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Abstract
Wildland (forest) firefighters are exposed to a wide
range of carcinogenic polycyclic aromatic
hydrocarbons (PAH) in forest fire smoke. PAH undergo
metabolic activation and can subsequently bind to
DNA. In this study, we investigated the association
between occupational and dietary PAH exposures and
the formation of WBC PAH-DNA adducts in a
population of wildland firefighters. An enzyme-linked
immunosorbent assay using an antiserum elicited
against benzo(a)pyrene-modified DNA was used to
measure PAH-DNA adducts in WBC obtained from 47
California firefighters at two time points, early and late
in the 1988 forest fire season. PAH-DNA adduct levels
were not associated with cumulative hours of recent
firefighting activity. However, firefighters who
consumed charbroiled food within the previous week
had elevated PAH-DNA adduct levels, which were
related to frequency of charbroiled food intake. These
findings suggest that dietary sources of PAH contribute
to PAH-DNA adduct levels in peripheral WBC and
should be evaluated when using this assay to assess
occupational and environmental PAH exposure.

Introduction
There are approximately 80,000 full-time and seasonal
wildland (forest or wilderness) firefighters in the United
States who are occupationally exposed to a wide range
of mutagens and carcinogens, such as PAH (1) in forest
fire smoke (2-5). The health consequences of this expo-
sure are unknown since neither mortality nor cancer
morbidity studies are available for wildland firefighters.

It has been proposed that monitoring workers
exposed to PAH for markers of biologically effective dose
may provide insight into PAH exposure (6). PAH undergo
metabolic activation (7) and subsequent bind to DNA,
forming DNA adducts that are hypothesized to constitute
an early step in the initiation of tumorigenesis (8). An
ELISA (9) that uses an antiserum elicited against
benzo(a)pyrene-modified DNA has been developed to
detect PAH-DNA adducts formed in human target tissues
and in peripheral WBC, which are readily accessible
surrogates for target tissue exposure. This assay has been
used to study WBC levels of PAH-DNA adducts in several
occupationally exposed populations including coke oven
workers and roofers (10-14), foundry workers (15), and
structural firefighters (16).

The purpose of this study was to determine whether or
not wildland firefighting is associated with elevated
PAH-DNA adduct levels in peripheral WBC of nonsmok-
ing firefighters. A second goal was to determine if con-
sumption of CB food is associated with elevated adduct
levels (17). The study was designed to measure adduct
levels and collect questionnaire information on work
practices and demographic and dietary information early
in a fire season and again, in the same group of firefight-
ers, late in the fire season after substantial exposure to
forest fire smoke had occurred.

Methods
Study Population. Subjects were firefighters assigned to
6 of the most active wildland fire stations in Region 2 of
the California Department of Forestry and Fire Protec-
tion, located in the foothills of the Sierra Nevada moun-
tains in northern California. Crews from surrounding
districts who were temporarily stationed at a fire station
under study also were included. Additional eligibility
criteria for participation in the study were being between
the ages of 18 and 49 years and being a nonsmoker (i.e.,
one who has never smoked or a former smoker who had
not smoked for at least 6 months prior to the study). The

The abbreviations used are: PAH, polycyclic aromatic hydrocarbons;
BP, benzo(a)pyrene; BPDE-DNA, DNA modified by reaction with BPDE
so that the major (>90%) DNA adduct is 7,8,9,10-tetrahydrobenzo(a)pyrene
deoxyguanosine; CB, charbroiled; ELISA, enzyme-linked immunosorbent assay.
study procedures were explained to all potential participants, and written informed consent was obtained from individuals agreeing to participate.

**First Survey and Sample Collection.** Sixty-nine individuals (85% of eligibles) were enrolled from late July 1988 through early August 1988 at several fire stations. Each completed a self-administered questionnaire on demographic features, recent potential dietary exposures to PAH, other dietary exposures that may influence PAH metabolism, and wildland firefighting work practices. This was reviewed with an investigator on-site. Daily firefighting activity on the fire line during the 4 weeks prior to the beginning of the study was recorded retrospectively by each participant with the help of fire station log records and diaries of fire crew captains. After they had completed the questionnaire, study subjects were given a diary to prospectively record daily hours of firefighting activity on the fire line during the following 8 weeks. A 40-ml peripheral blood sample was obtained from each participant, separated on-site into plasma, buffy coat, and RBC fractions, and immediately frozen on dry ice.

**Second Survey and Sample Collection.** Approximately 8 weeks after the first survey, the study team attempted to locate and reevaluate all 69 participants in the initial survey. Fifty-two of these individuals (75%) were successfully reevaluated either at their fire stations or in a fire base-camp. Of the 17 individuals unavailable for late season testing, 9 were on duty in another part of the state, 5 had left the California Department of Forestry and Fire Protection to attend school, and 3 were physically injured. None of these individuals had left active duty because of smoke inhalation, and this group was not significantly different with respect to age, race, sex, or occupational history in the forest service from those 52 study subjects successfully recontacted (data not shown). Fire fighting diaries were collected from all 52 individuals. A second questionnaire was self-administered and reviewed on-site. Blood samples were successfully obtained from 51 of the 52 individuals and processed as in the first survey.

**Laboratory Analysis.** DNA was extracted from the buffy coat samples using high-salt fractionation (18) followed by chloroform-isoamyl alcohol extraction. An adequate quantity of DNA for analysis by ELISA was extracted from 47 of the 51 matched early and late season blood samples. The PAH-DNA adduct content was analyzed by an ELISA that used rabbit antibody 33 elicited against DNA containing 7-R-N2-[10-(7,8,9,10-tetrahydrobenz[a]pyreneyl)]-deoxyguanosine, (diluted 1:70,000), goat-anti-rabbit IgG conjugated with alkaline phosphatase (diluted 1:400), and the fluorescent enzyme substrate 4-methylumbelliferyl phosphate, as reported previously (19). Human samples were compared to a BPDE-DNA standard modified in the same range as the human samples (4.4 fmol/µg DNA) (19). The mean of 3 DNA aliquots (triplicate wells) analyzed/sample was used for the final determination of adduct level. Since the antisera is capable of recognizing DNA samples modified with diol-epoxides of multiple PAH bound to DNA (20), and since human PAH exposure is generally to complex mixtures, the values for human samples, calculated by comparison to the BPDE-DNA standard, represent a spectrum of PAH-DNA adduct formation (21). The lower limit of detection was 0.04 fmol adduct/µg DNA and was based on standard curve linearity to 15% inhibition using 35 µg of DNA/well. Samples in the nondetectable range were assigned a value of 0.02 fmol adduct/µg DNA, one-half the lower limit of detection.

**Statistical Methods.** Hours of self-reported firefighting activity were used as an estimate for fire smoke exposure. A series of exposure variables was generated for each firefighter to assess the importance of proximity in time and magnitude of exposure on PAH-DNA adduct levels. Variables included cumulative hours of firefighting in the last 28, 21, 14, and 7 through 1 day(s) prior to blood collection. Additional occupational variables used in the analysis were number of seasons worked as a firefighter, minutes of exposure to diesel exhaust/day, and proportion of time at a fire that an individual used a cotton bandanna for respiratory protection.

The frequency of consumption of CB food in the previous 2 weeks and the number of days since CB food was last consumed were the primary variables used to evaluate dietary contribution to adduct levels. Since these variables were highly correlated, the two variables were used separately in multivariate models to avoid the problem of multicollinearity. Exposure to other sources of PAH or potential effect modifiers evaluated included history of past active cigarette smoking; years since cessation of smoking; passive smoke exposure; weekly alcohol consumption; frequency of grilling food; daily caffeine intake; and age, race, and sex.

Sixty % of a total of 94 adduct measurements were in the detectable range. Adduct levels could not be transformed into a normal distribution since 40% of the samples (the nondetectables) had the same value of one-half the limit of detection. Therefore two robust techniques were used to analyze the data: (a) linear regression using adduct levels transformed into ranks (22), an analytic procedure considered intermediate between a parametric and nonparametric technique; and (b) logistic regression in which adduct levels, the outcome variable, were dichotomized into nonelevated (≤0.2 fmol/µg DNA) or elevated (>0.2 fmol/µg DNA) levels. Selection of this cutoff point was based upon multiple (6 to 8) measurements of WBC PAH-DNA adduct levels made over 7-8 weeks in 4 human volunteers who did not have risk factors for elevated adduct levels (23); i.e., they were healthy, nonsmoking subjects without occupational exposure who specifically abstained from smoked, broiled, or charbroiled food. These samples were chosen for comparison because they were assayed by the same ELISA at the same time in the same laboratory as the samples from the current study. In addition, an upper range of 0.2 fmol/µg DNA is similar to that found in other studies that have measured peripheral WBC PAH-DNA adducts by ELISA with fluorescent detection (14, 15).

Summary values were calculated for each variable as means ± SE. The Wilcoxon signed rank test was used to measure cross-seasonal changes in both firefighting activity and adduct levels. Correlation analysis was performed by Spearman correlation unless otherwise specified. Early and late season data were combined and analyzed as a single longitudinal study using the method of Liang and Zeger (24) and the generalized estimating equations software (25), which adjusts the variance estimates of the regression coefficients for correlation of repeat outcome measurements made on the same indi-
vides greaten power and flexibility to perform bivaniate
adduct measurements was low $P_{tailed}$ were excluded from the analysis of that variable. Two-
only for subjects with complete data; individuals with
and multivaniate analysis. The software can analyze data
thus the adjusted variance estimates are generally similar
individual. In this study, the correlation between repeat
adduct measurements was low ($r = -0.06; P = 0.69$) and
thus the adjusted variance estimates are generally similar
to the unadjusted values. The combined data set provides
greater power and flexibility to perform bivariate and multivariate analysis. The software can analyze data
only for subjects with complete data; individuals with
missing questionnaire data from one of the two surveys
were excluded from the analysis of that variable. Two-
tailed $P$ values were calculated throughout.

Results

Descriptive Characteristics. Characteristics of the study subjects are shown in Table 1. Seventy-nine % of the
participants were male and 79% were Caucasian. The mean ($\pm SE$) age of the participants was 26.4 $\pm$ 1.0 years,
with 6.1 $\pm$ 0.7 seasons of firefighting experience. The
firefighters had 13.6 $\pm$ 0.2 mean years of education. The
10 former smokers (10 of 47) had accumulated 5.8 $\pm$ 3.3
time lifetime pack-years and had ceased smoking a mean of
8.4 $\pm$ 7.2 years prior to the study. Eighty-five % (40 of
the study population late in the fire season, there was
no change in adduct levels in the study group. The mean
adduct level in the early season was 0.1 1 $\pm$ 0.89
fmol/µg DNA compared to a mean adduct level late in
the fire season of 0.1 0 $\pm$ 0.014 fmol/µg DNA ($P = 0.93$).

The lack of association between firefighting activity
and adduct levels is demonstrated in Fig. 1, a box and
whisker plot (26) of adduct levels for individuals who had
low ($\leq$ 10 h), medium ($>10$ and $\leq 40$ h), and high ($>40$ h)
levels of firefighting activity during the previous 2 weeks.
No measure of firefighting was significantly associated
with adduct level in any unadjusted regression model
(Table 3). In addition, there was no pattern suggesting
that either recent or distant activity was more strongly
associated with adduct measurements. These results
were unaffected after adjustment of all other covariates
including frequency of CB food consumption.

Charbroiled Food Consumption. There was a high cor-
relation between the early and late season reports of CB
food consumption ($r = 0.48; P = 0.0006$). In the com-
bined data set, 24% of the study population had not
consumed CB food in the previous 2 weeks, 39% had
CB food 1 to 2 times, and 35% had CB food 3 or more
times in the previous 2 weeks. PAH-DNA adduct levels
increased as the frequency of CB food consumption
increased (Fig. 2). This association was significant when
tested by both linear and logistic regression (Table 3) and

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<td></td>
</tr>
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<tr>
<td>Male</td>
<td>37 (79)</td>
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<tr>
<td>Female</td>
<td>10 (21)</td>
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<tr>
<td>Caucasian</td>
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<td>30–39</td>
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<td>40–49</td>
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<td>6–10</td>
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<td>11–15</td>
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<td>16+</td>
<td>2 (4)</td>
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Fig. 1. PAH-DNA adduct level and recent firefighting activity (data combined from early and late seasons). a, hours of firefighting: low, 0–10 h (mean $\pm$ SE, 3.3 $\pm$ 0.5); medium, 11–40 h (22.5 $\pm$ 1.5); high, >40 h (112.7 $\pm$ 12.7). Box and whisker plot (Ref. 26): upper edge of box, highest 25th percentile of data; lower edge of box, 75th percentile. Whiskers indicate range of remaining data except for outliers. O, outlier defined as $>1.5$ and $<3.0 \times$ distance between 25th and 75th percentiles, $\Delta$, extreme outlier defined as $>3.0 \times$ distance between 25th and 75th percentiles. $^a$, mean; $\cdots$, median.
WBC PAH-DNA Adducts, Diet, and Occupation

Data combined from early and late seasons. Unadjusted coefficients presented. There were no substantial differences after adjustment for all other covariates tested (see Methods) including CB

For linear regression, as originally recorded. For logistic regression, coded as 0 (2 times) and 1 (>2 times in previous 2 weeks).

Logistic regression against adduct levels dichotomized into 0.2 fmol/gg DNA or >0.2 fmol/gg DNA.

d (Linear regression against adduct levels transformed into ranks. Linear regression against log transformed adduct levels gave similar results.

Cumulative hours of firefighting in previous 2 weeks
-0.026 0.044 0.55 1.0 0.99-1.01 0.64

Cumulative hours of firefighting in previous 4 weeks
-0.0011 0.011 0.93 1.0 0.99-1.0 0.47

Frequency of CB food consumption in previous 2 weeks
7.53 2.93 0.01 4.1 1.9-12.03 0.0088

Weeks since last consumed CB food
9.94 4.55 0.03 1.9 1.1-11.9 0.034

Table 3 Regression analysis of cumulative firefighting activity and CB food consumption on PAH-DNA adduct level, for 47 subjects

<table>
<thead>
<tr>
<th>Exposure Variable</th>
<th>Regression Techniqueb</th>
<th>Linearc</th>
<th>Logisticd</th>
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<tbody>
<tr>
<td></td>
<td>( \beta )</td>
<td>SE</td>
<td>P value</td>
</tr>
<tr>
<td>Cumulative hours of firefighting in previous 1 week</td>
<td>-0.03</td>
<td>0.082</td>
<td>0.71</td>
</tr>
<tr>
<td>Cumulative hours of firefighting in previous 2 weeks</td>
<td>-0.026</td>
<td>0.044</td>
<td>0.55</td>
</tr>
<tr>
<td>Cumulative hours of firefighting in previous 4 weeks</td>
<td>-0.0011</td>
<td>0.011</td>
<td>0.93</td>
</tr>
<tr>
<td>Frequency of CB food consumption in previous 2 weeks</td>
<td>7.53</td>
<td>2.93</td>
<td>0.01</td>
</tr>
<tr>
<td>Weeks since last consumed CB food</td>
<td>9.94</td>
<td>4.55</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a Data combined from early and late seasons.

b Unadjusted coefficients presented. There were no substantial differences after adjustment for all other covariates tested (see Methods) including CB

c Linear regression against adduct levels transformed into ranks. Linear regression against log transformed adduct levels gave similar results.

d Logistic regression against adduct levels dichotomized into ≤0.2 fmol/μg DNA or >0.2 fmol/μg DNA.

For example, individuals who had consumed CB food within the previous week were almost 4 times as likely to have elevated adduct levels (Odds ratio, 3.9; 95% confidence interval: 1.1–13.9).

The previous results suggest both a frequency and a time-related effect of CB food consumption on PAH-DNA adduct levels. In order to simultaneously explore the impact of both factors, frequency of CB food consumption and adduct levels was plotted for subjects who consumed CB food within the previous week and for individuals who consumed CB food between 1 and 2 weeks prior to phlebotomy (Fig. 4). Adduct levels for individuals who did not consume CB food during the previous 2 weeks were used as the baseline value. A positive dose-response relationship between frequency of CB food consumption and adduct levels was evident only for individuals who last consumed CB food within the previous week.

No other occupational, demographic, or dietary variable was associated with adduct levels in bivariate or multivariate models, either independently or through interaction with firefighting and CB food consumption.

Discussion

Among the 47 California wildland firefighters studied here, there was essentially no change in mean WBC PAH-DNA adduct level across the firefighting season despite a 5-fold greater average level of firefighting activity in the late season as compared to the early season. Furthermore, regression analyses found no association between recent or distant firefighting activity and adduct levels.

It is possible that the exposure variable used in this study, self-reported hours of firefighting, resulted in exposure misclassification. However, in a separate investigation performed on the same study population, this variable was strongly associated with a cross-seasonal decline in pulmonary function (27), suggesting it represents a reasonable measure of exposure to forest fire smoke.

The wildland firefighters in this study may have been exposed to PAH levels that were too low to cause a detectable increase in WBC PAH-DNA adduct levels.
Twenty personal breathing zone exposure measurements made at 2 fires during the 1988 fire season on firefighters working in the same area as the study subjects demonstrated low (28) and variable BP exposure (mean, 0.015 \( \mu g/m^2 \); range, <0.002–0.034 \( \mu g/m^2 \)) (29). However, Santella et al. (30) have recently reported a dose-response relationship between WBC PAH-DNA adducts in foundry workers exposed to BP levels that were also in the low occupational range (0.002–0.060 \( \mu g/m^2 \)). This suggests that the wildland firefighters were recently exposed to BP levels at the lower end of the range observed at the 2 fires described above (28), a level of exposure which did not have an impact on PAH-DNA adducts measured by this assay.

PAH-DNA adduct measurements made by different laboratories using different antibodies are only moderately comparable. Nevertheless, it is interesting to note that the mean ± SE adduct level (0.084 ± 0.016 fmol/\( \mu g \) DNA)\(^4\) in even the highest firefighter exposure category portrayed in Fig. 1 was similar to previously reported adduct levels measured by ELISA with fluorescent detection in populations without recent occupational or substantial environmental exposure, i.e., 0.083 ± 0.01 fmol/\( \mu g \) DNA in 44 unexposed controls in The Netherlands (14), 0.066 ± 0.028 fmol/\( \mu g \) DNA in 10 unexposed controls in Finland (15), 0.12 fmol/\( \mu g \) DNA in 9 foundry workers evaluated after 4 weeks of vacation in Finland (31), and 0.14 fmol/\( \mu g \) DNA in 13 unexposed rural subjects in Poland (32).

\(^4\)Crude PAH-DNA adduct level; mean PAH-DNA adduct level adjusted for CB food consumption by analysis of covariance was 0.091 ± 0.025 fmol/\( \mu g \) DNA (mean ± SE).

A limitation of this finding is its uncertain generalizability to wildland firefighting as a whole since it is not known if the exposure patterns experienced by individuals in the current study were representative of usual PAH exposure levels in this occupation. However, 1988 was one of the most active northern California fire seasons in the previous decade, and the study population was both recently and heavily exposed to forest fire smoke prior to blood sampling late in the season. Sixty-eight \% of the firefighters (32 of 47) had fought fires within the previous week, averaging 33 ± 6.5 cumulative hours of fire-line exposure during that time.

The implication of this finding for occupationally related cancer risk among wildland firefighters is unclear. Even low PAH levels may interact with other genotoxic compounds present in wood smoke, such as acrolein and formaldehyde, to produce increased cancer risks (5). Mortality and morbidity studies will be necessary to determine excess cancer patterns in this population.

The frequency of recent consumption of CB food was strongly associated with DNA adduct levels in both logistic regression against dichotomized adduct levels and in linear regression against adduct level rank. The effect was restricted to those subjects who had consumed CB food within the previous week, which is consistent with the kinetics of polynucleated WBC turnover (which constitute up to 60\% of peripheral WBC) in peripheral blood. After the last division in the bone marrow, polynucleated WBC spend 5 to 7 days in the maturation compartment of the bone marrow (33) where they can be exposed to blood-borne PAH and are theoretically at risk for the formation of adducts that will not be diluted by further cell divisions. Once in the peripheral blood, they have a half-life of less than 24 h, after which they migrate into interstitial areas and are no longer accessible by peripheral phlebotomy.
The magnitude of the dietary effect in the most heavily and recently exposed individuals is consistent with a previous report of adduct level increase in response to controlled feeding of CB hamburgers (23). In addition, an earlier study suggested an association between PAH-DNA adduct levels and consumption of CB food 3 or more times in the previous month (16). In contrast, a recent study reported the absence of an association between CB food consumption and WBC PAH-DNA adduct levels (30). However, a direct comparison cannot be made since that study assessed average weekly consumption of CB food during the previous 2 years, while the study reported here collected information about CB food consumption within the previous 2 weeks.

Nonsmoking status was an eligibility criteria for participation in this study. Since eligible subjects were identified with the assistance of crew captains, who live and work with their crews 24 h/day, it is unlikely that few if any of the study subjects were currently smoking. Furthermore, even if some subjects were currently smoking, it is unclear whether this exposure would have had a detectable impact on total WBC PAH-DNA adducts, given the inconsistency of previous studies (34).

Overall, these findings suggest that use of PAH-DNA adducts to evaluate low level occupational and environmental exposures should be accompanied by an assessment of recent dietary exposures as well, since all 3 sources of exposure may contribute to peripheral WBC PAH-DNA adduct levels (17). Unfortunately, estimating PAH dietary exposure is not straightforward since PAH are present in a wide variety of foods in addition to cooked meat, including some oils, grains, and vegetables (35). Even though CB food may constitute the major source of PAH exposure for people who frequently consume it, PAH from other food categories may predominate in populations with alternative eating habits (36-38) and prove difficult to assess by questionnaire alone. In fact, the measurement of WBC PAH-DNA adduct levels may prove useful in studies which seek to evaluate cancer risks from dietary PAH, which may be hazardous in and of themselves.

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References


Contribution of occupation and diet to white blood cell polycyclic aromatic hydrocarbon-DNA adducts in wildland firefighters.


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