A Study of Secondhand Tobacco Smoke Exposures in Bars from Three Regions in New Zealand: Results Prior to Implementation of Ban in Smoking in Bars

A Report to the New Zealand Ministry of Health

By

Jeff Fowles[1]
Annemarie Christophersen
Alistair Woodward[2]
Rod Lea[1]
Stuart Dickson[1]
Matthew Hosking[1]
Richard Berezowski[1]
Dinusha Fernando[1]

[1] Institute of Environmental Science and Research, Limited
[2] University of Auckland

April 2005

Report No. FW04104

A Study of Secondhand Tobacco Smoke Exposures in Bars from Three Regions in New Zealand: Results Prior to Implementation of Ban in Smoking in Bars

David Phillips
Science Programme Manager

Jeff Fowles, PhD
Project Leader

DISCLAIMER
This report or document ("the Report") is given by the Institute of Environmental Science and Research Limited ("ESR") solely for the benefit of the Ministry of Health, Public Health Service Providers and other Third Party Beneficiaries as defined in the Contract between ESR and the Ministry of Health, and is strictly subject to the conditions laid out in that Contract.

Neither ESR nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for use of the Report or its contents by any other person or organisation.

ACKNOWLEDGEMENTS

Many people contributed their time and support to this project. ESR would like to particularly thank Clare Bear (Ministry of Health) and the participants of the study.

This study was funded by the Ministry of Health.

CONTENTS

EXECUTIVE SUMMARY

Background

The Ministry of Health commissioned this scientific study to assess what level (if any) patrons in New Zealand bars are exposed to second-hand smoke (SHS), before and after changes to smoke-free legislation on 10 December 2004. The Smokefree Environments Amendment Act 2003 requires all workplaces, including restaurants and bars, to become smokefree from 10th December 2004. This follows the examples of Ireland, Norway, and a number of US States who have enacted similar changes in recent years. The purpose of the smokefree law is to protect workers and non-smoking patrons from the significant health risks of exposure to SHS.

Exposures to SHS in New Zealand contribute to approximately 400 premature deaths each year (Woodward and Laugesen, 2000), almost the same as the annual road toll. Until 10 December 2004, bars represented one of the most common public places for SHS exposures to occur.

The purpose of this study is to provide a quantifiable measurement of SHS exposure levels in New Zealand bars, before and after the smokefree law requires bars to be totally smokefree in indoor areas. This is the first national study of SHS exposure and builds on a pilot study completed in Wellington in 2003 (Woodward et al., 2005).

Part I of Project: baseline measurements

This paper reports on the results of Part I of the project. Two baseline measurements of SHS levels in 30 randomly selected bars during winter and spring 2004, prior to the smokefree law change. A final report will compare the results with two proposed follow-up measurements in the same venues during winter and spring 2005, after the smokefree law change.

Key results

The study involved measuring cotinine levels in non-smoker volunteers' saliva before and after a three hour visit to a randomly selected bar in Auckland, Wellington, and Southland. Cotinine is the main specific metabolite of nicotine and a well-established biomarker for SHS exposure.

In all bars, and in all volunteers, exposure to SHS was clearly established. A significant increase in saliva cotinine was seen after three hours in the bar (mean increase = 0.63 ng/mL; starting concentration = 0.24 ng/mL, ending concentration = 0.87 ng/mL, $p < 0.0001$).

The objective measures of SHS exposure correlated strongly with the volunteers' subjective observation of ventilation, air quality, and counts of lit cigarettes. One exception was where objective salivary markers indicated that even "seemingly smokefree" venues with "good ventilation" produced discernable levels of SHS

exposure. This indicates that patrons would not be able to fully detect the degree of their exposure to SHS in venues they perceived as being smokefree.

A seasonal difference in cotinine increases was seen, but only in venues rated as having "poor" ventilation, in which SHS were more pronounced in the winter months. For better ventilated venues, no seasonal difference was observed.

Regional mean SHS exposures fell in the following order: (Auckland < Wellington ($p = 0.27$) < Invercargill ($p = 0.025$)). The increases in Invercargill bars were more than double that seen in Auckland and approximately double that seen in Wellington).

Methods

Sampling sites: Thirty bars were randomly selected.

- 15 bars in Auckland
- 10 bars in Wellington, and
- 5 bars in Southland and Invercargill

Four bars from these regions were chosen to represent rural areas, 10 bars were in suburban/provincial areas, and the remainder were in urban centres. Five bars were in areas of high Maori and/or Pacific Island demographic representation.

Sampling times: Bars were visited on a Friday or Saturday night in July/August/September (winter), and again in October/November/December 2004.

Subjects: Each bar was visited by a group of 4-5 non-smokers who had volunteered for the study. All groups of volunteers spent a three-hour block of continuous time in the bar, recording time of arrival and departure. Each of the six groups of volunteers visited a total of five bars.

The volunteers in this study had pre-visit cotinine levels that confirmed their self-identification as non-smokers. In four instances out of 275 individual visits (1.4%), samples were discarded for the purposes of statistical analysis because the pre-visit cotinine level was > 4.0 ng/mL.

Saliva collection: Saliva samples (approximately 0.5 - 2 mLs) were provided immediately prior to entering the bar, and 5-15 minutes after leaving the bar. Saliva samples were stored in individually labelled Salivette tubes from a chilled container, and were stored at 4 C before being sent by courier to the ESR laboratory in Porirua for storage at –20 C and subsequent analysis

Data collection: Each group recorded the initial impression of air quality and ventilation, the number of observed lit cigarettes over three 10-minute intervals throughout the evening, and the number of patrons at each interval, in addition to any general comments about the venue relevant to bar attendance or air quality on the evening.

Analysis of the saliva samples was done using Liquid Chromatography with tandem Mass Spectrometry (LC-MS-MS).

1. INTRODUCTION AND BACKGROUND

1.1. Ethical Approval

Ethical approval was sought from the three centres where this research was proposed to be conducted. Conditional approval from all three sites, as fed back through the Wellington Ethics Committee was granted under reference No. 04/07/045. All subjects were given information sheets describing the risks of exposure to SHS, and signed consent forms for all volunteers were given to the study co-ordinators.

2. METHODOLOGY

2.1 Selection of Regions

Bar exposures were measured in Auckland, Wellington, and Invercargill. Both Auckland and Wellington centres represent a large proportion of the New Zealand population. Certain areas within these centres were sampled that have higher than average Maori and Pacific People resident demography. Invercargill was chosen to provide representation of a smaller population centre. Rural bars were also chosen from the three regions.

2.2 Selection of Bar Venues

Bars were selected randomly from three areas using lists of licenced premises obtained from the local Public Health Units. The random selection was stratified to include one or more 'rural' bars from each area. All venues were visited by one of the investigators prior to their inclusion. To be included, venues had to be classified as clearly representing bars/taverns, rather than venues that were primarily restaurants with, for example, a side waiting area serving alcoholic beverages. In addition, venues that had only recently opened (less than a year old), or had changed management in the past year were excluded in order to gain stable venues. Venues that had attendance that was primarily driven by the popularity of particular nightly events (music bands, etc) were excluded. Finally, venues had to be deemed to be safe and acceptable places to send groups of volunteers.

A description of selected bar venues is shown in Table 1. Individual venues are not identified because the study is of a random sample only.

2.3 Selection of Study Participants

Volunteer, non-smoking subjects, were recruited from within ESR Wellington and Auckland offices, and from local Public Health Units in Auckland and Wellington. In Invercargill, participants from a local teacher's college were recruited through help from Public Health South. The age range of all participants was 24-45 years old. Each group consisted of five individuals (three females and two males). Each group

of volunteers visited a total of five different venues over an eight-week period, on Friday or Saturday nights, spaced 1-2 weeks apart to ensure levels of salivary cotinine returned to baseline levels. Occasional substitutes in each group occurred due to various commitments for some participants.

2.4 Study Design

Fifteen bars were chosen from the Auckland area, 10 from Wellington, and five from Invercargill. A power calculation was based on a pilot study conducted in Wellington (Woodward et al., 2003). The results of this calculation were that a sample size of 5 individuals per venue would suffice to detect differences pre and post bar visit in salivary cotinine. In addition, the power calculation indicated that a venue number of 29-30 would suffice to detect significant variations between venues for the purposes of comparison.

Bars were visited in July/August/September 2004 (winter), and again in October/November/December (spring) on a Friday or Saturday night, for a three-hour stay between 1800 and 2400 hours. The average starting time in the study was 19:19 hours. During the visit, participants recorded the time of entry, the number of patrons and lit cigarettes in three 10-minute intervals evenly spaced throughout the three-hour visit. Volunteers attempted to neither seek out, nor avoid particularly smoky parts of the bar, but rather to try and capture a representative setting. All five volunteers stayed together throughout each three-hour bar visit.

Two follow-up cycles are scheduled to be completed at the same times in 2005. The second half of the study will control for the month, the night of the week, and, as much as possible, the time of day that the venue was sampled in this first half in order to make valid temporal comparisons in order to effectively evaluate the impact of the Smokefree Environments Amendment Act 2003 on levels of SHS exposure for patrons in New Zealand bars.

Saliva (0.5 – 2.0 mL) was provided into a Salivette plastic tube just prior to entering the venue, and 5-15 minutes after exiting the venue. The tubes were stored in a cooler until they could be placed in a –20C freezer for longer term storage. Studies in our laboratory show that cotinine in saliva is very stable, losing less than 10% after one week at room temperature (data not shown).

2.5 Analytical methods

Non-smokers, living in a non-smoking environment, carried out all extractions and extreme care was taken to ensure that contamination was eliminated. All glassware used in the analysis was pre-rinsed with methanol to ensure that no cotinine was present. Saliva (0.5 mL) was pipetted into a pre-rinsed 7 ml silanised culture tube. Each sample was spiked with 50 μL of cotinine D3 internal standard solution and capped immediately with caps with clean Teflon liners. The samples along with the extracted standards were then vortexed briefly and the internal standard was allowed to equilibrate for 5 minutes. These were basified with 0.5 mL 2.0 M potassium

carbonate, and then 3 mL ethyl acetate was added. The tubes were recapped and mixed for 15 minutes on the vortex mixer. The tubes were centrifuged and the ethyl acetate was transferred to clean culture tubes, which had been pre-rinsed with hexane. Glacial acetic acid (30 μL) was added to each tube, and then the ethyl acetate was evaporated just to dryness in Savant evaporator. The dry residue was reconstituted in 100 μL 10:90 acetonitrile and deionised water. The samples and standards were again capped with clean caps and vortexed briefly and then sonicated for 10 minutes. The samples were placed into micro inserts with rubber feet and immediately put in microvials and capped tightly.

Analyses were conducted using a Shimadzu 10AVP HPLC system attached to a Applied Biosystems API 300 Triple Quadrupole mass spectrometer equipped with a TurboIonSpray ion^{R} source. The HPLC column used was a Phenomenex Synergi^{TM} 4μm Polar, 75x2.0mm ID. The mobile phase was a gradient of acetonitrile and 5 millimolar ammonium acetate.

The mass spectrometer was used in the Multiple Reaction Monitoring mode. Transition ions monitored were at m/z 177→80 for cotinine and 180→80 for cotinine D3.

A nine point standard curve over a concentration range of 0 to 40 ng/mL was created by spiking 0.5 ml of Barnstead H_2O with cotinine standards. The analyses were carried out over seven separate runs on seven different days. As the lower end of the calibration was considered of greater significance, the calibration was weighted using a $1/\chi 2$ weighting. The mean correlation coefficient was 0.995.

The intraday reproducibility (five replicates) of the standard (0.3 ng/mL) had a CV of 9.4 %. The interday CV (3 days) was 14.5 %.

The detection limit was at least as good as 0.1 ng/mL of cotinine in 0.5 mL of saliva.

3. RESULTS

The individual bar results for mean cotinine increases after a three hour visit are shown in Table 1 in order of least to highest exposure to SHS. Bar names are not shown. Subjective ratings of air quality by the patrons are also shown (1 = seemingly smokefree, 2 = mildly smoky, 3 = moderately smoky, and 4 = severely smoky).

There was a eight-fold range of increases between the least smoky venue in Auckland and the smokiest venue in Invercargill.

Table 1. Mean increases in saliva cotinine in non-smoking patrons from randomly selected New Zealand bars in three regions (August – September 2004).

Bar Venue	Region	Cotinine Increase after 3 hours			Subjective Air Quality Rating		
		Winter	Spring	Overall	Winter	Spring	Overall
1	Auckland	0.305	0.085	0.195	1	2	1.5
2	Wellington	0.242	0.148	0.195	1	1	1
3	Auckland	0.326	0.114	0.220	1	2	1.5
4	Auckland	0.218	0.23	0.224	1.5	2.5	2
5	Auckland	0.398	0.17	0.284	2	1	1.5
6	Auckland	0.358	0.246	0.302	1	1	1
7	Auckland	0.386	---*	0.386	2	nr	2
8	Wellington	0.705	0.086	0.396	3	4	3.5
9	Auckland	0.417	---*	0.417	4	nr	4
10	Wellington	0.442	0.42	0.431	3	2	2.5
11	Auckland	0.504	0.3725	0.438	2	2	2
12	Auckland	0.605	0.325	0.465	nr	3	3
13	Auckland	0.654	0.362	0.508	2	1	1.5
14	Wellington	0.310	0.708	0.509	1	3	2
15	Wellington	0.535	0.506	0.521	1	1	1
16	Auckland	0.462	0.58	0.521	1.5	1	1.25
17	Auckland	0.650	0.456	0.553	3	nr	3
18	Auckland	0.696	0.508	0.602	1	2	1.5
19	Wellington	0.768	0.47	0.619	2	2	2
20	Wellington	1.344	0.055	0.700	2	1	1.5
21	Wellington	0.898	0.724	0.811	3	4	3.5
22	Auckland	1.498	0.136	0.817	3	2	2.5
23	Invercargill	0.922	0.764	0.843	4	4	4
24	Invercargill	1.166	0.594	0.88	4	4	4
25	Auckland	1.192	0.606	0.899	2	2	2
26	Wellington	1.153	0.65	0.903	4	2	3
27	Wellington	1.128	0.798	0.963	3	2	2.5
28	Invercargill	0.622	1.568	1.095	2	2	2
29	Invercargill	1.694	0.988	1.341	3	3	3
30	Invercargill	1.904	1.305	1.605	4	4	4
Total		0.747	0.499	0.627			

* = not sampled, due to logistical difficulties with volunteers
nr = not recorded

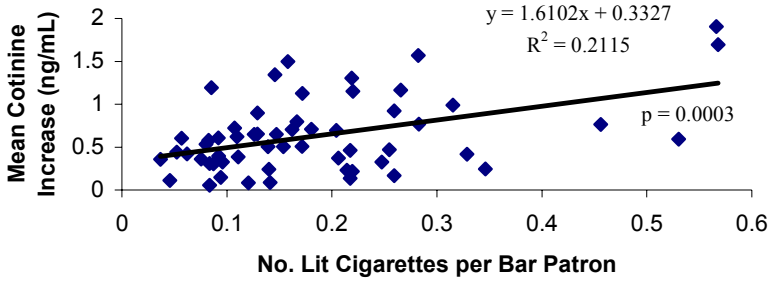
In all bars, and in all volunteers, a significant increase in saliva cotinine was seen after three hours in the bar (mean increase = 0.63 ng/mL; starting concentration = 0.24 ng/mL, ending concentration = 0.87 ng/mL, $p < 0.0001$). The increases in cotinine were greater in Invercargill bars. The difference between Auckland and Wellington bars was not statistically significant ($p < 0.27$). Increases in saliva cotinine in the Invercargill bars (1.27 and 1.04 ng/mL for winter and spring, respectively) were more than double those seen in Auckland (0.57 and 0.32 ng/mL, respectively, $p < 0.003$, 2-tailed t-test with unequal variance), and were double those

seen in Wellington (0.74 and 0.46 ng/mL, respectively, $p < 0.05$, 2-tailed t-test with unequal variance).

The average overall increase in cotinine was 0.75 ng/mL in winter vs 0.50 ng/mL in spring ($p = 0.025$, 2-tailed t-test with unequal variance).

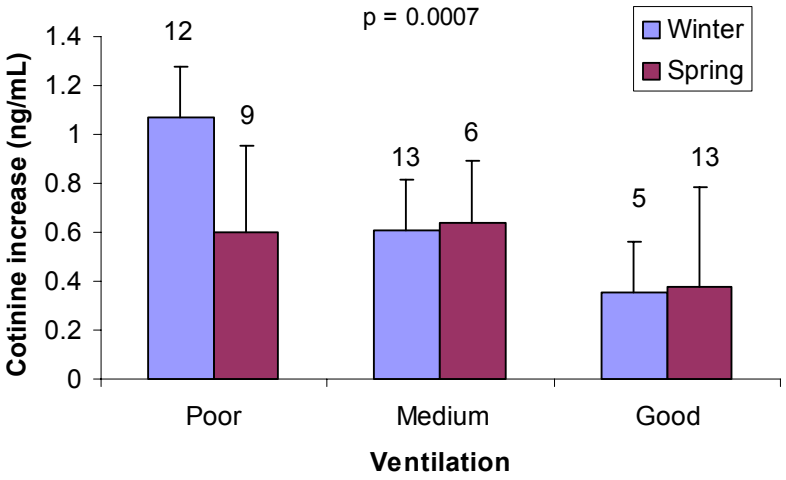
The increases in saliva cotinine were found to be correlated with the observed total number of lit cigarettes across three 10-minute counting intervals in the bar visit, and the correlation was similar when the cigarette count was adjusted for the total number of patrons (excluding the volunteers) in the bar (Figure 1).

Figure 1. Increase in Salivary Cotinine After 3 Hours vs No. Lit Cigarettes/Patron



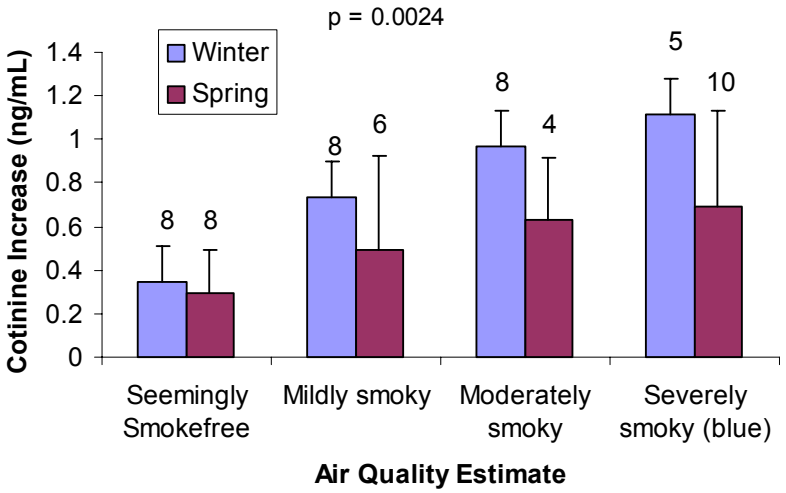
A clear relationship between patrons' perception of the venue's ventilation level and the degree of increase in saliva cotinine was observed (Figure 2). In Figure 2, means and standard deviations are shown. Increases in saliva cotinine were seen even in 'seemingly smokefree' venues with 'good' ventilation. The number of bars in a given category is shown on the graph. There was a statistically significant difference in cotinine increases with ventilation ratings, using one-way, two-tailed, ANOVA. The figure shows that 'poor' ventilation resulted in considerably greater SHS exposures in the winter months, whereas 'medium' and 'good' ventilation venues did not exhibit a seasonal difference in SHS exposures.

Figure 2. Saliva Cotinine vs Ventilation Quality



Similarly, study subjects filled in a questionnaire rating the perception of smokiness in the venue, on a scale of 1 to 4, as follows: 1 = seemingly smokefree, 2 = mildly smoky, 3 = moderately smoky, and 4= severely smoky (blue). Figure 3 shows that these groups' subjective ratings of air quality corresponded predictably with the degree of increase in saliva cotinine in the group ($p = 0.0024$ for one-way, two-tailed, ANOVA). Means and standard deviations are presented in Figure 3, and the number of venues in each category is shown on the graph. Figure 3 shows a consistent seasonal drop in SHS exposures in spring vs winter. Both seasons show that patrons in 'seemingly smokefree' venues were exposed to SHS during their visit.

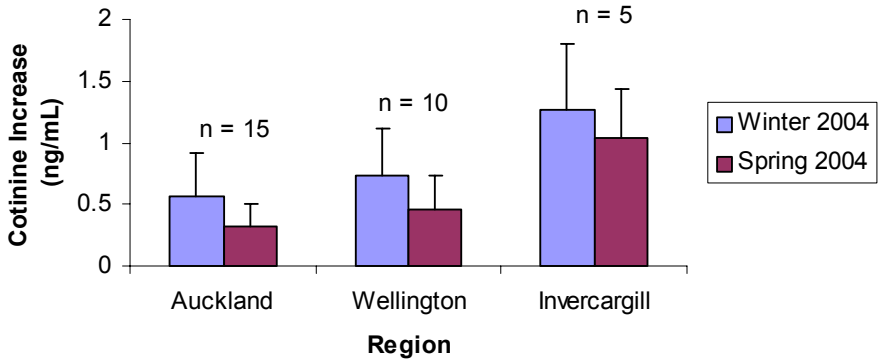
Figure 3. Saliva Cotinine vs Perceived Air Quality



Regional Variations

The pattern of cotinine increases across the three regions is shown in Figure 4. Auckland and Wellington were broadly comparable, while the increases in Invercargill were significantly greater than those seen in the other two areas regardless of season ($p < 0.05$).

Figure 4. Saliva Cotinine Increases in Three Regions



4. DISCUSSION

This is the first study in New Zealand to survey, on a national level, the quantitative amount of exposure to SHS in non-smoking bar patrons. This information forms a baseline exposure estimate in these venues for Part 2 of the project, which will measure levels of exposure to SHS during 2005 (smokefree changes take effect from 10 December 2004).

Effective Measure of SHS Exposure at Seemingly Smokefree Venues

The saliva cotinine analysis provides a quantitative and objective measurement that confirmed subjective measures of air quality in the study. However, it was evident that the saliva cotinine level was effective at quantifying SHS exposures even when individuals perceived venues to be "seemingly smokefree", and to have "good" ventilation. Thus, the analytical method provides a more accurate estimate of SHS exposures when the exposures are at the low end of the typical range.

This finding is significant as it shows that improved ventilation is unlikely to remove patron exposure to SHS in bars that allow smoking, even if non-smoking bar patrons fail to notice their own exposures. Subjective ratings of 'seemingly smokefree' corresponded to about 30 - 40% of the SHS exposure in a place that was "'severely smoky – blue'.

Regional Variation

The study covered urban, suburban, and rural areas in three centres and found similar relationships between subjective measures of air quality and salivary cotinine at each site. It is noteworthy that the less densely populated areas showed greater exposures to SHS. The 30 bars randomly selected in this study provided a good spectrum of SHS exposures, with mean cotinine increases spanning 8-fold, and also provided a corresponding range of subjective ratings of air quality and ventilation.

Seasonal Variation

Higher exposures were seen in the winter than in the spring, but because this was a one-time-only study it is impossible to say whether this is due to patron behaviour voluntarily changing as the deadline for the ban approached, or whether this represents a real seasonal difference.

Underestimate of SHS Exposure due to Delay in Peak Cotinine

The half-life of cotinine in various body fluids has been reported to range from 16-19 hours (Jarvis et al., 1988). On the other hand, nicotine has a half-life of roughly 2 hours depending on ethnicity, and a number of physiological states such as pregnancy (Dempsey et al., 2002). This means that the patrons in the current study would have had peak salivary cotinine levels approximately three to four hours after leaving the bar venue. However, this was not practical as many bar visits were late at night. Therefore the increases in cotinine reported in this study are underestimates of the actual exposures, and primarily reflect the exposures from the first 60-90 minutes in the bar.

The increase in salivary cotinine averaged 0.63 ng/mL at three hours, and it is unlikely that this represents the peak salivary level. Jarvis and colleagues found that

bar staff exhibited salivary cotinine levels on average of 9.28 ng/mL, which corresponded to an estimated nicotine intake of 0.6 mg (Jarvis et al., 1992). The exposures in the current study would suggest that a single 3-hour visit to a pub by a non-smoker results in an exposure that is substantially less, perhaps an order of magnitude less, than that experienced by bar staff. Given that the purpose of the Smoke-free Environments Amendment Act 2003 is to protect workers in particular from exposure to SHS, this study confirms that workers who spend even three hours in a smoky workplace face measurable levels of SHS exposure, and the health effects attendant upon such exposure.

5. REFERENCES

Dempsey D, Jacob P, and Benowitz N. 2002. Accelerated metabolism of nicotine and cotinine in pregnant smokers. *Journal of Pharmacology and Experimental Therapuetics* 301(2):594-598.

Jarvis MJ, Russell MA, Benowitz NL, Feyerabend C. 1988. Elimination of cotinine from body fluids: Implications for non-invasive measurement of tobacco smoke exposure. *American Journal of Public Health* 78(6):696-698.

Jarvis MJ, Foulds J, Feyerabend C. 1992. Exposure to passive smoking among bar staff. *British Journal of Addiction* 87(1):111-113.

Woodward A, and Laugesen M. 2000. Deaths in New Zealand attributed to secondhand cigarette smoke. A report to the NZ Ministry of Health.

Woodward A, Reid P, Fowles J, Dickson S, Berezowski R, and Hosking M. 2005. Increase in saliva cotinine after three hours exposure to second hand smoke in bars. *Australia and New Zealand Journal of Public Health* (in press).