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Assessment of Environmental Tobacco Smoke Exposure in U.S. Navy Submariners

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14. ABSTRACT

The effectiveness of the U.S. Navy and Marine Corps Tobacco Policy in protecting submariners from Environmental Tobacco Smoke (ETS) was evaluated. Exposure to ETS was measured using urinary cotinine as a biomarker. Subjects were recruited from 9 U.S. Navy submarines. Non-tobacco users were identified and provided two urine samples (1. pre-deployment, in harbor; 2. deployed, at sea) to quantify exposure to ETS and the magnitude of any change. Samples were analyzed using liquid chromatography - tandem mass spectrometry (LC-MS/MS). Overall, deployed cotinine levels were 2.1 times as much as in port levels in non-tobacco using submariners (95% CI: 1.8 to to 2.4, $p < 0.001$). This study provides evidence that non-tobacco using submariners were being exposed to ETS. Exposure was seen in all submarine classes and was not limited to personnel working in proximity to the smoking area.

15. SUBJECT TERMS

Submarine, Navy, Occupational, Environmental, Exposure, Environmental Tobacco Smoke, ETS, biological monitoring, smoking policy.

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The views expressed in this report are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. This work was done by U.S. Government employees as part of their official duties and therefore cannot be copyrighted. This work was supported by and/or funded by work unit number50703. The study protocol NSMRL.2007.0010 was approved by the Naval Submarine Medical Research Laboratory Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.

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CONTENTS

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SUMMARY

Problem.

Nicotine has been identified as a contaminant of submarine atmosphere during *Virginia* Class sea trials; however the extent to which non-tobacco using submariners are being exposed to environmental tobacco smoke (ETS) has not been adequately quantified.

Objective.

To quantify exposure to environmental tobacco smoke, in non-tobacco using submariners while underway by measuring any change in their level of urinary cotinine (a nicotine metabolite) after at least 10 days underway, when compared to exposures in port.

Findings.

Overall cotinine levels among deployed non-tobacco using submariners were twice as high as in port cotinine levels among non-tobacco using submariners (95% CI, 1.8-2.4, $p < 0.001$). Groups of subjects from six of the nine submarines that participated in the study showed a statistically significant increase in urinary cotinine ($p < 0.05$). A subgroup of submariners who reported no ETS exposure within 10 days prior to in port cotinine sampling had deployed cotinine levels 2.7 times as much as in port levels (95% CI, 2.2-3.3, $p < 0.001$).

Application.

Current U.S. Navy policy for submarines to allow smoking in designated spaces onboard should be reviewed.

BACKGROUND

The submarine's commanding officer is permitted to designate smoking areas aboard his submarine within certain guidelines.[1] The Nuclear Powered Submarine Atmosphere Control Manual[2] provides recommendations for placement of smoking areas but contains no evidence of the capability of atmosphere control equipment to remove ETS. In his 2006 report the United States Surgeon General concluded, "there is no risk-free level of exposure to secondhand smoke" and "separating smokers from nonsmokers, cleaning the air, and ventilating buildings cannot eliminate exposures of nonsmokers to secondhand smoke".[3] In addition this report comments on the massive and conclusive scientific evidence that documents the adverse health effects of involuntary smoking including cancer, cardiovascular disease and respiratory disease in healthy nonsmokers.

Nicotine is the principal alkaloid present in tobacco and is present in all tobacco products. The amount of nicotine present in cigarettes varies between 1-7% (6-12 mg nicotine/cigarette) and of this 15-25% enters the mainstream smoke and 75% enters the air as sidestream smoke.[4]

Non-smokers can absorb nicotine through their lungs. Consequently nicotine in air has been extensively used as a marker of exposure to ETS.[5] It should be noted that nicotine is not considered to be a toxicological component of ETS. When an individual is exposed to ETS over time their intake of nicotine reflects their exposure to the other constituents of ETS.[6] However, nicotine has a relatively short half-life (in the body in the order of two hours) and therefore is not suitable as a biomarker of exposure in non-smokers. Nicotine is metabolized in the body by hydroxylation to produce cotinine, which in turns is further metabolized to cotinine glucuronide, trans 3-hydroxyxotinine and trans 3-hydroxycotinine glucuronide.[7] Cotinine and its metabolites account for 70-80% of nicotine metabolism in humans.[8-11] Cotinine has a half-life in the body in the order of 16-20 hours.[12] Cotinine can be measured in blood, saliva, hair or urine. The presence of cotinine in body fluids is indicative of exposure to nicotine. Estimation of cotinine in blood, saliva or urine is currently the most widely accepted biomarker for the assessment of exposure to environmental tobacco smoke.[5] A more recent review concluded that urinary cotinine is a more reliable biomarker of exposure to ETS than saliva.[13] This has been further supported by a recent study of exposure to environmental tobacco smoke in Italian women. This concluded that salivary and urinary cotinine levels might be more applicable than plasma cotinine levels in epidemiological studies because of its higher sensitivity, higher inter-correlation and their non-invasive character.[14] Hair nicotine levels can also be used a marker of exposure to ETS.[6] However given the prevalence of very short hairstyles, particularly among junior enlisted sailors, hair is considered to have very limited utility as a marker of ETS exposure within the U.S. submarine force. Additionally it is invasive and may not be acceptable to study volunteers.

The concern that submariners were being exposed to ETS was supported by the identification of nicotine in the submarine's atmosphere in atmospheric sea trials.[15-19] A previous study of ETS exposure in U.S. submariners[20] found a small and non-statistically significant increase in urinary cotinine, depending on location of the smoking area and the individual's workstation (pre-deployment urinary cotinine levels ranged from 0.9-3.1 ng/ml and deployment levels from 0.9-4.6 ng/ml). The study had a number of limitations as it was of small scale and submariners were recruited from only 2 submarines of the same class. In retrospect, and in the light of subsequent guidance[3], this previous study's use of a threshold of 19 ng/ml of cotinine in urine for "significant exposure" to ETS cannot currently be justified. Exposures of this magnitude would now be considered representative of intermittent tobacco use or extreme exposure to ETS and are greater than the 15 ng/ml of cotinine in urine recently recommended to differentiate ETS exposure from active smoking in epidemiological studies.[21]

Given ongoing concerns over the adequacy of the policy in protecting non-smokers from exposure to ETS[22,23] and competing arguments regarding the validity of extrapolating advice from studies of "normal" workplaces with commercial HVAC systems[3] the Naval Sea Systems Command (NAVSEA) concluded that further research was required and tasked the Naval Submarine Medical Research Laboratory (NSMRL) to conduct this study.

METHODS

Subjects

Volunteers were recruited from the crews of 9 submarines (3 *Los Angeles*, 3 *Ohio*, 2 *Virginia*, and 1 *Seawolf* class). All 4 classes of submarine were sampled due to the potential for different ventilation systems and internal volumes affecting personnel exposures. Submarines from both the Atlantic and Pacific Fleets (SUBLANT and SUBPAC) were represented.

All volunteers were active duty male submariners. Researchers visited the submarine ports ensuring that the crew had been in harbor for a minimum of ten days so that baseline samples were representative of shore-side exposures. Researchers also recorded the location of the smoking area onboard.

Investigators briefed the crew and volunteers provided written consent and completed a 1 page self-administered questionnaire (Appendix 1) which collected basic demographic data including age, rank, years of service, duty station at sea, current and history of tobacco use, and any exposure to environmental tobacco smoke within the last 10 days. Three opinion questions regarding smoking aboard submarines were also included but are the focus of a separate paper. The questionnaire was used to identify non-tobacco users.

All who declared active use of a tobacco product, including nicotine replacement therapy (NRT), were excluded from the exposure assessment and were considered a tobacco user. Ex-smokers were allowed to participate only if they had abstained from all tobacco products and NRT for six months or more. All non-tobacco using submariners were considered eligible for inclusion in the study and a distinction was made between nonsmokers with and without self-reported ETS exposures ashore.

The study was approved by the Naval Submarine Medical Research Laboratory's Human Research Protection Program's Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.

Procedures

Non-tobacco using volunteers were asked to provide two urine samples, a pre-deployment sample (exposure in port) to measure exposures in a harbor environment and a deployed sample (at sea while dived) representative of the operational environment. Given that cotinine has half-life in the body of 16-20 hours[4,5,7] and that after 10 half-life the levels would be expected to have reached a steady state, a minimum of ten days in port was also required for pre-deployment samples. Independent Duty Corpsmen (IDCs) collected deployed samples at any time 10 days after the submarine had been dived and before the submarine surfaced. Samples were packaged and frozen at -20°C. When sample collection was complete they were packaged on dry ice and shipped by courier to the Clinical Pharmacology Laboratory at the University of California San Francisco (UCSF) and stored frozen until they were analyzed in pairs. Concentrations of cotinine and *trans*-3'-hydroxycotinine (3HC) (both nicotine metabolites) in urine were determined using recently improved methods (Jacob et al, unpublished data, 2010) for liquid chromatography - tandem mass spectrometry (LC-MS/MS). It is similar to methods used elsewhere[24] and has high sensitivity and specificity (detection limit 0.05 ng/ml for cotinine).[25]

A cutoff of 15 ng/ml of urinary cotinine concentration[21] was used to validate tobacco status, reducing the potential for misclassification bias. Any cotinine levels found below 0.05 ng/ml were replaced with a value of 0.05 ng/ml.

For valid samples, Pearson correlations were performed between the in port cotinine and in port 3HC levels; and the deployed cotinine and deployed 3HC levels to ensure samples were not mislabeled in processing or transit. Others have found 3HC and cotinine to correlate highly.[26]

Unlike the previous submarine study[20], the number of cigarettes smoked while underway was not counted, rather a proxy measure of ETS exposure by submarine was estimated based on the proportion of those surveyed (N=634) identified as a current smoker.

Statistical analyses

Urine cotinine levels were logarithmically transformed using the natural log, ln(x), to correct for positively skewed distributions. Log-transformed data were analyzed using the SPSS MIXED procedure (SPSS Inc., version 16, Chicago, IL) to examine change between in port and deployed cotinine levels. Separate models were constructed to examine ETS effects for all valid nonsmokers, and for a subgroup of nonsmokers who reported no prior in port ETS exposure in order to measure ETS effects absent of any baseline ETS. The antilogs of the means and mean differences and 95% confidence intervals (CI) were calculated. Results are presented as geometric mean (GM) cotinine concentrations and the ratio of the deployment/in port GM cotinine concentrations back in the original scale. When significant main effects or interactions occurred, Bonferroni[27] post hoc multiple comparison tests were employed. All statistical tests were non-directional and significant probability level was set at $p < 0.05$.

RESULTS

As shown in (fig 1), 634 volunteers completed the screening questionnaire and 329 non-tobacco users were eligible for the exposure study; of these, 239 non-tobacco using subjects (73% of those eligible) provided in port urine samples.

A total of 206 deployed urine samples were received and all samples were analyzed, in batches, for cotinine and 3HC in urine. The 33 unpaired in port samples were not included in the data analyses. Written reports on concentrations of cotinine and 3HC in urine were emailed to NSMRL and data were stored, by subject number, in an anonymised Micro Soft Access database in accordance with HIPAA requirements.[28]

Nine subjects had urinary cotinine levels >15 ng/ml in one or both samples. These individuals were excluded from any data analysis as probable intermittent smokers or users of smokeless tobacco. A total of 197 matched pairs of samples were considered valid and included in the data set for statistical analysis. This was an estimated 15% capture of all submariners assigned to the 9 submarines. Cotinine and 3HC levels sampled in port were highly correlated (Pearson $r = 0.86$; $p < 0.001$; n = 197) as were the deployed measurements (Pearson $r = 0.74$; $p < 0.001$; n = 197). Based on self-reports, 106/197 (54%) of these individuals reported some in port ETS exposure.

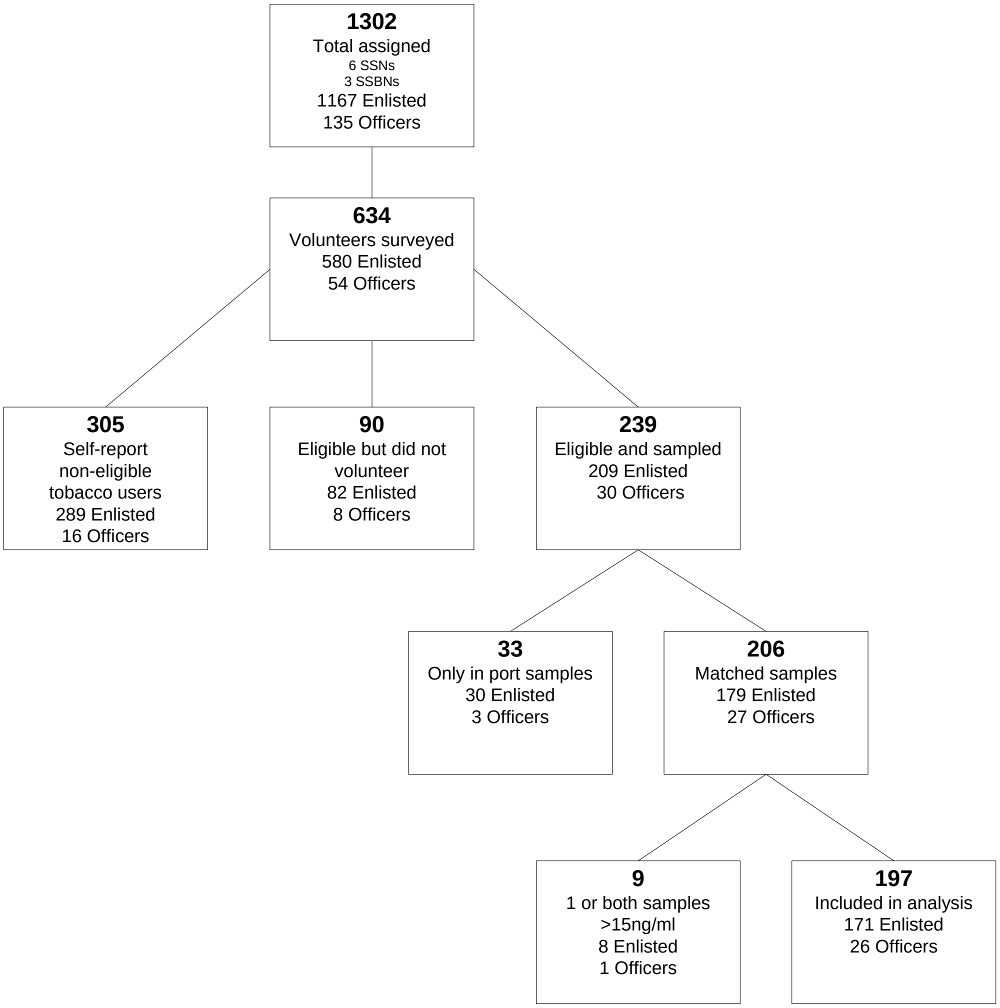

Figure 1. Flowchart of the recruitment process.

Subject characteristics

The median age of the 192 out of 197 submariners included in the data analyses who reported age was 26 years (range, 19-45 years). The median length of Navy service for all 197 participants was 5 years (range, 1-26 years), and median length of service in the submarine force was 3 years (range, 0-25 years). A total of 171 (87%) enlisted and 26 (13%) officers were included, and most (80%) were enlisted personnel with a rating in the E3 to E7 range.

The characteristics of each submarine sampled including number of smokers surveyed are shown in table 1. Based on self-reports, estimates of ETS exposure by submarine showed the highest proportion of smokers was aboard OHIO1 (52%), and an overall rate of 39%. Submarine by type is also listed and includes total sampled by hull.

Table 1. Survey counts, matched samples, and number of smokers by submarine.

Submarine ID[a]	Submarine Type	Officers assigned	Enlisted assigned	Total assigned	Surveyed No. (%)	Smokers No. (%)	Matched sample
VA1	SSN	14	120	134	49(37)	16(33)	20
VA2	SSN	14	120	134	73(54)	25(34)	32
LA1	SSN	16	127	143	69(48)	25(36)	23
LA2	SSN	16	127	143	56(39)	15(27)	21
LA3	SSN	16	127	143	65(45)	25(38)	27
SEA	SSN	14	126	140	47(34)	21(45)	10
OHIO1	SSBN	15	140	155	87(56)	45(52)	13
OHIO2	SSBN	15	140	155	112(72)	42(38)	29
OHIO3	SSBN	15	140	155	76(49)	32(42)	22
	Totals	**135**	**1167**	**1302**	**634(49)**	**246(39)**	**197**

[a]Submarine ID is also used to identify class, e.g. SEA represents a Seawolf class submarine.

Cotinine measurements

The distribution of the raw in port and deployed urinary cotinine levels is shown (fig 2). While most in port levels are clustered below 0.25 ng/ml, a positive shift is seen when compared with the deployed levels. Prior to log-transformations, both in port and deployed distributions were positively skewed.

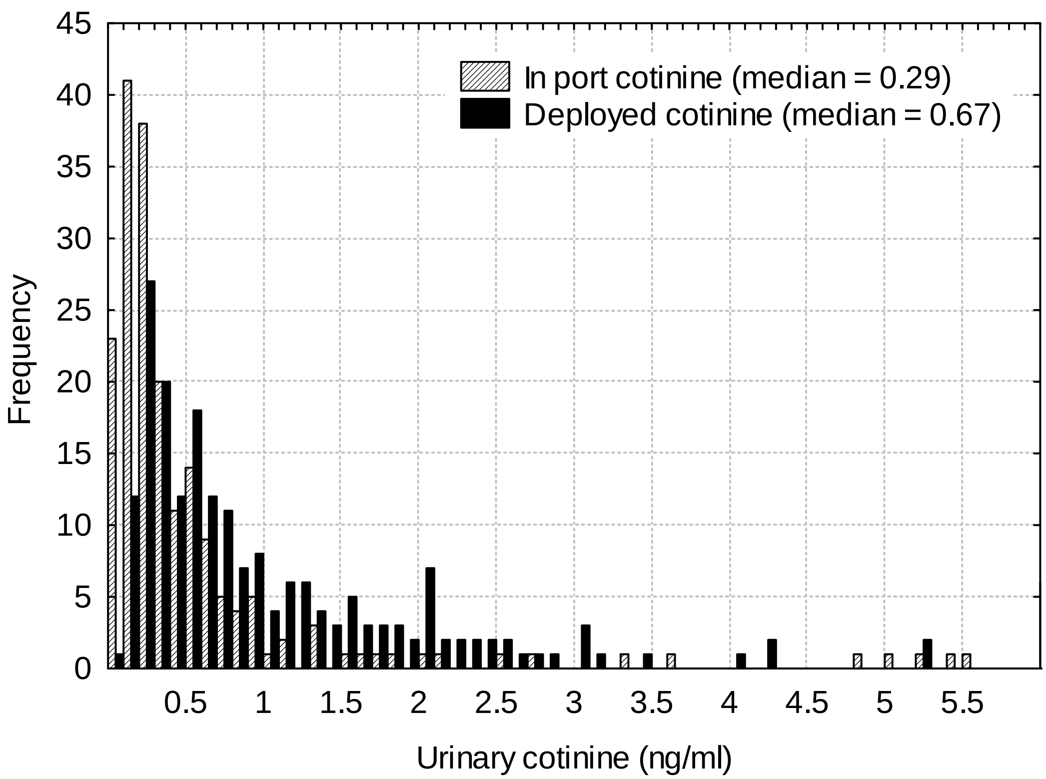

Figure 2. Distribution of urinary cotinine concentrations by sampling environment (n = 197). Five extreme in port values (i.e., 6.8, 7.0, 7.1, 7.7, 10.5 and 11.8 ng/ml) are excluded from graph.

Changes in Cotinine Levels

Log-transformed deployed cotinine levels were 2.1 times (95% CI, 1.8-2.4) higher than the in port levels found in non-tobacco using submariners ($F_{1,188} = 89.0$, $p < 0.001$). An interaction effect for deployment by submarine was also found ($F_{8,188} = 10.3$, $p < 0.001$) as a significant ($p < 0.05$) increase in urinary cotinine levels only occurred aboard 6 of the 9 submarines that participated in the study (fig 3). The LA3, OHIO2, and OHIO3 crews did not show significantly positive shifts in GM urinary cotinine levels when deployed. The GM deployment/in port ratios for the 6 boats showing significant shifts ranged from 1.5 on the LA1 to 5.8 on the VA2. While crews for both VA class submarines showed the largest GM increase in urinary cotinine levels, no similarities were evident among the LA or OHIO class crews.

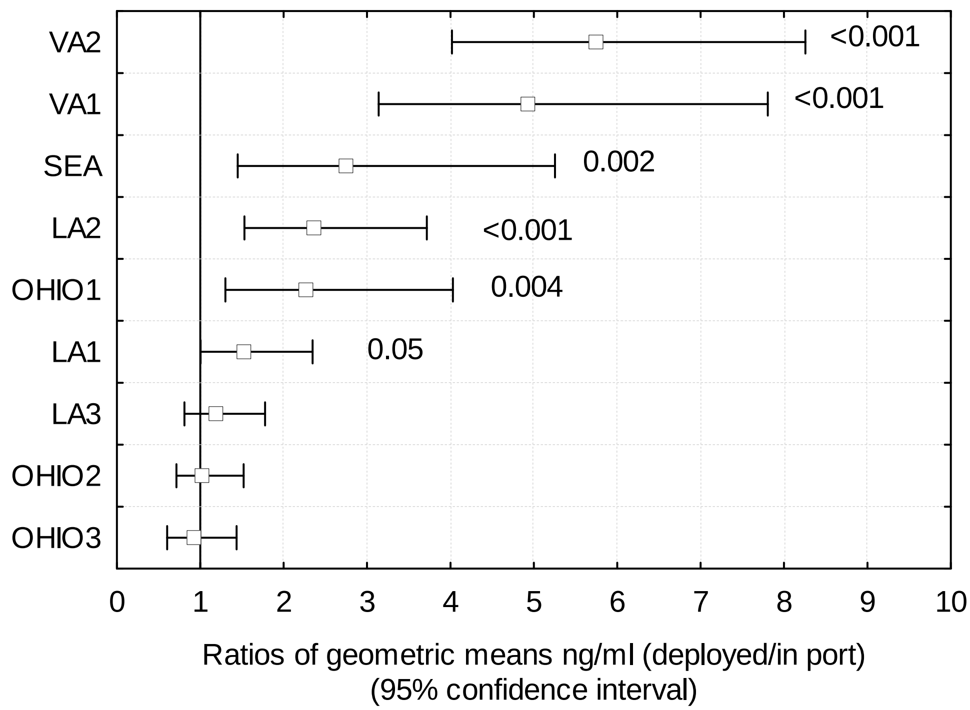

Figure 3. Group changes in urinary cotinine concentration by submarine. *P*-values are shown where changes were significant at $p < .05$. Submarines are plotted by GM ratio in descending order.

Comparing submarines by environment (i.e., in port versus deployed) showed significant differences occurred among both the in port and deployed cotinine measurements. The crews aboard the OHIO1 and LA3 had the two highest in port urinary cotinine levels (GM) and for both submarines; these levels were significantly higher than levels measured on crews aboard the LA1, LA2, VA1, and SEA (fig 4). The OHIO3 crew was also found to have significantly higher levels than the VA1 and SEA crews. No other in port comparison was significant.
For the deployment samples, the VA2 and OHIO1 crews had the highest cotinine levels (fig 5). The crew aboard the VA2 had levels significantly higher than all but the OHIO1 and VA1 crews. The OHIO1 crew was significantly higher than all but the two VA class crews and the LA3. The

third highest urinary cotinine levels were found on the VA1 and these were significantly higher than those measured on the OHIO2 and the LA1.

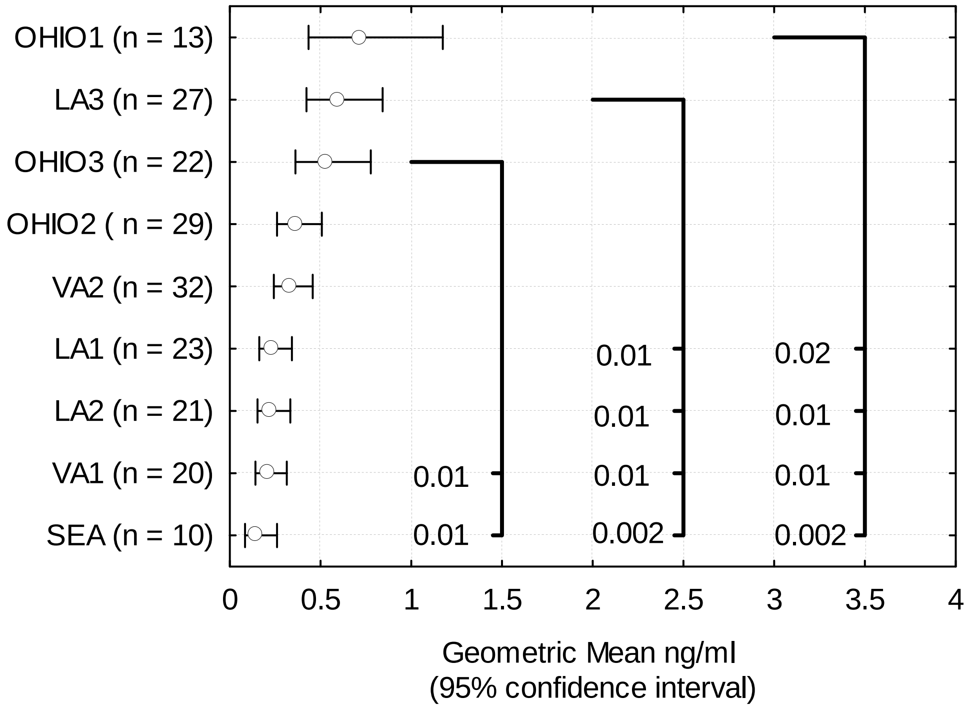

Figure 4. In port geometric mean values for urinary cotinine concentration by submarine. For significant comparisons, *p*-values are shown. Submarines are plotted by GM in descending order.

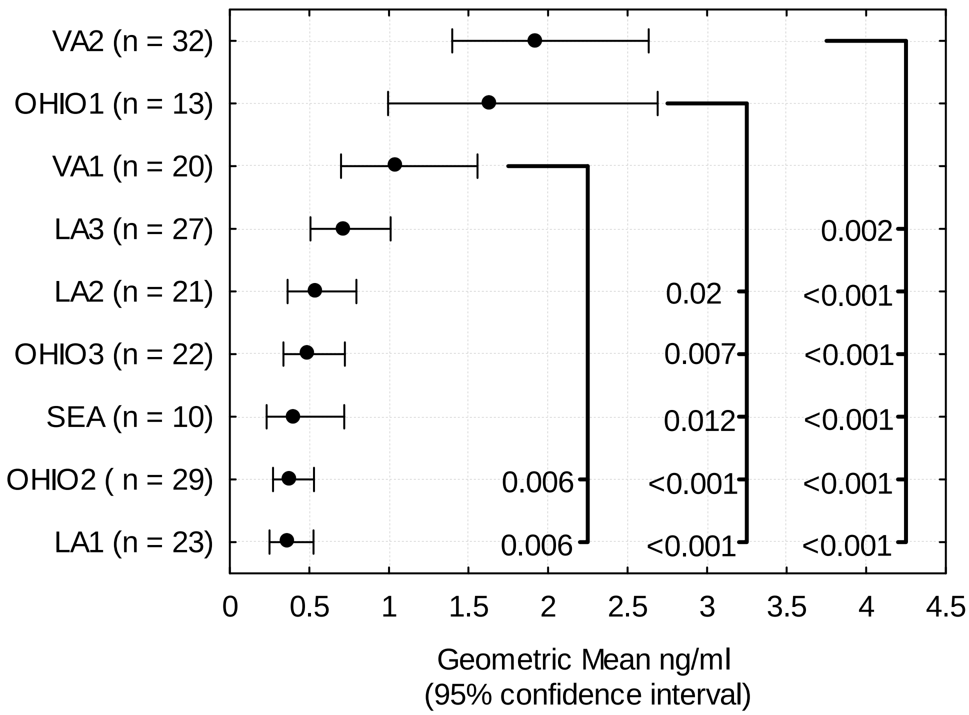

Figure 5. Deployed geometric mean values for urinary cotinine concentration by submarine. For significant comparisons, *p*-values are shown. Submarines are plotted by GM in descending order.

Changes in Cotinine Levels for Submariners without In Port ETS

An analysis on the subgroup of nonsmokers (n=91) who reported no prior in port ETS exposure for at least 10 days prior to in port cotinine sampling was done separately. This subgroup had deployed cotinine levels 2.7 (ratio of GM) times that of the in port levels (95% CI, 2.2-3.3, p <0.001). Available data for the SEA and LA3 were reduced to n = 5 and n = 2, respectively, thereby removing them from individual submarine in port versus deployment level comparisons. However, for the remaining submarines the changes in cotinine showed significantly ($p < 0.05$) higher levels for deployed samples for all but the OHIO3 crew who did not show a significant change (GM = 0.8, $p = 0.38$). The GM deployment/in port ratios for the 6 crews that showed significant increases with sufficient sample sizes ranged from 1.7 on the OHIO2 to 8.9 on the VA1.

Smoking prevalence and cotinine levels

Of those surveyed, OHIO1 had the highest proportion of smokers (52%) (table 1) and the highest (GM = 1.6 ng/ml) cotinine levels in its class when deployed. The same was true for the LA class submarines where the LA3 had the highest proportion of smokers (39%) and the highest (GM= 0.7 ng/ml) cotinine levels. In addition, for the 2 VA class submarines that were surveyed, the VA1 with the highest (GM = 1.9 ng/ml) cotinine concentrations also had a higher proportion of reported smokers (34%). However, when a Spearman rank ordered correlation was performed between proportion of smokers and the GM of deployed urinary cotinine concentrations, no significant relationship (Spearman ρ = -0.08; p = 0.83; n = 9) was found.

Cotinine levels and proximity to smoking area

Duty station at sea was matched to the smoking area onboard and proximity to smoking area by individual was categorized as being different, adjacent, close, or unknown. The vast majority (82%) of those sampled were assigned to a duty station ‘different’ from the designated smoking area. Of those who were determined to work adjacent or close to a smoking area (17%) their median increase in cotinine was 0.28 ng/ml whereas the median increase for those in duty station different from the smoking area was 0.22 ng/ml.

CONCLUSIONS AND DISCUSSION

This study was the first comprehensive study of ETS exposures in U.S. Navy submariners and the use of a well established, valid, sensitive and specific biomarker[3,6,29] to quantify U.S. Navy submariners' exposure to ETS.

Applying a conservative cutoff of 15 ng/ml cotinine in urine[21] excluded subjects who were potentially misclassified as non-tobacco users. The use of paired samples controlled for the effects of many potential confounders such as individual variations in nicotine metabolism rates, age, and minimized dietary intake effects as these may also influence the excretion of the nicotine metabolites.

While submarine ventilation systems are unique and incorporate highly specialized air cleaning equipment that is not typical of standard ventilation systems found in homes and workplaces, they did not prevent non-smoking submariners from being exposed to nicotine from environmental tobacco smoke. This study provides objective evidence that submarine atmosphere control equipment does not remove the entire nicotine component of ETS. While other constituents of ETS (which may contain in excess of 4000 chemical compounds)[30] were not measured; the submarine's atmosphere control equipment is not designed to remove tobacco smoke therefore other atmospheric contaminants caused by ETS are likely to be similarly distributed within this unique enclosed environment. It is thus highly likely that non-tobacco users are being exposed to the hazardous components of ETS.

In summary this study concludes that:

1. Overall, deployed cotinine levels were twice as high as in port cotinine levels, confirming that submariners are exposed to ETS while underway.
2. A subgroup of submariners without in port ETS exposure showed deployed cotinine levels nearly 3 times as high as their in port cotinine levels.
3. Comparisons between submarines of different classes and within a class, showed crews differed in their cotinine concentration levels while at sea and ashore.
4. Without valid estimates on the number of cigarettes being smoked underway, an explanation on the varying levels of ETS between submarine crews cannot be made.
5. Exposure to ETS was not limited to those whose watch station was in close proximity to the smoking area onboard suggesting that the ventilation system is distributing ETS throughout the submarine.

It is clear that administrative and engineering controls to eliminate ETS should be reevaluated. While there may be potential to improve engineering controls, this approach has not been successful in other settings.[31] The elimination of the hazard through the prohibition of smoking aboard represents the most effective and definitive control option to protect nonsmoking submariners from exposure to ETS. Such a policy may also have a wider public health impact as has been observed in other workplaces.[32]

To summarize the major findings, it is apparent that submariners are exposed to ETS underway in a manner that significantly increases the amount of nicotine markers in their body. It is also clear that this effect is present on all classes of submarines despite variations within and between classes.

This study did not attempt to quantify risk; as the U.S. Surgeon General advises that “there is no safe level of exposure to ETS”[3] and Navy and Marine Corps Tobacco Policy states “Where conflicts arise between the rights of non-tobacco users and the rights of tobacco users, the rights of non-tobacco users to a tobacco-free space shall prevail”. [1] Of note, the majority (57%) of never smoking submariners surveyed in the present study responded that smoking should not be allowed on submarines. Consequently, since the majority of nonsmokers preferred a smoke free submarine environment, and exposure to ETS is evident, the current submarine smoking policy is inconsistent with the overarching Navy Policy.

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Appendix I

Subject # ________

ASSESSMENT OF EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE

Please Print Clearly

Date: ____________ (mm/dd/yy) Submarine: ____________________

Last Name: ____________ First Name: ____________ SSN: ___ - __ - ____

Date of birth: ____________ (mm/dd/yy) Rank: ______ Rate: ________ Height: ______ Inches Weight: ______ Pounds

Number of years Navy service: ______ Number of years of submarine service: ______ Current Watch Station: __________

Time from last deployment: ____ ☐ weeks ☐ months ☐ years When not deployed in what state do you live: __________

Please answer the following using your best estimate

Current Tobacco Use Status

☐ Never smoked

☐ Ex-smoker or Ex-user of smokeless tobacco

Do you currently use nicotine replacement therapy (patch, gum, etc.)? ☐ Yes ☐ No

How long ago did you quit? ______ ☐ weeks ☐ months ☐ years

☐ Smokeless Tobacco

How long have you used smokeless tobacco? ______ ☐ weeks ☐ months ☐ years

☐ Snuff, pinches, dips, rubs, pouches

How much do you use per day? ☐ Less than 5 ☐ 6 - 10 ☐ 11 -15 ☐ 16 - 20 ☐ More than 20

☐ Chewing Tobacco, plugs, wads, chew

How much do you use per day? ☐ Less than 5 ☐ 6 - 10 ☐ 11 -15 ☐ More than 15

☐ Smoker

How long have you smoked? ______ ☐ weeks ☐ months ☐ years

How many cigarettes do you smoke a day? ☐ Less than 5 ☐ 6 - 10 ☐ 11 -15 ☐ 16 - 20 ☐ More than 20

How many cigars do you smoke a day? ☐ Less than 5 ☐ 6 - 10 ☐ 11 -15 ☐ 16 - 20 ☐ More than 20

How many pipefulls of tobacco do you smoke a day? ☐ Less than 2 ☐ 3 - 4 ☐ 5 - 6 ☐ More than 6

Have you been exposed to environmental tobacco smoke (second hand smoke) in a recreational setting within the last 10 days? This might include bars, restaurants, clubs, casinos, private parties, cars, other peoples homes. ☐ Yes ☐ No

Does your partner or anybody else you live with smoke? ☐ Yes ☐ No

The following questions are regarding your opinion/knowledge of smoking on U.S. Submarines:

Do you think smoking on submarines is ☐ A problem ☐ Not a problem

Do you think a ban on smoking on submarines would be

☐ Good ☐ Fair ☐ Don't Care ☐ Unfair ☐ Illegal

Are you aware of the current USN smoking policy for submarines? ☐ Yes ☐ No

Should smoking be allowed on submarines? ☐ Yes ☐ No

NSMRL 2007.0010 Urine Sample
Session ____________
Date ________ Time ________
Subject #
PRE EXPOSURE

NSMRL 2007.0010 Urine Sample
Session ____________
Date ________ Time ________
Subject #
POST EXPOSURE