**. INBIFO. Subreport IB 0500 / 3061 21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette type 2R1 on rats. 1982. Bates No. 2029190510/0618. <http://legacy.library.ucsf.edu/tid/uzg83e00>.
- 25 **Becker D, Romer E, Schnell P, et al**. Report A 0500/3069 21 - day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats histological changes of the larynx and the lung. 19 Mar 1984. Bates No. 2028797166/7195. <http://legacy.library.ucsf.edu/tid/aot73e00>.
- 26 **Romer E, Schnell P**. INBIFO. Subreport analytical chemistry A 0500/3069 21-day smoke inhalation study with mainstream and sidestream smoke standard reference cigarette 2R1 on rats histological changes of the larynx and the lung. 15 Feb 1984. Bates No. 2028797258/7386. <http://legacy.library.ucsf.edu/tid/coi73e00>.
- 27 **Romer E, Tewes F**. INBIFO. Subreport microbiology a 0500/3069 21 - day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats histological changes of the larynx and the lung. 15 Mar 1983. Bates No. 2026027752/7817. <http://legacy.library.ucsf.edu/tid/jxt73e00>.
- 28 **Romer E, Schnell P**. INBIFO. Subreport inhalation A 0500/3069 21 - day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats histological changes of the larynx and the lung. 15 Feb 1984. Bates No. 2026027633/7751. <http://legacy.library.ucsf.edu/tid/iym69e00>.
- 29 **Romer E, Terecesai A**. INBIFO. Subreport pathology A 0500/3069 21 - day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats histological changes of the larynx and the lung. 10 Oct 1983. Bates No. 2028797453/7725. <http://legacy.library.ucsf.edu/tid/xkz74e00>.
- 30 **Becker D, Romer E, Walk R**. INBIFO. Subreport biological chemistry A 0500 / 3081 21 - day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats sister chromatid exchange and chromosome aberrations in peripheral blood lymphocytes and pulmonary macrophages. 30 Mar 1984. Bates No. 2026033484/3629. <http://legacy.library.ucsf.edu/tid/lxm69e00>.

- 31 **Romer E**, Schnell P. INBIFO. Subreport inhalation A 0500 / 3081 21 - day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats sister chromatid exchange and chromosome aberrations in peripheral blood lymphocytes and pulmonary macrophages. 19 Oct 1984. Bates No. 2026033384/3483. <http://legacy.library.ucsf.edu/tid/inz74e00>.
- 32 **Romer E**, Teredesai A. INBIFO. Subreport pathology A 0500/3081 21-day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats sister chromatid exchange and chromosome aberrations in peripheral blood lymphocytes and pulmonary macrophages. 19 Oct 1984. Bates No. 2028936922/6952. <http://legacy.library.ucsf.edu/tid/eun73e00>.
- 33 **Romer E**, Speck M. INBIFO. Subreport analytical chemistry A 0500 / 3081 21 - day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats sister chromatid exchange and chromosome aberrations in peripheral blood lymphocytes and pulmonary macrophages. 5 Jan 1983. Bates No. 2026033336/3383. <http://legacy.library.ucsf.edu/tid/eyt73e00>.
- 34 **Gerstenberg B**, Romer E, Voncken P, et al. INBIFO. Report P 0500/3123 biological activity of sidestream smoke of standard reference cigarette 2R1 on rats and syrian hamsters 21-day inhalation study (whole body exposure) volume 2. 9 Feb 1988. Bates No. 2501833025/3380. <http://legacy.library.ucsf.edu/tid/fnd32d00>.
- 35 **Romer E**, Schnell P. INBIFO. Subproposal IH A 0500/3057 21-day smoke inhalation study with cigarette smoke of standard reference cigarette type 2R1 on rats biochemical and morphological free lung cell responses using various lavage methods (pt). Oct 1983. Bates No. 2501099835/9875. <http://legacy.library.ucsf.edu/tid/kuy32e00>.
- 36 **Kindt R**, Mull R, Romer E. INBIFO. Integrating proposal A 0500/3057 21-day inhalation study with main and sidestream cigarette smoke of standard reference cigarette 2R1 on rats biochemical and morphological free lung cell responses (pt). 31 Aug 1983. Bates No. 2029027157/7192. <http://legacy.library.ucsf.edu/tid/kze83e00>.
- 37 **Anon**. INBIFO. Subproposal biological chemistry A 0500/3057 21 - day inhalation study with main and sidestream cigarette smoke of standard reference cigarette 2R1 on rats biochemical and morphological free lung cell responses. 15 Aug 1983. Bates No. 1002948733/8768. <http://legacy.library.ucsf.edu/tid/vgu53e00>.
- 38 **Hackenberg U**, Kindt R, Pruhs D. INBIFO. Report P 0500/3108 21-day inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette 2R1 on rats phagocytic activity of free lung cells. 13 Dec 1985. Bates No. 2502060036/0428. <http://legacy.library.ucsf.edu/tid/dca32d00>.
- 39 **Becker D**, Gerstenberg B, Pruhs D. INBIFO. Report P 0500 / 3113 21-day inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats tpm concentration: 60 ug/l volume 1. 10 Jun 1986. Bates No. 2501832585/2822. <http://legacy.library.ucsf.edu/tid/dnd32d00>.
- 40 **Romer E**, Teredesai A, Tewes F. INBIFO. Report P 0500/3113 21-day inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats tpm concentration: 60 ug/l volume 2. 10 Jun 1986. Bates No. 2501832823/3024. <http://legacy.library.ucsf.edu/tid/end32d00>.
- 41 **Becker D**, Romer E, Schnell P. INBIFO. Report A 0500/3081 21-day smoke inhalation study with mainstream and sidestream smoke of standard reference cigarette 2R1 on rats sister chromatid exchange and chromosome aberrations in peripheral blood lymphocytes and pulmonary macrophages. 6 Feb 1985. Bates No. 2028798068/8126. <http://legacy.library.ucsf.edu/tid/got73e00>.
- 42 **INBIFO**. Report A 0500/3067 mutagenicity study of sidestream and mainstream condensate of standard reference cigarette 2r1 on salmonella typhimurium strains TA 98 and TA 100. Oct 1982. Bates No. 2029141043/1048. <http://legacy.library.ucsf.edu/tid/uqm73e00>.
- 43 **Gerstenberg B**, Kuhn D, Romer E, et al. INBIFO. Report P 0500/3068 skin tumorigenicity of mainstream and sidestream whole smoke condensate of standard reference cigarette 2R1 80 - week dermal application study with CD1(I)CR)BR and B6C3F1 mice. 26 Oct 1987. Bates No. 2026051118/1619. <http://legacy.library.ucsf.edu/tid/sgk85e00>.
- 44 **Gerstenberg B**, Romer E. INBIFO. Report P 0500/3068 skin tumorigenicity of mainstream and sidestream whole smoke condensate of standard reference cigarette 2R1 80-week dermal application study with CD1(CR) BR and B6C3F1 mice volume 2. 26 Oct 1987. Bates No. 2501945805/5937. <http://legacy.library.ucsf.edu/tid/wib32d00>.
- 45 **Romer E**, Teredesai A, Thomas C. INBIFO. Subreport pathology P 0500/3068 skin tumorigenicity of mainstream and sidestream whole smoke condensate of standard reference cigarette 2R1 80 - week dermal application study with CD1(I)CR)BR and B6C3F1 mice. 26 Oct 1987. Bates No. 2028796662/7009. <http://legacy.library.ucsf.edu/tid/dnt73e00>.
- 46 **No Author**. INBIFO. Proposal A 0500/3071 29-day dermal application of impaction trap mainstream and sidestream condensates of standard reference cigarette type 2R1 on CRL:CD1(ICR)BR and B6C3F1 mice determination of epidermal ornithine decarboxylase and aryl hydro carbon monooxygenase. 18 Jan 1983. Bates No. 1002945110/5415. <http://legacy.library.ucsf.edu/tid/yfu53e00>.
- 47 **Kuhn D**, Romer E. INBIFO. Subreport animal treatment P 0500/3071 29-day dermal application of impaction trap mainstream and sidestream condensates of standard reference cigarette type 2R1 on CD1 and B6C3F1 mice determination of epidermal ornithine decarboxylase and aryl hydrocarbon monooxygenase (pt). 27 Aug 1984. Bates No. 2029099417/9803. <http://legacy.library.ucsf.edu/tid/dao37e00>.
- 48 **Kuhn D**, Romer E, Speck M, et al. INBIFO. Report A 0500/3070 acute toxicity of mainstream and sidestream condensate (impaction trap collection) of the standard reference cigarette 2R1 after a single dermal application on mice. 19 Oct 1984. Bates No. 2026028027/8123. <http://legacy.library.ucsf.edu/tid/lxt73e00>.
- 49 **Heil M**, Oey J, Romer E, et al. INBIFO. Report P 0500/3099 1-day cytotoxicity in human lung PAN-L3 cells after exposure to diluted mainstream and sidestream whole smoke of standard reference 2R1. 7 Dec 1984. Bates No. 2028799774/9877. <http://legacy.library.ucsf.edu/tid/bpt73e00>.
- 50 **Becker D**, Romer E, Walk R. INBIFO. Documentation P. 0500/5015 spontaneous and mitogen-stimulated proliferation of lymphocytes drive for rats exposed to mainstream and sidestream smoke of standard reference cigarette 2R1. 19 Mar 1985. Bates No. 2505433265/3333. <http://legacy.library.ucsf.edu/tid/gum15c00>.
- 51 **Kuby J**. *Immunology*, 2nd ed. New York: WH Freeman & Co, 1994.
- 52 **Romer E**. INBIFO. Report P 0500/3104. 1985. Bates No. 2501581117/1151. <http://legacy.library.ucsf.edu/tid/coh39e00>.
- 53 **INBIFO**. Report P 0500/3104 acute inhalation study with sidestream cigarette smoke of standard reference cigarette 2R1 and ammonia vapor on rats. 28 Jun 1984. Bates No. 2022826000/6038. <http://legacy.library.ucsf.edu/tid/pig27e00>.
- 54 **Reininghaus W**, Romer E, Schnell P, et al. INBIFO. Proposal P 0500/3104 acute inhalation study with sidestream cigarette smoke of standard reference cigarette 2R1 and ammonia vapor on rats (pt). 27 Jun 1984. Bates No. 2501844748/4830. <http://legacy.library.ucsf.edu/tid/red32d00>.
- 55 **Gerstenberg B**, Pruhs D, Romer E, et al. INBIFO. Documentation P 0500/5061 effect of subacute inhalation of formaldehyde, acetaldehyde, acrolein, ammonia, isoprene and a mixture of these aldehydes with ammonia on CO<sub>2</sub>-exhalation of rats. 19 Aug 1986. Bates No. 2505432685/2801. <http://legacy.library.ucsf.edu/tid/abn15c00>.
- 56 **Alarie Y**. Sensory irritation of the upper airways by airborne chemicals. *Toxicol Appl Pharmacol* 1973;**24**:279-97.
- 57 **Gerstenberg B**, Romer E. INBIFO. Subreport analytical chemistry P 0500/3124 three day inhalation study with sidestream whole smoke, gas/vapor phase, particle phase and recombined sidestream smoke of standard reference cigarette 2R1 on rats. 14 Jan 1987. Bates No. 2028984864/4923. <http://legacy.library.ucsf.edu/tid/ajy73e00>.
- 58 **Pruhs D**, Romer E. INBIFO. Subreport inhalation P 0500 / 3124 3-day inhalation study with sidestream whole smoke, gas/vapor phase, particle phase and recombined sidestream smoke of standard reference cigarette 2R1 on rats. 3 Sep 1986. Bates No. 2501033600/3784. <http://legacy.library.ucsf.edu/tid/zhu32e00>.
- 59 **Gerstenberg B**, Pruhs D, Romer E, et al. INBIFO. Subreport - laufzettel (A) p 0500/3124 3-day inhalation study with sidestream whole smoke, gas/vapor phase, particle phase and recombined sidestream smoke of standard reference cigarette 2R1 on rats (pt). 13 Jan 1987. Bates No. 2501929191/9441. <http://legacy.library.ucsf.edu/tid/uzb32d00>.
- 60 **Romer E**, Teredesai A. INBIFO. Subreport pathology P 0500/3124 3-day inhalation study with sidestream whole smoke, gas/vapor phase, particle phase, and recombined sidestream smoke of standard reference cigarette 2R1 on rats (pt). 19 Nov 1987. Bates No. 2501603476/3775. <http://legacy.library.ucsf.edu/tid/zec32e00>.
- 61 **Romer E**, Stinn W. INBIFO. Subreport inhalation P 0500/3124 irritative activity of sidestream whole smoke, gas/vapor phase, particle phase, and recombined sidestream smoke of standard reference cigarette 2R1: 3-day inhalation study in rats (pt). 15 Jan 1988. Bates No. 2501928486/8675. <http://legacy.library.ucsf.edu/tid/ozb32d00>.
- 62 **Kelly JT**, Kimbell JS, Asgharian B. Deposition of fine and coarse aerosols in a rat nasal mold. *Inhal Toxicol* 2001;**13**:577-88.
- 63 **Romer E**, Teredesai A. INBIFO. Subdocumentation P 0500/5062. 1987. Bates No. 2028985267/5517. <http://legacy.library.ucsf.edu/tid/gne69e00>.
- 64 **Romer E**, Teredesai A. INBIFO. Subreport pathology P 0500/3126 3-day inhalation study with sidestream smoke of standard reference cigarette 2R1, nicotine, sidestream smoke of nontobacco cigarette X6D6MW without and with added nicotine and with formaldehyde, acetaldehyde, acrolein, and ammonia on rats. Aug 1988. Bates No. 2501595493/5892. <http://legacy.library.ucsf.edu/tid/flc32e00>.
- 65 **Gerstenberg B**, Romer E, Voncken P. INBIFO. Subreport analytical chemistry P 0500/3126 3 - day inhalation study with sidestream smoke of standard reference cigarette 2R1, nicotine, sidestream smoke of nontobacco cigarette X6D6MW without and with formaldehyde, acetaldehyde, acrolein, and ammonia on rats. Jun 1987. Bates No. 2028984472/4546. <http://legacy.library.ucsf.edu/tid/wce69e00>.
- 66 **INBIFO**. Study P 0500/3126 three-day inhalation study with sidestream smoke of standard reference cigarette 2R1, nicotine, sidestream smoke of nontobacco cigarette X6D6MW without and with added nicotine and with formaldehyde, acetaldehyde, acrolein and ammonia on rats (pt). 1987. Bates No. 2028984723/4763. <http://legacy.library.ucsf.edu/tid/fce69e00>.
- 67 **Bachmayer D**, Gerstenberg B, Pruhs D, et al. INBIFO. Report p 0500/3114 21-day inhalation study with sidestream cigarette smoke of standard reference cigarette 2R1 on rats tpm concentrations: 2, 5 and 17 ug/l volume 1.8 Dec 1986. Bates No. 2501832143/2363. <http://legacy.library.ucsf.edu/tid/bnd32d00>.
- 68 **Romer E**, Teredesai A, Tewes F. INBIFO. Report P 0500/3114 21-day inhalation study with sidestream. 8 Dec 1986. Bates No. 2501832364/2584. <http://legacy.library.ucsf.edu/tid/cnd32d00>.
- 69 **Gerstenberg B**, Pruhs D, Romer E. INBIFO. Report P 0500/3125 biological activity of sidestream smoke of standard reference cigarette 2R1 21 - day inhalation study (nose-only exposure) on rats and syrian hamsters volume 1

- volume 2. 8 Aug 1988. Bates No. 2026041559/1835. <http://legacy.library.ucsf.edu/tid/ebu73e00>.
- 70 **Gerstenberg B**, Haussmann H. INBIFO. Integrating report P 0500/3127 biological activity of the sidestream smoke of the standard reference cigarette 2R1 on rats and syrian hamsters 90-day inhalation study (nose-only exposure) with a 21-day day recovery period. 1988. Bates No. 2062040405/0479. <http://legacy.library.ucsf.edu/tid/xvz43e00>.
  - 71 **Bachmayer D**, Gerstenberg B. INBIFO. Report p 0500/3123 biological activity of sidestream smoke of standard reference cigarette 2R1 on rats and syrian hamsters 21 - day inhalation study (whole body exposure) volume 1. 9 Feb 1988. Bates No. 2026018591/8675. <http://legacy.library.ucsf.edu/tid/tgu73e00>.
  - 72 **Bachmayer D**, Gocke W, Kindt R, Pruhs D, Romer E, Schepers G. INBIFO. Report P 0500/3123 biological activity of sidestream smoke of standard reference cigarette 2R1 on rats and syrian hamsters 21-day inhalation study (whole body exposure) volume 3. 9 Feb 1988. Bates No. 2501833822/4200. <http://legacy.library.ucsf.edu/tid/ind32d00>.
  - 73 **Romer E**, Teredesai A, Tewes F. INBIFO. Report P 0500/3123 biological activity of sidestream smoke of standard reference cigarette 2R1 on rats and syrian hamsters 21-day inhalation study (whole body exposure) volume 4. 9 Feb 1988. Bates No. 2501833512/3821. <http://legacy.library.ucsf.edu/tid/hnd32d00>.
  - 74 **Gerstenberg B**, Pruhs D, Romer E, *et al*. INBIFO. Report p 0500/3125 biological activity of sidestream smoke of standard reference cigarette 2R1 21-day inhalation study (nose-only exposure) on rats and syrian hamsters volume 1 volume 2. 8 Aug 1988. Bates No. 2026041559/1835. <http://legacy.library.ucsf.edu/tid/ebu73e00>.
  - 75 **Lofroth G**, Lazaridis G. Environmental tobacco smoke: comparative characterization by mutagenicity assays of sidestream and mainstream cigarette smoke. *Environ Mutagen* 1986;**8**:693-704.
  - 76 **Ling PI**, Lofroth G, Lewtas J. Mutagenic determination of passive smoking. *Toxicol Lett* 1987;**35**:147-51.
  - 77 **Chortyk OT**, Chamberlain WJ. A study on the mutagenicity of tobacco smoke from low-tar cigarettes. *Arch Environ Health* 1990;**45**:237-44.
  - 78 **Mohtashamipur E**, Mohtashamipur A, Germann PG, *et al*. Comparative carcinogenicity of cigarette mainstream and sidestream smoke condensates on the mouse skin. *J Cancer Res Clin Oncol* 1990;**116**:604-8.
  - 79 **Mohtashamipur E**, Steinforth T, Norpoth K. Comparative bone marrow clastogenicity of cigarette sidestream, mainstream and recombined smoke condensates in mice. *Mutagenesis* 1988;**3**:419-22.
  - 80 **Haley NJ**, Adams JD, Alzofon J, *et al*. Uptake of sidestream smoke by Syrian golden hamsters. *Toxicol Lett* 1987;**35**:83-8.
  - 81 **Witschi H**, Espiritu I, Maronpot RR, *et al*. The carcinogenic potential of the gas phase of environmental tobacco smoke. *Carcinogenesis* 1997;**18**:2035-42.
  - 82 **Melkonian G**, Cheung L, Marr R, *et al*. Mainstream and sidestream cigarette smoke inhibit growth and angiogenesis in the day 5 chick chorioallantoic membrane. *Toxicol Sci* 2002;**68**:237-48.
  - 83 **Fowles J**, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tobacco Control* 2003;**12**:424-30.
  - 84 **Teredesai A**, Pruhs D. *Histopathological findings in the rats and hamster respiratory tract in a 90-day inhalation study using fresh sidestream smoke of the standard reference cigarette 2R1*. Washington DC: ILSI Press, 1994.
  - 85 **Haussmann HJ**, Anskait E, Becker D, *et al*. Comparison of fresh and room-aged cigarette sidestream smoke in a subchronic inhalation study on rats. *Toxicol Sci* 1998;**41**:100-16.
  - 86 **Haussmann HJ**, Gerstenberg B, Goeke W, *et al*. 12-month inhalation study on room-aged cigarette sidestream smoke in rats. *Inhalation toxicology* 1998;**10**:663-97.
  - 87 **Schlage WK**, Bulles H, Friedrichs D, *et al*. Tobacco smoke-induced alterations of cytokeratin expression in the rat nasal activity cavity following chronic inhalation of room-aged sidestream smoke. *Toxicol Lett* 1998;**96-97**:309-18.
  - 88 **Stabbert R**, Schepers G, Stinn WS, *et al*. Hemoglobin adducts in rats chronically exposed to room-aged cigarette sidestream smoke and diesel engine exhaust. *Adv Exp Med Biol* 2001;**500**:153-6.
  - 89 **Neilsen K**, Glantz SA. A tobacco industry study of airline cabin air quality: dropping inconvenient findings. *Tobacco Control* 2004;**13**(suppl 1):i20-9.
  - 90 **California Office of Environmental Health Hazard Assessment**. *The determination of acute reference exposure levels for airborne toxicants*. Oakland, California: California Office of Environmental Health Hazard Assessment; 1989 March, 1999.
  - 91 **California Environmental Protection Agency, Board AR**. *Draft Technical support document - part A. Proposed identification of environmental tobacco smoke as the toxic air contaminant*. Oakland, California: California Environmental Protection Agency; 2003 December 2003.
  - 92 **United States Federal Trade Commission**. *Federal Trade Commission cigarette report for 2001*. Washington DC: FTC, 2001, 2003.

## The Lighter Side



29 05 04 UNIVERSAL PRESS SYNDICATE

The death of ABC News anchorman Peter Jennings in August leads to an upsurge in reporting on the link between smoking and lung cancer in the US. The cartoonist Tony Auth sees echoes of the Vietnam War Memorial, with an even more impressive death toll. © Tony Auth. Universal Press Syndicate.