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14. ABSTRACT This study examined whether exposure to cadmium (Cd) from dietary or environmental sources increases the risk of breast cancer. We examined this hypothesis using information collected from the California Teachers Study (CTS) cohort, a group of approximately 130,000 female school employees living in California followed for breast cancer since 1995. Information collected by questionnaire included residential addresses, exposure to tobacco smoke, and food and beverage consumption. We assessed levels of dietary and environmental exposure by linking these collected data with available information on Cd residue levels in foods and beverages and environmental sources of Cd pollution near women's residences. In addition, we used existing urine samples provided by a subset of 296 women in the CTS to identify predictors of urinary Cd concentrations, which is considered a good measure of cumulative lifetime exposure. In this analysis, estimates of dietary Cd intake and environmental Cd exposure were not associated with urinary Cd concentrations and we observed only modest agreement between repeat measurements of urinary Cd concentrations in the exposure sub-study. We then evaluated whether dietary and environmental exposure to Cd increased the risks of estrogen-receptor (ER) subtypes of breast cancer in the entire CTS cohort. We observed an increased risk for ER-negative breast cancer associated with residential proximity to high vehicular traffic density and with residence in a census tract with an elevated Cd concentration in ambient air."We observed a modest increase in risk for ER-positive breast cancer, particularly among women with larger body size (BMI ≥ 30 kg/m2). We failed to observe an association between dietary Cd intake and breast cancer risk. This final report summarizes the work conducted over the entire research period.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusion.....	10
References.....	11
Personnel Receiving Pay from the Research Effort.....	11
Supporting Data.....	15
Appendices.....	29

INTRODUCTION

The objective of this study was to examine whether exposure to cadmium (Cd) from dietary or environmental sources increases the risk of breast cancer. We examined this hypothesis using information collected from the California Teachers Study (CTS) cohort, a group of approximately 130,000 female school employees living in California followed for breast cancer since 1995. Information collected by questionnaire included residential addresses, exposure to tobacco smoke, and food and beverage consumption. We assessed levels of dietary and environmental exposure by linking these collected data with available information on Cd residue levels in foods and beverages and environmental sources of Cd pollution near women's residences. In addition, we used existing urine samples provided by a subset of 296 women in the CTS to identify predictors of urinary Cd concentrations, which is considered a good measure of cumulative lifetime exposure. We then evaluated whether dietary and environmental exposure to Cd increased the risk of breast cancer in the entire CTS cohort.

This final report summarizes the findings of this project.

BODY

Aim 1. Estimate exposure to Cd from dietary and environmental sources for all participants in the CTS cohort.

These distributions have been updated to include incident invasive breast cancer cases through 2009. Table 1 lists the distributions of demographic and personal characteristics of women enrolled in the CTS cohort. Table 2 lists the distributions of Cd exposure from environmental sources for all eligible CTS subjects, including from traffic density (vehicle kilometers traveled within 300 m), industrial Cd emissions (kg/km within 5 km), and estimated outdoor Cd concentration of the residential census tract (ng/m³). Table 3 lists the distributions of daily Cd dietary intake (µg/day) for all eligible CTS subjects. In addition to the unadjusted total, we also list the calorie-adjusted intake (adjusted for daily calories excluding alcohol ÷ 1,000) as well as the calorie-adjusted intake derived using the residual method.

Aim 2. Evaluate the contribution of dietary and environmental sources to total Cd exposure based on urinary Cd concentrations, for 304 validation sub-study participants.

- a) Measure the Cd concentration in 24-hour urine samples provided by 304 validation sub-study participants and in repeat samples from 176 of the participants.
- b) Calibrate Cd exposure estimates with measured urinary concentrations using mixed-effects models.
- c) Estimate total exposure to Cd based on the calibration model for all participants in the CTS.

The analyses and findings of this study aim are described in the journal manuscript in Appendix 1. This manuscript, entitled “Reproducibility and determinants of urinary cadmium concentrations among women in Northern California” was accepted for publication in September 2012 by Environmental Health Perspectives. As described in the 2011 annual report, because of the largely null associations between urinary Cd concentrations and estimated dietary and environmental Cd exposures, we did not have statistically-significant parameter estimates from the mixed-effects models that would have served as weights for the dietary and environmental exposures to estimate total Cd exposure (Aim 2c). As a result, the risk analyses in Aim 3 focused only on dietary and environmental Cd exposures.

Aim 3. Estimate the effects of total, dietary, and environmental exposure to Cd on breast cancer incidence in the CTS from 1996 to 2005.

Since the 2011 annual report, we have updated results to account for breast cancer cases ascertained through 2009. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

The following risk analyses of dietary exposure are based on calorie-adjusted dietary Cd intake estimated using the residual method. Effect estimates based on unadjusted and calorie-adjusted intake were similar to those derived based on residual-method-derived Cd intake. Because of the availability of estrogen-receptor (ER) status and a priori information that these breast cancer types have different etiologies, we conducted analyses stratified by ER-status.

Table 4 lists hazard ratios (HRs) and 95% confidence intervals (CIs) for ER-positive breast cancer and quintiles of daily dietary Cd intake. The first column is adjusted only for total daily calories. Here, we observed an increased risk associated with dietary Cd in the highest quintile (HR = 1.12; 95% CI: 0.99-1.26) compared with the lowest quintile; HRs across the quartiles suggested a monotonic exposure-response trend (p-trend =

0.02). The second column presents HRs from a model adjusted for total daily calories and the following confounding variables: parity (no, yes) and age at first full term pregnancy (continuous), history of benign breast disease (no, yes), family history of breast cancer (no, yes, adopted), alcohol consumption in the year prior to baseline (none, <20 g/d, 20+ g/d), menopausal status and hormone therapy (HT) use at baseline (premenopausal, peri-/post-menopausal: never HT, current E+P, current E alone, past HT), BMI at baseline (continuous), height at baseline (continuous), and smoking status (never, former, current). In this model, we observed slightly lower HRs compared with the calorie-only-adjusted model but still observed a suggestion of a monotonic exposure-response pattern (p-trend = 0.09).

However, we were concerned that dietary patterns may additionally confound the observed trend. In a previous analysis, we identified five dietary patterns in the CTS cohort using principal components analysis: plant-based, high-protein/high-fat, high-carbohydrate, ethnic, and salad-and-wine (Chang et al., 2008). Evaluating each of these dietary patterns as potential confounders of the dietary Cd and ER-positive breast cancer association, only the salad-and-wine dietary pattern appeared to significantly change the magnitude of the effect estimates. The third column of Table 4 lists HRs for quintiles of dietary Cd, adjusted for all previously listed covariates and the salad-and-wine dietary pattern. These HRs suggest that there is no association between dietary Cd (p-trend = 0.58). This result is not surprising, given the fact that while leafy green vegetables are an important dietary source of Cd, they are also rich in antioxidants and other beneficial nutrients. Consequently, a true adverse effect of dietary Cd on risk may be offset by the beneficial effects of other nutrients, thus leading to the observed null result when adjusting for the salad-and-wine dietary pattern.

We evaluated whether the salad-and-wine dietary pattern modified the effect of dietary Cd on ER-positive breast cancer risk by comparing levels of these two exposures to a common reference group of women with low dietary Cd intake (< 8.23 µg/day) and a low salad-and-wine dietary pattern score (< 25th percentile). Table 5 lists HRs by level of these two exposures. By level of salad-and-wine dietary pattern, we observed elevated risk in the medium (25th–<75th percentile) and high (≥ 75th percentile). However, HRs for dietary Cd intake within the medium and high levels of salad-and-wine dietary pattern did not appear to differ with one another. Thus, we did not see any evidence of an interaction (p-interaction = 0.64).

Table 6 lists HRs and 95% confidence intervals (CIs) for ER-negative breast cancer and quintiles of daily dietary Cd intake. The first column is adjusted only for total daily calories. Here, we observed a negative association between dietary Cd and risk, where the rate is 75% (95% CI: 56–100%) the rate in the in the highest quintile compared with

the lowest quintile (p-trend = 0.03). The second column presents HRs from a model adjusted for total daily calories and the following confounding variables: birthplace (North American born, not North American born), age at menarche (continuous from ≤ 9 to 17+), history of benign breast disease (no, yes), family history of breast cancer (no, yes, adopted), average lifetime (high school to age 54) moderate physical activity (hours per week; continuous), alcohol consumption in the year prior to baseline (none, any), menopausal status and hormone therapy use at baseline (premenopausal, peri-/post-menopausal: never hormone therapy, ever hormone therapy), BMI at baseline (continuous), and continuous factor scores for the following dietary factors in the year prior to baseline: “high protein and high fat”, “high carbohydrate”, and “ethnic”. HRs in this model were similar to those observed in the minimally-adjusted model (p-trend=0.03). However, additional adjustment for antioxidant intake from vegetables (ORAC_OH) eliminated the exposure-response pattern (p-trend = 0.49).

Similar to the joint analysis of dietary Cd and the salad-and-wine dietary pattern for the risk of ER-positive breast cancer, we evaluated the joint effect of dietary Cd and antioxidants from vegetables on the risk of ER-negative breast cancer using a common reference group of women in the lowest tertiles of low dietary Cd intake and antioxidant (ORAC_OH) score (Table 7). By tertile of level of antioxidant score, we observed reduced risks in the higher tertiles. However, HRs for dietary Cd intake within the medium and high levels of antioxidants did not appear to differ with one another, suggesting no interaction. Similar to observation about the dietary Cd and the salad-and-wine dietary pattern for ER-positive breast cancer, the fact that Cd and antioxidants both come from leafy green vegetables contributes to the challenge of identifying the independent effect of Cd intake.

Based on these findings, we failed to see any evidence of an association between dietary Cd intake and the risk of ER-positive or ER-negative breast cancer. We are currently developing a manuscript on these results. These null findings are consistent with those reported in a recent study of dietary Cd intake and postmenopausal breast cancer risk in the US VITamins And Lifestyle (VITAL) cohort (Adams et al., 2012). Similar to our study, dietary Cd intake was assessed by linking cohort participants’ food-frequency questionnaires with Cd concentrations in food and beverage items obtained from the Total Diet Study. However, a study of women enrolled in the Swedish Mammography Cohort observed an increased risk in breast cancer associated with dietary Cd intake (Julin et al., 2012).

Analyses of environmental sources of Cd exposure were also updated to include breast cancer cases ascertained through 2009. For these analyses, we estimated effects on the risks of ER-positive and ER-negative breast cancer in the entire CTS cohort and three

subpopulations: women resided in the same residential address since baseline (non-movers), women who reported never smoking in their lifetime (never smokers), and non-moving never-smoking women. Models in these tables were first minimally adjusted for age and race/ethnicity, and then for the following additional variables: family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, BMI, menopausal status/hormone therapy combined, smoking status, smoking pack-years, home environmental tobacco smoke exposure.

Stratifying by ER-status (Table 8), for outdoor Cd concentrations, we observed a positive association with ER-negative breast cancer, particularly among women who never smoked and never moved (p-trend = 0.006). Among these women, we observed a 55% increase in risk (95% CI: 10–119%) in the highest quartile of exposure compared to the lowest. We observed similar patterns for the entire CTS cohort and the other subpopulations of women who never smoked and women who did not move. In contrast, we did not observe any associations between ER+ breast cancer risk and outdoor Cd concentrations within the entire CTS cohort or the other subpopulations.

We additionally stratified this analysis of ER-subtypes by menopausal status. Table 9 lists HRs for outdoor Cd concentrations and ER-positive breast cancer. Among pre/peri-menopausal women in the total cohort, we observed a monotonic exposure-response trend (p-trend = 0.02). This was also observed among non-movers and never-smokers, although with less precision. Among post-menopausal women, we did not observe a similar trend in the entire cohort (p-trend = 0.54), although we observed significant increases in risk in the second (HR = 1.13; 95% CI: 1.01-1.27) and third (HR = 1.14; 95% CI: 1.02-1.28) quartiles compared with the lowest quartile, respectively. For ER-negative breast cancer (Table 10), we did not observe any differences in trends between pre/peri-menopausal and post-menopausal women. This was most evident among non-moving never-smoking women, where we observed positive exposure-response trends for both pre/peri-menopausal (p = 0.08) and post-menopausal women (p = 0.03).

We examined whether there were any potential modifiers of the effect of outdoor Cd concentrations on the risk of ER-positive breast cancer. Only body mass index (BMI) suggested a potential interaction. Table 11 lists HRs for outdoor Cd concentration within three strata of BMI (<25, 25-29, ≥30 kg/m²). Only among women with BMI ≥ 30 kg/m² did we observe an exposure-response trend; this was observed in the entire CTS cohort (p-trend = 0.008) as well as the other subpopulations of women. When we used a common reference group of women in the lowest quartile of exposure with BMI <25 kg/m², this exposure-response trend persisted in the highest category of BMI (Table 12). The p-values for interaction suggested that the effect of outdoor Cd concentration was

modified by BMI in the total cohort ($p = 0.15$) as well as among never-smokers ($p = 0.08$) and non-movers/never-smokers ($p = 0.09$).

For quartiles of traffic density by ER-subtype (Table 13), we observed a monotonic exposure-response trend for ER- negative breast cancer among never-smoking ($p = 0.07$) and non-moving never-smoking women (p -trend = 0.06). Among non-moving never-smoking women, the HR for the highest quartile of exposure compared with the lowest quartile was 1.41 (95% CI: 1.00-1.99). No association was observed between traffic density and ER-positive breast cancer, and there was no evidence of heterogeneity by menopausal status or body size for this subtype.

Table 14 lists HRs by ER-subtype for industrial Cd emissions (kg/kg within 5 km). We observed no association with this exposure for either subtype.

These findings were presented in August 2012 at the 24th Conference of the International Society for Environmental Epidemiology in Columbia, SC. We are developing a manuscript describing these findings on the effects of environmental sources of Cd on the ER-subtypes of breast cancer.

KEY RESEARCH ACCOMPLISHMENTS, FINAL REPORT

- Completion of assessments of environmental Cd exposure and dietary Cd intake in the CTS cohort.
- Identification of predictors of urinary Cd concentrations in the exposure validation sub-study. The manuscript of these findings has been accepted for publication by Environmental Health Perspectives.
- Completion of analyses of the effects of Cd from dietary intake on breast cancer risk. We are developing a manuscript describing these findings.
- Completion of analyses of the effects of Cd from environmental exposures on breast cancer risk. These findings were presented at the 24th Conference of the International Society for Environmental Epidemiology in Columbia, SC. We are developing a manuscript describing these findings.

REPORTABLE OUTCOMES

There are several reportable outcomes arising from project activities. These include one publication, an oral presentation, two poster presentations, and two funded grants. These are listed below:

Publication:

Gunier RB, Horn-Ross PL, Canchola AJ, Duffy CN, Reynolds P, Hertz A, Garcia E, Rull RP. Reproducibility and determinants of urinary cadmium concentrations among women in Northern California. *Environmental Health Perspectives*. 2012, in press (manuscript in Appendix 1).

Oral presentation:

Rull RP, Goldberg D, Gunier RB, Hertz A, Horn-Ross PL, Canchola A, Reynolds P. Environmental cadmium exposure and the risks of estrogen-receptor positive and negative breast cancer. Presented at the 24th Conference of the International Society for Environmental Epidemiology, August 28, 2012, Columbia, South Carolina (abstract in Appendix 2).

Poster presentations:

Gunier RB, Rull RP, Hertz A, Canchola A, Horn-Ross P, Reynolds P. Urinary cadmium concentrations among female teachers from Northern California.

Presented at:

- 1) Joint Conference of the International Societies for Environmental Epidemiology and Exposure Assessment, August 28-September 1, 2010, Seoul, Korea.
- 2) 6th Department of Defense Breast Cancer Research Program Era of Hope Conference, August 2-5, 2011, Orlando, Florida (abstract in Appendix 3).

Funded grants:

1. National Institute of Environmental Health Sciences Grant No. 1 R01 ES018841 (6/1/2010 – 4/30/2013): Dietary and Environmental Exposure to Cadmium and the Risk of Endometrial Cancer (abstract in Appendix 5)
2. California Breast Cancer Research Program Grant No. 17IB-0016 (10/1/2011 – 3/31/2013): Cadmium, Age at Menarche, and Early Puberty in Girls (abstract in Appendix 5)

In addition, in part based on this work, Dr. Rull was appointed as an Assistant Professor in Epidemiology at the University of Nevada, Reno in July 2012.

CONCLUSION

The multiple sources of exposure complicate the evaluation of the effects of Cd on the risk of breast cancer. Our findings contribute to the growing number of epidemiologic reports on this topic, particularly on the effects of Cd from environmental sources. We

observed increases in risk for ER-negative breast cancer associated with outdoor Cd concentration and vehicular traffic density. We observed a modest increase in risk for ER-positive breast cancer, particularly among women with larger body size (BMI $\geq$ 30 kg/m²). Consistent with a recent US study of dietary Cd exposure and breast cancer risk, we observed no association in the CTS cohort. Our finding of modest agreement between repeat measurements of urinary Cd concentrations in the exposure sub-study contributes to the growing body of knowledge of the reliability and reproducibility of this biomarker.

REFERENCES (EXCLUDING THOSE LISTED IN MANUSCRIPT IN APPENDIX 1)

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Chang ET, Lee VS, Canchola AJ, Dalvi TB, Clarke C a, Reynolds P, et al. Dietary patterns and risk of ovarian cancer in the California Teachers Study cohort. *Nutrition and cancer*. 2008; 60:285–91.

Julin B, Wolk A, Bergkvist L, Bottai M, Akesson A. Dietary cadmium exposure and risk of postmenopausal breast cancer: a population-based prospective cohort study. *Cancer research*. 2012; 72:1459–66.

PERSONNEL RECEIVING PAY FROM THE RESEARCH EFFORT

Rudolph Rull
Pamela Horn-Ross
Peggy Reynolds
Robert Gunier
Andrew Hertz
Alison Canchola
Debbie Goldberg
David Nelson
Christine Duffy
Erika Garcia

SUPPORTING DATA (UNPUBLISHED)

Table 1. Characteristics among women with no prior history of breast cancer through 2009 and who resided in California at the time of the baseline questionnaire (California Teachers Study cohort, N = 114,253).

Table 2. Distributions of environmental Cd exposures from environmental sources among women with no prior history of breast cancer through 2009 and who resided in California at the time of the baseline questionnaire (California Teachers Study cohort, N = 114,253).

Table 3. Distributions of daily dietary Cd intake among women with no prior history of breast cancer through 2009 who resided in California at the time of the baseline questionnaire with complete dietary data (California Teachers Study cohort).

Table 4. Hazard ratios and 95% confidence intervals for ER-positive breast cancer (n = 2,385) and quintiles of calorie-adjusted dietary Cd intake^a, California Teachers Study cohort (N = 85,509).

Table 5. Hazard ratios and 95% confidence intervals for ER-positive breast cancer (n = 2,385) by tertile of calorie-adjusted dietary Cd intake^a and interquartile category of salad-and-wine dietary pattern using a common reference category, California Teachers Study cohort (N = 85,509).

Table 6. Hazard ratios and 95% confidence intervals for ER-negative breast cancer (n = 409) and quintiles of calorie-adjusted dietary Cd intake^a, California Teachers Study cohort (N = 84,865).

Table 7. Hazard ratios and 95% confidence intervals for ER-negative breast cancer (n = 409) by tertile of calorie-adjusted dietary Cd intake^a and tertile of antioxidant intake from vegetables using a common reference category, California Teachers Study cohort (N = 84,865).

Table 8. Hazard ratios and 95% confidence intervals for breast cancer by quartile of estimated outdoor Cd concentration, by ER-subtype, California Teachers Study cohort.

Table 9. Hazard ratios and 95% confidence intervals for ER-positive breast cancer by quartile of estimated outdoor Cd concentration, by menopausal status, California Teachers Study cohort.

Table 10. Hazard ratios and 95% confidence intervals for ER-negative breast cancer by quartile of estimated outdoor Cd concentration, by menopausal status, California Teachers Study cohort.

Table 11. Hazard ratios and 95% confidence intervals for ER-positive breast cancer by quartile of estimated outdoor Cd concentration, by category of body mass index, California Teachers Study cohort.

Table 12. Hazard ratios and 95% confidence intervals for ER-positive breast cancer by quartile of estimated outdoor Cd concentration, by category of body mass index, using a common reference group (lowest exposure quartile and BMI<25 kg/m²), California Teachers Study cohort.

Table 13. Hazard ratios and 95% confidence intervals for breast cancer by quartile of traffic density (vehicle km traveled within 300m), by ER-subtype, California Teachers Study cohort.

Table 14. Hazard ratios and 95% confidence intervals for breast cancer and industrial Cd emissions (kg/km within 5 km), by categories of exposure, California Teachers Study cohort.

Table 1. Characteristics among women with no prior history of breast cancer through 2009 and who resided in California at the time of the baseline questionnaire (California Teachers Study cohort, N = 114,253). **(UNPUBLISHED)**

Characteristics	Cases		Non-cases		Total	
	N	%	N	%	N	%
Total	5,098	100	109,155	100	114,253	100
Race/ethnicity						
Non-Hispanic White	4,532	89	93,970	86	98,502	86
Black	125	2	2,947	3	3,072	3
Hispanic	136	3	4,868	4	5,004	4
Asian/Pacific Islander	177	3	4,006	4	4,183	4
Other	128	3	3,364	3	3,492	3
Family history of breast cancer						
No	4,046	79	92,452	85	96,498	84
Yes	872	17	12,518	11	13,390	12
Unknown	180	4	4,185	4	4,365	4
Age at menarche (years)						
<=11	1,202	24	24,083	22	25,285	22
12-13	2,825	55	61,078	56	63,903	56
>=14	1,000	20	22,288	20	23,288	20
Unknown	71	1	1,706	2	1,777	2
Age at first full-term pregnancy (years)						
Nulliparous	1,176	23	28,912	26	30,088	26
<25	1,388	27	27,830	25	29,218	26
25-29	1,570	31	31,765	29	33,335	29
>=30	871	17	18,460	17	19,331	17
Unknown	93	2	2,188	2	2,281	2
Breast feeding history (months)						
Nulliparous	921	18	22,356	20	23,277	20
Pregnant without a live birth	252	5	6,423	6	6,675	6
None	996	20	17,369	16	18,365	16
<6	921	18	19,042	17	19,963	17
6-11	709	14	14,683	13	15,392	13
>=12	1,187	23	26,570	24	27,757	24
Unknown	112	2	2,712	2	2,824	2
Physical activity (hours/week)						
0.00-0.50	1,822	36	32,490	30	34,312	30
0.51-2.00	1,613	32	34,731	32	36,344	32
2.01-3.50	789	15	19,141	18	19,930	17
3.51-5.00	430	8	10,403	10	10,833	9
>5.00	394	8	11,592	11	11,986	10
Unknown	50	1	798	1	848	1

Characteristics	Cases		Non-cases		Total	
	N	%	N	%	N	%
Alcohol consumption (g/day)						
None	1,492	29	35,082	32	36,574	32
<20	2,815	55	59,745	55	62,560	55
>=20	541	11	8,284	8	8,825	8
Unknown	250	5	6,044	6	6,294	6
Body mass index (kg/m ²)						
16.0-24.9	2,791	55	64,157	59	66,948	59
25.0-29.9	1,382	27	25,665	24	27,047	24
30.0-54.8	702	14	14,552	13	15,254	13
Unknown/outlier	223	4	4,781	4	5,004	4
Menopausal status & hormone therapy (HT) use						
Pre-menopausal	1,196	23	45,062	41	46,258	40
Peri/post-menopausal & no HT use	628	12	12,868	12	13,496	12
Peri/post-menopausal & past HT use	377	7	7,448	7	7,825	7
Peri/post-menopausal & current HT use	2,109	41	29,191	27	31,300	27
Unknown	788	15	14,586	13	15,374	13
Smoking status						
Never	2,985	59	72,597	67	75,582	66
Former	1,742	34	30,390	28	32,132	28
Current	325	6	5,468	5	5,793	5
Unknown	46	1	700	1	746	1
Environmental tobacco smoke residential exposure						
None	781	15	21,315	20	22,096	19
Childhood only	1,287	25	29,307	27	30,594	27
Adulthood only	932	18	18,378	17	19,310	17
Both childhood and adulthood	1,784	35	34,032	31	35,816	31
Unknown	314	6	6,123	6	6,437	6

Characteristics	Cases	Non-cases	Total
	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	57.6 (11.9)	52.6 (14.6)	52.8 (14.5)
Total pack-years of smoking	17.5 (18.4)	15.0 (17.6)	15.1 (17.6)
Average number of cigarettes smoked per day	13.6 (10.4)	12.5 (10.2)	12.6 (10.3)
Total years since quit smoking	20.2 (11.3)	19.3 (11.5)	19.4 (11.5)

Table 2. Distributions of environmental Cd exposures from environmental sources among women with no prior history of breast cancer through 2009 and who resided in California at the time of the baseline questionnaire (California Teachers Study cohort, N = 114,253).

(UNPUBLISHED)

Exposure	Cases	Non-cases	Total
Traffic density (vehicle kilometers traveled within 300 m)			
N	5,070	108,630	113,700
Mean (SD)	2,561 (4,512)	2,517 (4,469)	2,519 (4,471)
25 th percentile	223	227	227
Median	1,220	1,172	1,174
75 th percentile	3,064	2,996	2,999
Industrial Cd emissions (kg/km within 5 km)			
N	5,098	109,155	114,253
Mean (SD)	5.49 (75.03)	4.63 (95.09)	4.67 (94.29)
25 th percentile	0.00	0.00	0.00
Median	0.00	0.00	0.00
75 th percentile	0.11	0.13	0.13
Estimated outdoor cadmium concentration (ng/m ³)			
N	5,098	109,153	114,251
Mean (SD)	0.27 (0.34)	0.27 (0.32)	0.27 (0.32)
25 th percentile	0.16	0.15	0.15
Median	0.21	0.21	0.21
75 th percentile	0.29	0.29	0.29

Table 3. Distributions of daily dietary Cd intake among women with no prior history of breast cancer through 2009 who resided in California at the time of the baseline questionnaire with complete dietary data (California Teachers Study cohort).

(UNPUBLISHED)

Daily dietary Cd intake	N	Mean	Standard Deviation	Minimum	25 th percentile	Median	75 th percentile	Maximum
Unadjusted	105,682	10.36	4.52	0.40	7.16	9.67	12.72	49.17
Calorie-adjusted (adjusted for daily calories excluding alcohol ÷ 1,000)	105,682	6.99	2.49	0.62	5.29	6.54	8.19	36.36
Calorie-adjusted using the residual method	105,682	9.96	3.41	0.85	7.62	9.40	11.66	47.13

Table 4. Hazard ratios and 95% confidence intervals for ER-positive breast cancer (n = 2,798) and quintiles of calorie-adjusted dietary Cd intake^a, California Teachers Study cohort (N = 85,509). **(UNPUBLISHED)**

Dietary Cd ^a	Cases Person-years		Minimally adjusted ^b			Fully adjusted ^c			Fully adjusted + salad & wine dietary pattern ^d		
			HR ^e	95% CI	p-trend	HR ^e	95% CI	p-trend	HR ^e	95% CI	p-trend
<7.24	437	217,366	1.0			1.0			1.0		
7.24-8.69	467	215,787	0.98	0.86-1.12		0.96	0.85-1.10		0.95	0.83-1.08	
8.70-10.16	575	215,131	1.09	0.96-1.23		1.06	0.93-1.20		1.03	0.91-1.17	
10.17-12.32	632	212,650	1.11	0.98-1.26		1.08	0.96-1.23		1.04	0.92-1.19	
≥12.33	687	208,104	1.12	0.99-1.26	0.02	1.08	0.95-1.22	0.09	1.01	0.89-1.16	0.58

^a Calorie adjusted using the residual method based on calories excluding alcohol.

^b Adjusted for total calories (continuous).

^c Additionally adjusted for parity (no, yes) and age at first full term pregnancy (continuous), history of benign breast disease (no, yes), family history of breast cancer (no, yes, adopted), alcohol consumption in the year prior to baseline (none, <20 g/d, 20+ g/d), menopausal status and HT use at baseline (premenopausal, peri-/post-menopausal: never HT, current E+P, current E alone, past HT), BMI at baseline (continuous), height at baseline (continuous) and smoking status (never, former, current).

^d Additionally adjusted for a factor score measuring consumption of a 'salad and wine' dietary pattern in the year prior to baseline (continuous).

^e Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 5. Hazard ratios and 95% confidence intervals for ER-positive breast cancer (n = 2,798) by tertile of calorie-adjusted dietary Cd intake^a and interquartile category of salad-and-wine dietary pattern using a common reference category, California Teachers Study cohort (N = 85,509). **(UNPUBLISHED)**

Dietary Cd ^a (tertiles)	Salad-and-wine dietary pattern								
	Low (<25th percentile)			Med (25th-<75 th percentile)			High (≥75th percentile)		
	Cases	HR ^b	95% CI	Cases	HR ^b	95% CI	Cases	HR ^b	95% CI
<8.23	248	1.00	reference	401	1.10	0.94-1.29	97	1.17	0.92-1.50
8.23-10.76	161	1.16	0.95-1.42	521	1.12	0.96-1.31	263	1.18	0.98-1.42
≥10.77	69	0.97	0.74-1.27	460	1.14	0.97-1.33	578	1.24	1.06-1.46

^a Calorie-adjusted using the residual method based on calories excluding alcohol.

^b Adjusted for total calories (continuous), parity (no, yes) and age at first full term pregnancy (continuous), history of benign breast disease (no, yes), family history of breast cancer (no, yes, adopted), alcohol consumption in the year prior to baseline (none, <20 g/d, 20+ g/d), menopausal status and HT use at baseline (premenopausal, peri-/post-menopausal: never HT, current E+P, current E alone, past HT), BMI at baseline (continuous), height at baseline (continuous) and smoking status (never, former, current). HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 6. Hazard ratios and 95% confidence intervals for ER-negative breast cancer (n = 486) and quintiles of calorie-adjusted dietary Cd intake^a, California Teachers Study cohort (N = 84,865). **(UNPUBLISHED)**

Dietary Cd ^a	Cases Person-years		Minimally adjusted ^b			Fully adjusted ^c			Fully adjusted + antioxidants from vegetables ^d		
			HR ^e	95% CI	p-trend	HR ^e	95% CI	p-trend	HR ^e	95% CI	p-trend
<7.24	99	216,131	1.0			1.0			1.0		
7.24-8.69	96	214,337	0.90	0.68-1.20		0.88	0.66-1.17		0.92	0.69-1.22	
8.70-10.16	97	213,797	0.83	0.62-1.10		0.81	0.60-1.07		0.86	0.64-1.16	
10.17-12.32	94	211,076	0.75	0.57-1.00		0.74	0.55-0.99		0.82	0.60-1.12	
≥12.33	100	206,120	0.75	0.56-1.00	0.03	0.73	0.54-0.98	0.03	0.88	0.62-1.27	0.49

^a Calorie-adjusted using the residual method based on calories excluding alcohol.

^b Adjusted for total calories (continuous).

^c Additionally adjusted for birthplace (North American born, not North American born), age at menarche (continuous from ≤9 to 17+), history of benign breast disease (no, yes), family history of breast cancer (no, yes, adopted), average lifetime (high school to age 54) moderate physical activity (hours per week; continuous), alcohol consumption in the year prior to baseline (none, any), menopausal status and hormone therapy use at baseline (premenopausal, peri-/post-menopausal: never hormone therapy, ever hormone therapy), BMI at baseline (continuous), a factor score measuring consumption of a 'high protein and high fat' dietary pattern in the year prior to baseline (continuous), a factor score measuring consumption of a 'high carbohydrate' dietary pattern in the year prior to baseline (continuous) and a factor score measuring consumption of an 'ethnic' dietary pattern in the year prior to baseline (continuous).

^d Additionally adjusted for antioxidant intake from vegetables (ORAC_OH, calorie-adjusted using the residual method, continuous) and its interaction with BMI and menopausal status/hormone therapy.

^e Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 7. Hazard ratios and 95% confidence intervals for ER-negative breast cancer (n = 486) by tertile of calorie-adjusted dietary Cd intake^a and tertile of antioxidant intake from vegetables using a common reference category, California Teachers Study cohort (N = 84,865).

(UNPUBLISHED)

Dietary Cd ^a (tertiles)	Tertiles of antioxidants from vegetables (ORAC_OH)								
	Cases	<u><2.12</u>		Cases	<u>2.12-2.88</u>		Cases	<u>≥2.89</u>	
		HR	95% CI		HR	95% CI		HR	95% CI
<8.23	113	1.00	reference	45	0.84	0.60-1.19	9	0.59	0.30-1.17
8.23-10.76	42	0.70	0.49-1.01	79	0.84	0.63-1.12	40	0.67	0.47-0.98
≥10.77	14	0.94	0.54-1.66	51	0.72	0.51-1.02	93	0.60	0.45-0.81

^a Calorie-adjusted using the residual method based on calories excluding alcohol.

^b Adjusted for total calories (continuous), birthplace (North American born, not North American born), age at menarche (continuous from ≤9 to 17+), history of benign breast disease (no, yes), family history of breast cancer (no, yes, adopted), average lifetime (high school to age 54) moderate physical activity (hours per week; continuous), alcohol consumption in the year prior to baseline (none, any), menopausal status and hormone therapy use at baseline (premenopausal, peri-/post-menopausal: never hormone therapy, ever hormone therapy), BMI at baseline (continuous), a factor score measuring consumption of a 'high protein and high fat' dietary pattern in the year prior to baseline (continuous), a factor score measuring consumption of a 'high carbohydrate' dietary pattern in the year prior to baseline (continuous) and a factor score measuring consumption of an 'ethnic' dietary pattern in the year prior to baseline (continuous). HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 8. Hazard ratios and 95% confidence intervals for breast cancer by quartile of estimated outdoor Cd concentration, by ER-subtype, California Teachers Study cohort.

(UNPUBLISHED)

Exposure quartile (ng/m ³)	ER-positive			ER-negative		
	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)
Total Cohort						
< 0.15	27,214	851	1.0	26,522	159	1.0
0.15-0.20	27,247	959	1.11 (1.01, 1.22)	26,429	141	0.88 (0.70, 1.10)
0.21-0.28	27,240	951	1.11 (1.01, 1.22)	26,458	169	1.06 (0.85, 1.31)
≥ 0.29	27,217	886	1.08 (0.98, 1.19)	26,507	176	1.13 (0.91, 1.41)
			p-trend=0.13			p-trend=0.12
Non-movers						
< 0.15	17,351	579	1.0	16,868	96	1.0
0.15-0.20	16,752	683	1.18 (1.05, 1.31)	16,164	95	0.99 (0.75, 1.32)
0.21-0.28	16,943	682	1.16 (1.04, 1.30)	16,373	112	1.16 (0.88, 1.53)
≥ 0.29	17,107	610	1.07 (0.96, 1.20)	16,620	123	1.31 (1.00, 1.72)
			p-trend=0.30			p-trend=0.03
Never smokers						
< 0.15	18,580	514	1.0	18,162	96	1.0
0.15-0.20	18,538	580	1.13 (1.00, 1.27)	18,053	95	0.98 (0.74, 1.31)
0.21-0.28	18,633	562	1.09 (0.97, 1.23)	18,179	108	1.11 (0.84, 1.46)
≥ 0.29	19,049	548	1.08 (0.95, 1.22)	18,616	115	1.20 (0.92, 1.58)
			p-trend=0.35			p-trend=0.13
Non-movers/never smokers						
< 0.15	11,782	358	1.0	11,479	55	1.0
0.15-0.20	11,240	411	1.16 (1.01, 1.34)	10,890	61	1.13 (0.78, 1.62)
0.21-0.28	11,340	402	1.11 (0.97, 1.29)	11,013	75	1.37 (0.97, 1.95)
≥ 0.29	11,851	381	1.06 (0.91, 1.22)	11,553	83	1.55 (1.10, 2.19)
			p-trend=0.61			p-trend=0.006

^a Adjusted for age, race, family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, BMI, menopausal status/hormone therapy combined, smoking status, smoking pack-years, home environmental tobacco smoke exposure. HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 9. Hazard ratios and 95% confidence intervals for ER-positive breast cancer by quartile of estimated outdoor Cd concentration, by menopausal status, California Teachers Study cohort.

(UNPUBLISHED)

Exposure quartile (ng/m ³)	Pre/peri-menopausal			Post-menopausal		
	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)
Total Cohort						
< 0.15	11,802	218	1.0	13,128	546	1.0
0.15-0.20	11,525	238	1.15 (0.95, 1.38)	13,556	642	1.13 (1.01, 1.27)
0.21-0.28	11,614	251	1.21 (1.01, 1.45)	13,444	636	1.14 (1.02, 1.28)
≥ 0.29	11,956	255	1.24 (1.03, 1.49)	13,138	554	1.04 (0.92, 1.17)
			p-trend=0.02			p-trend=0.54
Non-Movers						
< 0.15	6,912	147	1.0	8,919	372	1.0
0.15-0.20	6,259	156	1.15 (0.91, 1.44)	9,025	470	1.23 (1.07, 1.41)
0.21-0.28	6,180	170	1.27 (1.01, 1.58)	9,280	466	1.19 (1.04, 1.37)
≥ 0.29	6,518	165	1.22 (0.97, 1.52)	9,183	391	1.03 (0.89, 1.19)
			p-trend=0.06			p-trend=0.83
Never smokers						
< 0.15	9,043	152	1.0	8,046	302	1.0
0.15-0.20	8,953	161	1.11 (0.89, 1.39)	8,210	376	1.22 (1.04, 1.42)
0.21-0.28	8,963	182	1.25 (1.01, 1.56)	8,211	342	1.11 (0.95, 1.29)
≥ 0.29	9,423	178	1.20 (0.97, 1.50)	8,208	320	1.05 (0.90, 1.23)
			p-trend=0.06			p-trend=0.88
Non-movers/never smokers						
< 0.15	5,254	105	1.0	5,518	209	1.0
0.15-0.20	4,806	101	1.04 (0.79, 1.37)	5,489	277	1.31 (1.10, 1.57)
0.21-0.28	4,676	122	1.27 (0.98, 1.66)	5,667	253	1.16 (0.97, 1.40)
≥ 0.29	5,131	115	1.14 (0.87, 1.49)	5,759	229	1.05 (0.87, 1.27)
			p-trend=0.17			p-trend=0.98

^a Adjusted for age, race, family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, BMI, smoking status, smoking pack-years, home environmental tobacco smoke exposure. HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 10. Hazard ratios and 95% confidence intervals for ER-negative breast cancer by quartile of estimated outdoor Cd concentration, by menopausal status, California Teachers Study cohort. **(UNPUBLISHED)**

Pre/peri-menopausal				Post-menopausal		
Exposure quartile (ng/m ³)	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)
Total Cohort						
< 0.15	11,626	42	1.0	12,677	95	1.0
0.15-0.20	11,327	40	0.96 (0.62, 1.49)	12,997	83	0.85 (0.63, 1.14)
0.21-0.28	11,413	50	1.22 (0.80, 1.84)	12,909	101	1.04 (0.79, 1.38)
≥ 0.29	11,754	53	1.29 (0.85, 1.94)	12,686	102	1.09 (0.82, 1.45)
			p-trend=0.14			p-trend=0.32
Non-movers						
< 0.15	6,787	22	1.0	8,604	57	1.0
0.15-0.20	6,127	24	1.18 (0.66, 2.10)	8,611	56	0.95 (0.66, 1.38)
0.21-0.28	6,039	29	1.42 (0.81, 2.47)	8,886	72	1.21 (0.85, 1.72)
≥ 0.29	6,389	36	1.75 (1.02, 2.99)	8,862	70	1.22 (0.86, 1.73)
			p-trend=0.03			p-trend=0.14
Never smokers						
< 0.15	8,922	31	1.0	7,795	51	1.0
0.15-0.20	8,822	30	0.96 (0.58, 1.59)	7,888	54	1.06 (0.72, 1.55)
0.21-0.28	8,812	31	0.99 (0.60, 1.64)	7,935	66	1.27 (0.88, 1.83)
≥ 0.29	9,284	39	1.26 (0.78, 2.04)	7,951	63	1.25 (0.86, 1.82)
			p-trend=0.33			p-trend=0.16
Non-movers/never smokers						
< 0.15	5,166	17	1.0	5,337	28	1.0
0.15-0.20	4,722	17	1.08 (0.55, 2.13)	5,248	36	1.29 (0.79, 2.12)
0.21-0.28	4,573	19	1.20 (0.62, 2.33)	5,461	47	1.64 (1.02, 2.63)
≥ 0.29	5,043	27	1.70 (0.92, 3.15)	5,575	45	1.62 (1.00, 2.61)
			p-trend=0.08			p-trend=0.03

^a Adjusted for age, race, family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, BMI, smoking status, smoking pack-years, home environmental tobacco smoke exposure. HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 11. Hazard ratios and 95% confidence intervals for ER-positive breast cancer by quartile of estimated outdoor Cd concentration, by category of body mass index, California Teachers Study cohort. **(UNPUBLISHED)**

Exposure quartile (ng/m ³)	BMI<25 kg/m ²			BMI 25-29 kg/m ²			BMI≥30 kg/m ²		
	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)
Total Cohort									
< 0.15	16,244	464	1.0	6,471	258	1.0	3,458	96	1.0
0.15-0.20	16,432	528	1.11 (0.98, 1.26)	6,207	258	1.02 (0.86, 1.22)	3,404	124	1.28 (0.98, 1.67)
0.21-0.28	16,082	514	1.11 (0.98, 1.26)	6,403	240	0.92 (0.77, 1.10)	3,553	156	1.60 (1.24, 2.06)
≥ 0.29	15,152	454	1.10 (0.96, 1.25)	6,688	243	0.93 (0.78, 1.12)	4,230	153	1.37 (1.06, 1.77)
			p-trend=0.18			p-trend=0.28			p-trend=0.008
Non-movers									
< 0.15	10,186	323	1.0	4,246	172	1.0	2,245	62	1.0
0.15-0.20	9,873	374	1.13 (0.98, 1.32)	3,962	188	1.13 (0.92, 1.39)	2,184	89	1.48 (1.07, 2.05)
0.21-0.28	9,735	377	1.16 (1.00, 1.35)	4,173	171	0.95 (0.77, 1.18)	2,301	107	1.68 (1.23, 2.31)
≥ 0.29	9,210	302	1.03 (0.88, 1.21)	4,368	172	0.96 (0.77, 1.19)	2,813	113	1.51 (1.11, 2.07)
			p-trend=0.60			p-trend=0.38			p-trend=0.01
Never smokers									
< 0.15	11,260	279	1.0	4,274	151	1.0	2,336	61	1.0
0.15-0.20	11,388	323	1.15 (0.98, 1.35)	4,111	163	1.11 (0.89, 1.38)	2,254	73	1.19 (0.84, 1.67)
0.21-0.28	11,141	311	1.13 (0.96, 1.33)	4,269	131	0.85 (0.67, 1.07)	2,388	101	1.63 (1.18, 2.25)
≥ 0.29	10,763	279	1.11 (0.94, 1.31)	4,570	148	0.93 (0.74, 1.17)	2,922	100	1.35 (0.98, 1.86)
			p-trend=0.27			p-trend=0.19			p-trend=0.03
Non-movers/never smokers									
< 0.15	6,997	197	1.0	2,813	107	1.0	1,505	38	1.0
0.15-0.20	6,738	227	1.15 (0.95, 1.39)	2,586	119	1.17 (0.90, 1.52)	1,439	49	1.34 (0.87, 2.05)
0.21-0.28	6,590	229	1.17 (0.97, 1.42)	2,720	93	0.84 (0.63, 1.11)	1,528	69	1.81 (1.21, 2.71)
≥ 0.29	6,451	181	1.02 (0.83, 1.25)	2,959	109	0.93 (0.71, 1.22)	1,944	76	1.57 (1.06, 2.34)
			p-trend=0.77			p-trend=0.22			p-trend=0.01

^a Adjusted for age, race, family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, menopausal status/hormone therapy combined, smoking status, smoking pack-years, home environmental tobacco smoke exposure. HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 12. Hazard ratios and 95% confidence intervals for ER-positive breast cancer by quartile of estimated outdoor Cd concentration, by category of body mass index, using a common reference group (lowest exposure quartile and BMI<25 kg/m²), California Teachers Study cohort. **(UNPUBLISHED)**

Exposure quartile (ng/m ³)	BMI<25 kg/m ²			BMI 25-29 kg/m ²			BMI≥30 kg/m ²		
	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)
Total Cohort (p-interaction=0.15)									
< 0.15	16,244	464	1.0	6,471	258	1.27 (1.09, 1.48)	3,458	96	0.95 (0.76, 1.18)
0.15-0.20	16,432	528	1.11 (0.98, 1.26)	6,207	258	1.31 (1.12, 1.52)	3,404	124	1.20 (0.98, 1.46)
0.21-0.28	16,082	514	1.12 (0.98, 1.27)	6,403	240	1.18 (1.01, 1.38)	3,553	156	1.48 (1.23, 1.77)
≥ 0.29	15,152	454	1.10 (0.97, 1.25)	6,688	243	1.19 (1.02, 1.39)	4,230	153	1.29 (1.07, 1.55)
Non-movers (p-interaction=0.35)									
< 0.15	10,186	323	1.0	4,246	172	1.19 (0.99, 1.43)	2,245	62	0.85 (0.65, 1.12)
0.15-0.20	9,873	374	1.14 (0.98, 1.32)	3,962	188	1.35 (1.13, 1.62)	2,184	89	1.22 (0.96, 1.54)
0.21-0.28	9,735	377	1.17 (1.00, 1.35)	4,173	171	1.15 (0.96, 1.39)	2,301	107	1.40 (1.13, 1.75)
≥ 0.29	9,210	302	1.03 (0.88, 1.21)	4,368	172	1.15 (0.95, 1.38)	2,813	113	1.28 (1.03, 1.59)
Never smokers (p-interaction=0.08)									
< 0.15	11,260	279	1.0	4,274	151	1.30 (1.06, 1.58)	2,336	61	1.02 (0.77, 1.35)
0.15-0.20	11,388	323	1.15 (0.98, 1.35)	4,111	163	1.44 (1.18, 1.75)	2,254	73	1.23 (0.95, 1.60)
0.21-0.28	11,141	311	1.14 (0.97, 1.34)	4,269	131	1.11 (0.90, 1.36)	2,388	101	1.62 (1.29, 2.04)
≥ 0.29	10,763	279	1.12 (0.94, 1.32)	4,570	148	1.20 (0.98, 1.46)	2,922	100	1.38 (1.10, 1.74)
Non-movers/never smokers (p-interaction=0.09)									
< 0.15	6,997	197	1.0	2,813	107	1.27 (1.00, 1.61)	1,505	38	0.89 (0.63, 1.26)
0.15-0.20	6,738	227	1.15 (0.95, 1.39)	2,586	119	1.49 (1.18, 1.87)	1,439	49	1.16 (0.85, 1.59)
0.21-0.28	6,590	229	1.18 (0.97, 1.43)	2,720	93	1.08 (0.84, 1.39)	1,528	69	1.53 (1.16, 2.01)
≥ 0.29	6,451	181	1.02 (0.83, 1.24)	2,959	109	1.19 (0.94, 1.51)	1,944	76	1.39 (1.06, 1.82)

^a Adjusted for age, race, family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, menopausal status/hormone therapy combined, smoking status, smoking pack-years, home environmental tobacco smoke exposure. HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 13. Hazard ratios and 95% confidence intervals for breast cancer by quartile of traffic density (vehicle km traveled within 300m), by ER-subtype, California Teachers Study cohort. (UNPUBLISHED)

Exposure quartile (vkt/km ²)	ER-positive			ER-negative		
	N	Cases	HR ^a (95% CI)	N	Cases	HR ^a (95% CI)
Total Cohort						
< 227.9	27,077	905	1.0	26,323	151	1.0
227.9-1,172.5	27,104	841	0.95 (0.86, 1.04)	26,422	159	1.07 (0.86, 1.34)
1,172.6-2,993.0	27,101	925	1.03 (0.94, 1.13)	26,340	164	1.11 (0.89, 1.39)
≥ 2,993.1	27,110	960	1.06 (0.96, 1.16)	26,317	167	1.13 (0.90, 1.41)
			p-trend=0.9			p-trend=0.28
Non-movers						
< 227.9	18,555	674	1.0	17,981	100	1.0
227.9-1,172.5	15,730	559	0.95 (0.85, 1.06)	15,273	102	1.17 (0.89, 1.55)
1,172.6-2,993.0	16,263	630	1.02 (0.91, 1.14)	15,742	109	1.21 (0.92, 1.59)
≥ 2,993.1	17,606	691	1.03 (0.92, 1.15)	17,030	115	1.20 (0.92, 1.57)
			p-trend=0.9			p-trend=0.19
Never smokers						
< 227.9	18,842	562	1.0	18,369	89	1.0
227.9-1,172.5	18,923	513	0.93 (0.82, 1.05)	18,516	106	1.22 (0.92, 1.61)
1,172.6-2,993.0	18,693	545	0.99 (0.88, 1.11)	18,255	107	1.23 (0.93, 1.63)
≥ 2,993.1	18,001	573	1.07 (0.95, 1.21)	17,538	110	1.32 (0.99, 1.75)
			p-trend=0.9			p-trend=0.07
Non-movers/never smokers						
< 227.9	12,842	417	1.0	12,484	59	1.0
227.9-1,172.5	10,782	353	0.98 (0.85, 1.13)	10,497	68	1.34 (0.95, 1.91)
1,172.6-2,993.0	11,010	376	1.00 (0.87, 1.15)	10,705	71	1.35 (0.96, 1.91)
≥ 2,993.1	11,580	406	1.03 (0.90, 1.18)	11,250	76	1.41 (1.00, 1.99)
			p-trend=0.9			p-trend=0.06

^a Adjusted for age, race, family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, BMI, menopausal status/hormone therapy combined, smoking status, smoking pack-years, home environmental tobacco smoke exposure. HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

Table 14. Hazard ratios and 95% confidence intervals for breast cancer and industrial Cd emissions (kg/km within 5 km), by categories of exposure, California Teachers Study cohort. **(UNPUBLISHED)**

Population	ER-positive		ER-negative	
	Cases	HR ^a (95% CI)	Cases	HR ^a (95% CI)
Total cohort (N = 105,935)	3,117		545	
<75 th percentile		1.0		1.0
75 th -89 th percentile		1.01 (0.91, 1.12)		0.95 (0.75, 1.22)
≥90 th percentile		0.99 (0.88, 1.12)		0.81 (0.59, 1.10)
Non-movers (N = 66,057)	2,180		358	
<75 th percentile		1.0		1.0
75 th -89 th percentile		1.01 (0.89, 1.15)		1.15 (0.85, 1.54)
≥90 th percentile		0.97 (0.84, 1.13)		0.93 (0.65, 1.35)
Never smokers (N = 73,024)	1,869		343	
<75 th percentile		1.0		1.0
75 th -89 th percentile		1.01 (0.88, 1.15)		1.14 (0.85, 1.52)
≥90 th percentile		1.08 (0.93, 1.26)		0.66 (0.43, 1.01)
Non-movers and never smokers (N = 44,955)	1,313		228	
<75 th percentile		1.0		1.0
75 th -89 th percentile		1.03 (0.87, 1.21)		1.37 (0.96, 1.95)
≥90 th percentile		1.03 (0.86, 1.23)		0.89 (0.55, 1.43)

^a Adjusted for age, race, family history of breast cancer, age at menarche, pregnancy history, breast feeding history, physical activity, alcohol consumption, BMI, menopausal status/hormone therapy combined, smoking status, smoking pack-years, home environmental tobacco smoke exposure. HRs estimated using Cox regression with age (in days) as the time-scale and stratified by age (in years) at baseline.

APPENDIX 1

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Reproducibility and Determinants of Urinary Cadmium Concentrations among Women in Northern California

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Abbreviations:

AADT = annual average daily traffic

Cd = cadmium

CTS = California Teachers Study

FFQ = food frequency questionnaire

GIS = geographic information system

GM = geometric mean

LOD = limit of detection

R^2 = coefficient of determination

U-Cd = urinary cadmium

VKT = vehicle kilometers traveled

ABSTRACT

Background: Cadmium is a toxic metal associated with increased morbidity and mortality.

Urinary cadmium concentration is considered a biomarker of long-term exposure.

Objectives: Our objectives were to evaluate the within-person correlation among repeat samples and identify predictors of urinary cadmium concentrations.

Methods: Urinary cadmium concentrations (ug/L) were measured in 24-hour urine samples collected from 296 women enrolled in the California Teachers Study in 2000 and a 24-hour sample collected 3 – 9 months later from 141 of the participants. Lifestyle and sociodemographic characteristics were obtained via questionnaires. The Total Diet Study database was used to quantify dietary cadmium intake. We estimated environmental cadmium emissions near participants' residences using a geographic information system.

Results: The geometric mean urinary cadmium concentration was 0.27 ug/L and the range was 0.1– 3.6 ug/L. The intraclass correlation among repeat samples from the same individual was $r=0.50$ resulting in a 50% attenuation bias. The use of a single 24-hour urine specimen to characterize Cd exposure in a case-control study with a true odds ratio of 2.0 would result in an observed odds ratio of 1.4. Urinary cadmium concentration increased with creatinine, age and lifetime pack-years of smoking among ever smokers or lifetime intensity-years of passive smoking among nonsmokers, while it decreased with greater alcohol consumption and number of previous pregnancies. These factors explained 42 – 44% of the variability in urinary cadmium concentrations.

Conclusion: Urinary cadmium levels varied with several individual characteristics and a single measurement of urinary cadmium did not accurately reflect medium to long-term body burden.

BACKGROUND

Cadmium (Cd) is a toxic, bioaccumulating, and somewhat persistent metal released into the environment during mining operations, industrial processes, and as a byproduct of oil combustion (ATSDR 1999). Non-occupational Cd exposure, assessed using urinary Cd levels, has been associated with kidney disease (Suwazono et al. 2006; Jarup and Akesson 2009), cardiovascular disease (Peters et al. 2010), dental caries (Arora et al. 2008), decreases in bone mineral density (Satarug and Moore 2004), and increased mortality (Menke et al. 2009; Nawrot et al. 2008). Cd is classified as a human lung carcinogen (IARC 1993), and has been associated with increased overall cancer mortality (Adams et al. 2012) and the incidence of breast (Gallagher et al. 2010; Julin et al. 2012; McElroy et al. 2006) and endometrial cancers (Akesson et al. 2008).

Cd is stored in the liver and kidneys, has a biological half-life of 10–30 years, and is absorbed via inhalation and ingestion (ATSDR 1999). Absorption of Cd in the gastrointestinal tract is poor (3-10%) while absorption from the deep areas of the lung is high (50-90%), suggesting that inhalation may be an important route of exposure (Waalkes 2003). The principal source of exposure for smokers in non-industrial settings is inhalation of cigarette smoke (CDC 2005) as smoking may double the daily intake of Cd compared with not smoking (ATSDR 1999). For nonsmokers, the principal source of Cd is ingestion of contaminated plant-based foods (CDC 2005). Women generally have higher internal Cd levels than men because depleted iron stores and iron deficiency, common among women of childbearing age, increases the intestinal uptake of Cd (Vahter et al. 2002).

Numerous studies have cited urinary Cd (U-Cd) concentration as a reliable measure of cumulative lifetime exposure (Julin et al. 2011; McElroy et al. 2006; Nawrot et al. 2006).

Accurate assessment of long-term Cd exposure is important because U-Cd levels have been associated with health outcomes thought to have a long latency period such as cancer and cardiovascular disease. However, short-term Cd exposure levels could also be important for studies of prenatal exposure and developmental effects in children. U-Cd concentrations have been shown to be correlated with age (Hellstrom et al. 2004), ²⁶gender (Hellstrom et al. 2004), iron deficiency (Berglund et al. 1994), parity (Akesson et al. 2002), smoking status (Hellstrom et al. 2004; Ikeda et al. 2005), second-hand smoke (Willers et al. 2005), and dietary intake of Cd (Adams et al. 2011; Choudhury et al. 2001; Julin et al. 2011; Shimbo et al. 2000). However, most of these exposure studies have relied on single spot urine samples instead of repeated 24-hour urine collections. In addition, sources of Cd exposure among non-occupationally exposed and mostly nonsmoking women have not been well characterized. The objective of this study was to identify determinants of urinary Cd using repeat 24-hour urine collections and exposure information from a variety of sources including self-reports, environmental databases, and a dietary contaminant database in a sample of women enrolled in the largely non-smoking California Teachers Study (CTS) cohort.

METHODS

Study population and questionnaire data

Our study population consisted of 296 women participating in a measurement sub-study of the CTS cohort. The cohort includes 133,479 women who were active or retired public school teachers or administrators in 1995 (Bernstein et al. 2002). The sub-study, conducted in 2000, included a random sample of CTS participants who resided in the sub-study area (i.e., western Alameda, Santa Clara, San Mateo, Santa Cruz, Monterey, or northern San Benito counties in California) and were aged 85 years or younger at baseline in 1995-96 (Gunier et al. 2006; Horn-

Ross et al. 2008). Of the 484 women invited to participate, 328 (68%) agreed, 138 refused and 18 were not interviewed for other reasons. All participants provided written informed consent and this research was approved by the Institutional Review Board of the Cancer Prevention Institute of California. Of the 328 participants, 304 (93%) provided a 24-hour urine specimen. Our analysis is based on 296 of these women with adequate urine volume available for cadmium analysis, 122 of whom were urban and 174 of whom were rural residents. Of the 157 women asked, 141 (90%) provided a second 24-hour urine specimen; these samples were collected 3, 6, or 9 months following the initial sample. Both the original and repeat specimens were analyzed for U-Cd.

We used self-administered questionnaires to collect information on age, height, weight, parity, duration of breastfeeding and active smoking history when the cohort was established in 1995-96 as well as additional questions on the source, setting, timing and dose of passive smoking exposures from a second survey mailed in 1997. For nonsmokers, we used a measure of lifetime intensity-years of passive smoking based on a qualitative description of smoke intensity (a little smoky, fairly smoky or very smoky) and duration of exposure in years (Reynolds et al. 2009). Usual diet and alcohol consumption during the past year and current residential address were obtained at the time of urine sample collection in 2000.

Sample collection and laboratory analysis

Each sub-study participant received a collection kit and was instructed to collect all urine produced in the 24-hour period starting. The samples were collected and stored at -20°C up to two weeks until they were thawed, aliquotted and frozen at -70°C. Approximately 9 years elapsed between the sample collection and the analysis for Cd concentrations.

Urinary cadmium concentrations ($\mu\text{g/L}$) were measured using inductively-coupled plasma/ mass spectrometry at a certified commercial laboratory (Pacific Toxicology Laboratories; Chatsworth, CA). The limit of detection (LOD) for U-Cd was $0.1 \mu\text{g/L}$. Low and high Cd control standards were included in each batch to evaluate assay accuracy and precision. The within-batch coefficient of variation was $<10\%$ and between-batch coefficient was $<15\%$. Creatinine concentrations (g/L) were measured using a modified-rate Jaffe method and were highly correlated with creatinine concentrations measured in 2000 at another laboratory (intraclass correlation coefficient= 0.88).

Environmental and dietary exposure assessment

We estimated potential environmental exposure to Cd at the participants' geocoded residences in 2000 using a geographic information system (GIS) and three available databases for industrial emissions, ambient air concentrations, and vehicle traffic. The geocoded residential locations were also assigned urban or rural classifications based on the 2000 U.S. Census. To estimate exposure to Cd emissions from industrial and commercial facilities, we used 1995 data from the California Air Toxics Emissions Data System which provides latitude/longitude coordinates and annual emissions in pounds self-reported by each facility (CARB 1998). We estimated the distance between a residence and all facilities within five kilometers with reported Cd emissions. Geocoded residences were also linked by census tract to estimated Cd concentrations in ambient air in 1999 from the National Air Toxics Assessment (USEPA 2006). These concentrations were derived using an atmospheric dispersion model that combined emissions inventories with local meteorology (Rosenbaum et al. 1999).

To estimate potential exposure to Cd from vehicle emissions, we obtained traffic count data for 2000 from the California Highway Performance and Monitoring System (CDOT 2007). These

data provide the annual average daily traffic (AADT), the average number of vehicles per day traveling in both directions on major roads. For each participant's residence, we calculated traffic density by summing the vehicle kilometers of travel (VKT) within a 300 meter radius buffer by multiplying the AADT by the length of the road segment for each road segment with AADT values within the buffer, then dividing by the buffered area (0.28 km^2) to obtain VKT per day per square kilometer (Gunier et al. 2003). We used a 300 meter radius because this approximates the distance at which particulate pollutant concentrations approach background levels (Zhou and Levy 2007) .

Dietary Cd intake was assessed via an early version of the 103-item Block95 food frequency questionnaire (FFQ) (Block et al. 1986; Block et al. 1990; Horn-Ross et al. 2008). For each food item, frequency of consumption (categories from never to once/day or 5+/day depending on the item) and usual portion size (small, medium, or large relative to a given standard medium portion) were assessed for the previous year (i.e., 1999). The FFQs were self-administered and checked by study staff for completeness. FFQ items were assigned Cd values based on the Total Diet Study market basket surveys conducted between 1991 and 2004 (USFDA 2006). Dietary Cd was not estimated for 3 participants who did not complete the food frequency questionnaire and 6 participants whose reported food consumption was judged to be implausibly low (<600 calories/day) or high (>5,000 calories/day).

Statistical analysis

For seven samples with U-Cd concentration below the limit of detection (LOD), we assigned a concentration equivalent to the $\text{LOD} \div \sqrt{2}$ ($0.07 \text{ } \mu\text{g/L}$). We calculated the creatinine-adjusted U-Cd (U-CdCr) levels ($\mu\text{g/g-Cr}$) by dividing the U-Cd concentrations ($\mu\text{g/L}$) by the creatinine concentrations (g/L). We multiplied U-Cd concentration ($\mu\text{g/L}$) by the total volume of urine

collected during the 24-hour period (L/day) to estimate daily Cd output ($\mu\text{g/day}$). Potential explanatory variables for the variation in U-Cd concentrations included age at the time of urine sample collection (2000) rescaled so that the youngest person had an age of zero years, body mass index (kg/m^2 ; a measure of weight independent of height) and body surface area ($((\text{weight}(\text{kg})^{0.425} \times \text{height}(\text{cm})^{0.725} \times 0.007184)$; a measure of body size reflecting muscle mass) (Ruggieri and Rocca 2010), parity (i.e., number of full-term pregnancies), total duration of breastfeeding (months), oral contraceptive use (ever/never), and hormone replacement therapy (ever/never) as of 1995-96; lifetime active and passive smoking history (through 1997); usual alcohol consumption (g/d), dietary Cd intake ($\mu\text{g/d}$), and environmental indicators of potential exposure from traffic, industrial and commercial sources as of 1999. Because the distribution of U-Cd was skewed, we used the non-parametric Kruskal-Wallis test to make univariate categorical comparisons of the U-Cd distribution from the first urine sample collected from each participant ($n=296$) and demographic, dietary and environmental characteristics.

For regression models, we used a natural-log transformation to normalize the U-Cd distribution. Variance components models with random intercepts for each participant were used to determine the intraclass correlation coefficient of U-Cd concentrations from repeated samples collected from the same individual. We calculated the ratio of the within- and between-person variance and the attenuation bias that would result from measurement error in a study using a single measure of U-Cd to estimate exposure (Loomis and Kromhout 2004).

We used linear mixed-effects models with random intercepts to identify significant determinants of U-Cd levels and estimate the amount of variability in measured levels explained by the model while accounting for the correlation among repeat samples collected from the same individual (Peretz et al. 2002). We included creatinine concentration as a predictor in our models with

unadjusted U-Cd levels as the dependent variable instead of using U-CdCr as the dependent variable because this allows for an evaluation of the relationship between U-Cd and other predictor variables independent of urinary creatinine concentration (Barr et al. 2005). Backwards stepwise elimination regression was employed to evaluate potential explanatory variables for inclusion in the models from questionnaire data that were related to U-Cd concentrations from the univariate analyses ($p < 0.2$). In the final models, significant predictors ($p < 0.1$) were maintained along with the environmental and dietary exposure Cd estimates. To estimate the effects of passive tobacco smoke exposure, we created a separate model restricted to women who never smoked ($n = 163$). We performed a 10-fold cross-validation to evaluate the fit of our models by setting aside 10% of the data and rerunning the models (Shao 1993). All analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA) and STATA version 11 (STATA Corp., College Station, TX, USA).

RESULTS

Table 1 presents the characteristics of the study participants. Participants were on average 55 years of age, had a slightly greater than ideal body mass (median = 25.1 kg/m^2), had an average body surface area of 1.8 m^2 , a median of two full-term pregnancies, breastfed for a total of three months and most had never smoked (68%). Traffic density ($0 - 427,000 \text{ VKT/km}^2$) and industrial Cd emissions ($0 - 1,760 \text{ kg}$) ranged over several orders of magnitude, while participants had approximately 2- to 3-fold variations in the interquartile ranges for estimated dietary Cd intake ($7.9 - 14 \text{ ug/day}$) and estimated Cd concentrations in ambient air ($0.09 - 0.28 \text{ ng/m}^3$). The geometric means of U-Cd concentration, U-CdCr concentration and 24-hour U-Cd output from the first urine sample were $0.27 \text{ } \mu\text{g/L}$, $0.38 \text{ } \mu\text{g/g}$ and $0.46 \text{ } \mu\text{g/day}$, respectively.

The overall intraclass correlation coefficient among the 141 participants with repeated urine samples was 0.50 for U-Cd concentration and 0.42 for U-CdCr concentration, indicating moderate within-person correlation over time. Correlations were similar whether the time between repeat urine sample collection was 3, 6 or 9 months ($\rho=0.51$, 0.59 and 0.42 respectively). Based on the overall within- and between-person variance components (0.221 and 0.216 , respectively; a ratio of 1.0), measurement error resulting from the use of a single 24-hour U-Cd sample to estimate exposure would result in a 50% attenuation bias of the regression coefficient towards the null while the use of two or four U-Cd samples would result in 33% and 20% attenuation bias respectively.

Table 2 presents selected results from non-parametric univariate analyses of self-reported characteristics and U-Cd levels in the first urine sample ($n=296$). U-Cd levels increased significantly with both age and cumulative pack-years of smoking, and the relationship was stronger for U-CdCr concentrations ($p<0.0001$). The geometric mean U-CdCr concentration among those with 20 or more pack-years of smoking ($0.57 \mu\text{g/g}$) was 63% higher than the geometric mean levels among never-smokers ($0.35 \mu\text{g/g}$). Participants that reported consuming 20 g of alcohol (approximately 2 drinks) or more per day had significantly lower U-Cd concentrations than participants that did not drink alcohol. Increasing parity was also related to lower U-Cd levels and the relationship was stronger for U-CdCr levels ($p<0.0002$). Larger body surface area was associated with lower unadjusted U-Cd concentrations and weakly associated with U-CdCr levels ($p=0.08$), while higher body mass index was related to U-CdCr ($p=0.08$) but not unadjusted U-Cd concentrations. Duration of breastfeeding ($p=0.03$) and ever use of hormone replacement therapy ($p=0.02$) were associated with U-CdCr but not unadjusted U-Cd. Never using oral contraceptives was associated with higher unadjusted ($p=0.005$) and U-CdCr

concentrations ($p=0.001$). In univariate analyses, there was no relationship between U-Cd or U-CdCr levels and passive smoking, estimated dietary intake or potential exposure to environmental sources of Cd (Table 3).

Table 4 provides the percentage change in U-Cd concentrations from the final mixed-effects models with smoking as a predictor variable for all participants (Model 1) and among never-smokers with passive smoking intensity-years as a predictor variable (Model 2). The variance explained (R^2) was similar at 42 – 44% for both models. The greatest variability in U-Cd concentrations was explained by creatinine concentration (27%) and age (8%). In models with an interaction term between age and creatinine, we observed evidence of an interaction between these variables (p -interaction=0.09), suggesting that U-Cd levels increase more with creatinine levels as age increases. Total pack-years of smoking among all participants and total lifetime intensity of passive smoking among nonsmokers were also positively associated with U-Cd. Each year in age was associated with a 1.4% increase in U-Cd concentration and each pack year of active smoking was associated with a 1% increase. Among former smokers ($n=70$ participant and 97 samples), the number of years since smoking stopped was associated ($p=0.01$) with a 1.5% decrease per year in U-Cd concentration. Increasing parity and alcohol intake were associated with lower U-Cd concentrations. Dietary and environmental estimates of Cd exposure were not significant predictors of U-Cd concentrations in this population.

Models with creatinine-adjusted U-Cd or 24-hour U-Cd output as the dependent variable produced parameter estimates similar to those for U-Cd concentration (results not shown). Cross-validation showed that the models were not over fit, with the same independent variables significant in each subset of the data, similar regression coefficients ($\pm 10\%$) and overall adjusted R^2 values (40 – 46%).

DISCUSSION

In this analysis, we observed only a moderate level of within-person correlation for repeated measures of U-Cd concentrations (unadjusted $\rho=0.50$; creatinine-adjusted $\rho=0.42$) from samples collected 3 – 9 months apart, suggesting that a single U-Cd measurement does not accurately represent lifetime Cd body burden. This result from repeat 24-hour urine samples is within the range of correlations ($r = 0.4 - 0.6$) observed from the few studies that measured U-Cd in repeat morning void samples (Ikeda et al. 2006; Mason et al. 1998; Yamagami et al. 2008). For an epidemiologic study of the effect of Cd where a single measurement of U-Cd would be used to characterize exposure, this observed level of within-person correlation for repeated samples leads to exposure misclassification with an estimated attenuation bias of approximately 50% such that a “true” odds ratio of 2.0 would be reduced to an observed value of 1.4. This attenuation is similar to that observed for pesticide exposure misclassification on estimates of disease risk (Blair et al. 2011). U-CdCr levels in this study ($GM=0.38 \mu\text{g/g}$) were nearly identical to levels from other studies in the U.S. in women of similar age ($GM=0.28 - 0.36 \mu\text{g/g}$) that have observed associations between higher U-Cd and increased risk of cancer and cardiovascular mortality (Adams et al. 2012; McElroy et al. 2006; Menke et al. 2009).

In this population of California women without occupational exposure to Cd and a very low prevalence of current smoking (3%), we identified several specific factors that predicted U-Cd concentrations. Age and lifetime pack-years of smoking were positively associated with U-Cd; these findings are consistent with previous studies (Adams et al. 2011; Ikeda et al. 2005; McElroy et al. 2007a; Richter et al. 2009). The association with age, however, may be due to age-related changes in renal physiology such as lower Cd excretion among older individuals due to reduced tubular reabsorption capacity (Bernard 2004; Vahter et al. 2004; Jarup and Akesson

2009) as well as lower absorption of Cd in older women due to postmenopausal increases in serum ferritin (Milman et al. 1992; Jian et al. 2009). Each pack-year of smoking increased U-Cd concentrations approximately 1% in our study of both pre- and postmenopausal women compared with a 2% increase observed in a study of only premenopausal women (Adams et al. 2011). Lifetime intensity of passive smoke exposure was also associated with U-Cd among never-smokers in our population. One study observed a significant correlation between urinary cotinine and U-Cd among children (Willers et al. 2005) while another study of women found no association between U-Cd and self-reported recent passive smoke exposure or number of locations where women were exposed (McElroy et al. 2007a).

We observed a weak negative relationship between parity and U-Cd concentration, a finding consistent with a recent study of premenopausal women (Adams et al. 2011). Other studies observed a positive association between U-Cd and parity and attributed this trend to potential iron deficiency during pregnancy that leads to a greater absorption of Cd (Akesson et al. 2002; McElroy et al. 2007b). Average daily alcohol consumption was inversely associated with U-Cd in our study population; this contradicts previous studies that reported no association (Gil et al. 2011; McElroy et al. 2007b; Peters et al. 2010). Consistent with previous studies, body surface area, a measure of muscle mass, was inversely associated with U-Cd in univariate models (Dhooge et al. 2010; McElroy et al. 2007b; Suwazono et al. 2005); however, this association was not observed for body surface area or body mass index, a measure of adiposity, in adjusted models.

Studies of populations consuming food contaminated with Cd have observed positive associations between dietary Cd intake and urinary Cd levels (Ikeda et al. 2006; Yamagami et al. 2006) as have several other studies in low-exposure populations (Choudhury et al. 2001; Julin et

al. 2011; Shimbo et al. 2000). However, consistent with our findings, other studies of women with low Cd exposure have observed no association between U-Cd levels and either dietary Cd intake (Vahter et al. 1996) or the consumption of specific food items (McElroy et al. 2007b). In a study of non-smoking women, Adams et al. (2011) observed an association between U-Cd and usual consumption of tofu and cooked cereals. The Total Diet Study, from which we obtained our estimated of dietary Cd, did not include Cd levels in tofu or other soy products. However, tofu was not largely consumed in our population. While some studies measured Cd in duplicate food samples (Vahter et al. 1996; Shimbo et al, 2000; Julin et al. 2011) and others relied on food-frequencies questionnaires and TDS data (Choudhury et al. 2001; and the present study), no clear pattern between methodologies and results was apparent. Variation in Cd absorption related to iron stores is another possible explanation for the mixed findings between dietary and urinary Cd (Vahter et al., 1996; McElroy et al. 2007). In addition, Cd levels in food are dependent on soil levels as evidenced by a study in Japan that showed a two-fold variation in Cd levels from various locations (Shimbo et al. 2000).

We did not observe associations between estimated Cd exposures from environmental sources and U-Cd. Our study area, which included urban, suburban, and rural regions of the state, was not known to have Cd contamination and had relatively low Cd emissions compared to areas of California with a greater concentration of industrial sources. Furthermore, the ambient levels of Cd observed in the study area are considered to be low and not thought to be a major source of exposure to the general population (ATSDR 1999). Our GIS-derived exposure estimates were based on residential location only and did not account for time spent in other locations (e.g., workplace), wind direction or meteorology. Nonetheless, the amount of variance in U-Cd concentrations explained by our mixed-effects regression models ($R^2=42-44\%$) is similar to that

observed in a study ($R^2=40\%$) of non-occupationally exposed women that used a single measure of U-CdCr (McElroy et al. 2007b).

Limitations of this analysis include the relatively low and limited range of U-Cd levels and estimated Cd exposure from smoking and environmental sources among our study participants. However, these women are representative of a large portion of the California population (Bernstein et al. 2002) and these findings can thus be generalized to that significant segment of the population. Our estimates of potential environmental exposure were based on a single year of data and may not accurately reflect historical cumulative exposure through air. Urine samples were stored for about 9 years at $-70\text{ }^{\circ}\text{C}$ and were not collected specifically for U-Cd analysis, therefore potential contamination of the urine collection containers could not be ruled out that could contribute to the observed within- and between-person variation in measured concentrations (McElroy et al. 2007b). Other than tobacco smoke, we were unable to identify sources of exposure that were associated with U-Cd levels. We did not measure iron status in our participants which can be an important factor influencing gastrointestinal uptake of Cd (Akesson et al. 2002; Gallagher et al. 2011; Julin et al. 2011; Satarug et al. 2010). We also did not have any information on renal function such as measures of glomerular filtration that were positively and paradoxically associated with U-Cd in recent studies, suggesting a potential reverse causality (Weaver et al. 2011a; Weaver et al. 2011b; Chaumont et al. 2012). The strengths of this study include use of 24-hour urine samples (Akerstrom et al. 2012), the ability to evaluate within-person variation in U-Cd levels for about half of the study subjects; comprehensive questionnaire data related to dietary, reproductive and lifestyle factors; and the estimation of Cd exposure from outdoor sources.

CONCLUSIONS

These results suggest that urinary cadmium levels increase with age and exposure to tobacco smoke and that a single measurement of urinary cadmium does not accurately reflect medium to long-term (i.e. 6 – 9 month average) body burden.

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Table 1. Distributions of demographic, lifestyle, and geographic factors and laboratory data from the initial urine samples provided by participants.

Variable (units)	N	Min	25 th	50 th	75 th	Max	Mean±SD
Self-reported data							
Age (years)	296	31	47	54	62	84	55±12
Body mass index (kg/m ²)	293	16	23	25	29	61	27±5.9
Body surface area (m ²)	293	1.2	1.7	1.8	1.9	2.7	1.8±0.2
Parity (full-term pregnancies)	291	0	0	2	3	6	1.7±1.4
Breastfeeding (months)	290	0	1	3	5	9	3.2±2.3
Smoking (pack-years)	296	0	0	0	1.0	61	3.9±10
Passive smoking ^a (intensity-years)	171	0	4.0	21	40	203	30±35
Dietary Cd intake (µg/day)	287	2.5	7.9	11	14	28	11±4.2
Geographic exposure data							
Industrial emissions ^b (kg)	296	0	0	0	0.003	1,760	18±146
Outdoor air (ng/m ³)	296	0.05	0.09	0.15	0.28	0.65	0.20±0.13
Traffic density ^c (VKT/km ²)	296	0	0	7,000	33,900	427,000	27,500±55,200
Urinary concentrations							
Unadjusted Cd (µg/L)	296	0.1	0.2	0.3	0.4	2.0	0.27±1.9 ^d
Creatinine (g/L)	296	0.1	0.5	0.7	1.0	2.5	0.71±1.6 ^d
Creatinine-adjusted Cd (µg/g)	296	0.1	0.3	0.4	0.5	1.5	0.38±1.8 ^d
Cd output (µg/day)	295	0.1	0.3	0.5	0.7	2.6	0.46±1.7 ^d

^a Among never-smokers responding to a 1997 questionnaire about exposure to second-hand smoke (n=171).

^b Cadmium emissions within five kilometers of residence.

^c VKT = vehicle kilometers traveled per square kilometer within 300 meters of residence.

^d Geometric mean and geometric standard deviation.

Table 2. Selected host and lifestyle characteristics and 24-hour urinary cadmium concentrations from initial visit unadjusted and adjusted for creatinine.

Characteristic	n	%	Unadjusted Cd (µg/L)			Creatinine-adjusted Cd (µg/g)		
			GM (µg/L)	Kruskal-Wallis p-value ^a	Linear trend p-value ^b	GM (µg/g)	Kruskal-Wallis p-value ^a	Linear trend p-value ^b
Age (years)								
31 – 39	30	10%	0.26	0.11	0.01	0.30	<0.0001	<0.0001
40 – 49	63	21%	0.23			0.31		
50 – 59	114	39%	0.27			0.38		
60 – 84	89	30%	0.30			0.45		
Smoking (pack-years)								
0 (never)	207	70%	0.26	0.02	0.01	0.35	0.0002	<0.0001
0.1 – 4.9	40	13%	0.25			0.40		
5.0 – 19.9	26	9%	0.27			0.43		
≥ 20	23	8%	0.41			0.57		
Passive smoking (intensity-years) ^c								
< 4	42	25%	0.26	0.15	0.70	0.34	0.27	0.53
4 – 20	44	27%	0.31			0.40		
21 – 40	43	25%	0.24			0.34		
> 40	37	23%	0.28			0.37		
Alcohol (g/day)								
None	96	32%	0.32	0.0005	0.0001	0.44	0.0006	0.0001
< 20	174	59%	0.25			0.36		
≥ 20	26	9%	0.19			0.29		
Parity (full-term pregnancies)								
0	75	26%	0.28	0.65	0.02	0.41	0.39	0.0002
1 – 2	143	49%	0.27			0.37		
3	43	15%	0.24			0.35		
> 3	30	10%	0.25			0.37		
Total duration of breastfeeding (months)								
≤ 1	81	27%	0.28	0.37	0.17	0.41	0.03	0.05
2 – 3	81	27%	0.29			0.40		
4 – 5	87	29%	0.25			0.33		
> 5	47	16%	0.25			0.36		
Oral contraceptive use								
Ever	201	72%	0.25	0.0005	-	0.35	0.0001	-
Never	80	28%	0.32			0.44		
Hormone replacement therapy								
Ever	141	48%	0.27	0.31	-	0.38	0.02	-
Never	154	52%	0.27			0.37		
Body mass index (kg/m ²)								
< 25.0	145	50%	0.28	0.30	0.14	0.40	0.15	0.08
25.0 – 29.9	82	28%	0.24			0.35		
≥ 30.0	66	22%	0.28			0.36		
Body surface area (m ²)								
< 1.65	73	25%	0.31	0.04	-	0.42	0.08	-
≥ 1.65	223	75%	0.25			0.36		

^a Non-parametric test using the Kruskal-Wallis one-way analysis of variance by ranks.

^b Linear test for trend with natural logarithm-transformed concentrations adjusted for age.

^c Among never-smokers responding to the 1997 questionnaire about exposure to second-hand smoke (n=171).

Table 3. Dietary and environmental characteristics and 24-hour urinary cadmium concentrations from initial visit unadjusted and adjusted for creatinine.

Characteristic	n	%	Unadjusted Cd (µg/L)			Creatinine Adjusted Cd (µg/g)		
			GM (µg/L)	Kruskal-Wallis p-Value ^a	Linear trend p-Value ^b	GM (µg/g)	Kruskal-Wallis p-Value ^a	Linear trend p-Value ^b
Dietary cadmium intake (µg/day)								
< 7.9	71	25%	0.28	0.72	0.30	0.38	0.82	0.98
7.9 – 10.6	72	25%	0.27			0.37		
10.6 – 13.7	73	25%	0.27			0.37		
> 13.7	71	25%	0.25			0.38		
Urban or rural residence								
Urban	119	40%	0.28	0.12	0.17	0.40	0.08	0.42
Rural	177	60%	0.26			0.36		
Estimated outdoor cadmium concentration (ng/m ³)								
< 0.1	94	32%	0.25	0.37	0.29	0.36	0.34	0.31
0.1 – 0.3	142	48%	0.27			0.38		
> 0.3	60	20%	0.29			0.39		
Traffic density (vehicle kilometers traveled per square kilometer)								
0	101	34%	0.27	0.78	0.82	0.37	0.64	0.54
1 – 7,000	48	16%	0.27			0.36		
7,001 – 70,000	118	40%	0.26			0.38		
> 70,000	29	10%	0.29			0.41		
Industrial cadmium emissions (kg within 5 kilometer)								
0	203	69%	0.26	0.23	0.06	0.37	0.57	0.23
0.001 – 20	52	18%	0.27			0.38		
> 20	41	14%	0.30			0.40		

^a Non-parametric test using the Kruskal-Wallis one-way analysis of variance by ranks.

^b Linear test for trend with natural logarithm-transformed concentrations adjusted for age.

Table 4. Estimated adjusted percentage change and 95% confidence interval (95% CI) in 24-hour urinary cadmium concentration (µg/L) associated with potential predictors.

Variable (categories if applicable)	Model 1 (all participants) % change ^a (95% CI)	R ²	Model 2 (never-smokers) % change ^a (95% CI)	R ²
Number of (Samples/subjects)	412/285		233/161	
Creatinine (per 0.1 g/L)	15 (12, 19)**	0.27	14 (11, 19)**	0.27
Age (per year)	1.4 (0.9, 1.9)**	0.35	1.1 (0.40, 1.8)*	0.35
Smoking (per lifetime pack-year)	1.1 (0.5, 1.6)**	0.37	Not Included	
Passive smoking (per lifetime intensity-year)	Not Included		0.2 (0.0, 0.4)*	0.37
Total full-term pregnancies (per pregnancy)	-4.6 (-8.6, -0.5)*	0.39	-3.9 (-8.6, 1.0)	0.39
Alcohol intake (0, <20, ≥20 g/day)	-16 (-23, -7.4) **	0.40	-16 (-24, -4.9)*	0.40
Cadmium in air (per 0.1 ng/m ³)	-1.4 (-7.9, 5.5)	0.40	1.0 (-7.4, 11)	0.41
Industrial emissions – within 5 km (per 10-fold increase in kg)	2.5 (-1.9, 7.1)	0.41	1.5 (-4.4, 7.7)	0.41
Traffic density – within 300m (per 10-fold increase in VKT/km ²)	-1.9 (-4.9, 1.2)	0.41	-2.2 (-5.8, 1.5)	0.42
Dietary cadmium intake (per µg/day)	-0.1 (-1.4, 1.4)	0.42	-0.9 (-2.5, 0.7)	0.44

^a % change = [exp(β)-1]*100

*p<0.05; **p<0.001

APPENDIX 2

Oral presentation abstract:

Rull RP, Goldberg D, Gunier RB, Hertz A, Horn-Ross PL, Canchola A, Reynolds P. Environmental cadmium exposure and the risks of estrogen-receptor positive and negative breast cancer. Presented at the 24th Conference of the International Society for Environmental Epidemiology, August 28, 2012, Columbia, South Carolina.

ISEE 2012 Abstract: Environmental Cadmium Exposure and the Risks of Estrogen-Receptor Positive and Negative Breast Cancer

Rull RP, Goldberg D, Gunier RB, Hertz A, Horn-Ross P, Canchola A, Reynolds P

BACKGROUND

Cadmium is a toxic metal that exhibits potent estrogen-like activity. Exposure to cadmium occurs from smoking, diet and inhalation of polluted air. Previous case-control studies reported elevated risks of breast cancer associated with urinary concentrations of cadmium, a biomarker of body burden, but did not estimate effects by estrogen-receptor (ER) subtype.

OBJECTIVES

Our objectives were to characterize exposures to cadmium from environmental sources in the California Teachers Study (CTS) cohort of over 130,000 women and evaluate whether these exposures increased the risk of breast cancer by ER subtype.

METHODS

Based on CTS participants' geocoded residential addresses, we: a) estimated cadmium emissions from industrial sources within 5 kilometers, b) estimated vehicular traffic density within 300 meters, and c) assigned modeled ambient air concentrations of cadmium at the census-tract level. Cases of breast cancer diagnosed between 1996 and 2009 and ER status were identified in the CTS via linkage with the California Cancer Registry. Hazard ratios (HRs) were estimated using Cox proportional-hazards regression. To minimize exposure misclassification due to residential mobility and cadmium exposure from cigarette smoking, we conducted analyses restricted to women who did not change addresses during follow-up and never smoked.

RESULTS

We observed elevated risks of ER-negative breast cancer associated with residence in the highest quartiles of cadmium concentration in air (HR: 1.7; 95% confidence interval=1.1—2.6) and traffic density (HR: 1.4; 95% confidence interval=1.0—2.6). These exposures were not associated with ER-positive breast cancer.

CONCLUSIONS

These results suggest that cadmium exposure may contribute to ER-negative breast cancer.

APPENDIX 3

Poster presentation abstract:

Gunier RB, Rull RP, Hertz A, Canchola A, Horn-Ross P, Reynolds P. Urinary cadmium concentrations among female teachers from Northern California. Presented at the 6th Department of Defense Breast Cancer Research Program Era of Hope Conference, August 2-5, 2011, Orlando, Florida.

Urinary Cadmium Concentrations among Female Teachers from Northern California

Authors: Robert Gunier, Rudy Rull, Andrew Hertz, Alison Canchola, Pamela Horn-Ross, Peggy Reynolds

Background: Cadmium is a toxic metal associated with kidney disease and increased mortality. It has been classified as a probable human carcinogen, demonstrated to have estrogenic properties, and associated with breast cancer in previous case-control studies. Exposure to cadmium occurs from smoking, diet and inhalation of air polluted from combustion, mining, and manufacturing. Excretion of cadmium in urine is widely considered a biomarker of lifetime exposure. Urinary cadmium concentration has been associated with age, smoking status, body surface area, parity, and household income in previous studies. Our objectives were to identify predictors of urinary cadmium concentrations and determine the within-person correlation among repeat samples.

Methods: We collected a 24-hour urine sample from 298 women enrolled in the California Teachers Study in 2000 and a second 24-hour sample from 141 participants approximately three, six, or nine months later. Urinary cadmium concentrations ($\mu\text{g/L}$) were determined by inductively-coupled plasma/mass spectrometry. Age, body mass index, smoking status, passive smoking, dietary intake, alcohol consumption, parity, and several reproductive factors were obtained by interview. Environmental cadmium exposure from vehicular traffic and from industrial and commercial emission sources around the address of residence as well as modeled outdoor air concentrations were estimated using a geographic information system. Dietary cadmium intake was assessed by linking data from a food-frequency questionnaire with the Total Diet Study database. We used mixed-effects models to estimate the within-person correlation between repeat measurements and identify predictors of urinary cadmium levels.

Results: The arithmetic mean cadmium concentration was 0.3 micrograms per liter ($\mu\text{g/L}$) (standard deviation = 0.2 $\mu\text{g/L}$) and the range was 0.1 to 2.0 $\mu\text{g/L}$. The intra-class correlation among repeat samples from the same individual was 0.5. Urinary cadmium concentration increased with age, creatinine concentration, lifetime pack-years of smoking, lifetime intensity of passive smoking among non-smokers, and decreased with greater alcohol consumption and number of previous pregnancies. These factors explained 44% of the variability in urinary cadmium concentrations. However, cadmium exposures from environmental or dietary sources did not appear to be associated with urinary concentrations.

Conclusion: These results suggest that a single measurement of urinary cadmium concentration does not accurately assess lifetime exposure. Although our estimates of environmental and dietary exposure were not associated with urinary cadmium levels, we will evaluate whether these exposures are associated with breast cancer risk. If increased risks are observed with estimated cadmium exposure, our results could serve as the impetus for future regulatory actions to mitigate cadmium exposure and ultimately reduce the burden of breast cancer in women.

APPENDIX 4

Scientific Abstract:

National Institute of Environmental Health Sciences Grant No. 1 R01 ES018841: Dietary and Environmental Exposure to Cadmium and the Risk of Endometrial Cancer

PROJECT SUMMARY/ABSTRACT

Exposure to high levels of circulating estrogens unopposed by progestins is the primary cause of endometrial cancer. However, little is known about whether environmental contaminants that mimic the effects of estrogen increase the risk of this disease. Cadmium is a trace metal released into air and soil as a byproduct of industrial processes and is perhaps the most potent of these estrogenic contaminants with respect to endometrial cancer etiology. Major sources of non-occupational exposure to Cd include cigarette smoke, diet, and inhalation of ambient air contaminated by industrial processes and combustion of fossil fuels. This proposed study will test the emerging hypothesis that greater levels of Cd exposure increase endometrial cancer risk by utilizing existing data on dietary intake, residence, smoking history, and other risk factors and urine specimens from 356 women diagnosed with endometrial cancer and 683 matched controls enrolled in the Nutrition, Estrogens and Endometrial Cancer in Teachers (NEET) study, a nested case-control study within the California Teachers Study (CTS) cohort. The availability of these data, urine specimens for the measurement of Cd concentration—a classic measure of chronic exposure, and existing databases of environmental and dietary sources of Cd, will allow us to conduct a comprehensive assessment of exposure that incorporates a myriad of sources and evaluates the relative contribution of each source. This will also allow us to assess whether any observed elevations in risk are heterogeneous across exposure sources. The specific aims of this study will be to: 1) characterize exposure to Cd from dietary and environmental sources for all cases and controls in the NEET study; 2) evaluate the contributions of dietary intake and exposure from environmental sources on Cd concentrations measured in urine; and 3) estimate the effects of dietary, environmental, and total Cd exposure on the risk of endometrial cancer.

The emerging evidence that Cd is a potential risk factor for endometrial cancer suggests a future direction for minimizing dietary and environmental exposures to this toxic metal. This study offers a unique and timely opportunity to improve our understanding of whether Cd plays an etiologic role in the development of this cancer and identify important, and potentially modifiable, sources of exposure to this metal.

APPENDIX 5

Scientific Abstract:

California Breast Cancer Research Program Grant No. 17IB-0016: Cadmium, Age at Menarche, and Early Puberty in Girls

Background and overall topic: Women who experience their first menstrual period (i.e., menarche) before the age of 12 years have an increased risk of breast cancer. It has been estimated that each one-year decrease in age at menarche is associated with a 5-10% elevation in risk of this disease. This association is consistent with the hypothesis that the earlier establishment of ovulatory cycles which in turn increases the the period during which breast cells are most mitotically active and susceptible to tumorigenic somatic events. Early menarche has also been associated with higher cumulative exposure to estrogens.

Over the past two decades the average age at menarche has been declining in the US and Europe. While the causes of early menarche and pubertal development are largely unknown, emerging evidence from animal and in vitro studies suggest that increasing exposures to estrogenic environmental chemicals may be contributing to this trend. Cadmium (Cd), a trace metal released into air and soil as a byproduct of industrial processes, is perhaps the most potent of these estrogenic contaminants. Previous epidemiologic studies have observed an association between a higher body burden of Cd and breast cancer risk. While the major sources of non-occupational exposure to Cd in adults include cigarette smoke, diet, and inhalation of ambient air contaminated by industrial processes and combustion of fossil fuels, recent discoveries of Cd in children's toys and jewelry have led to public concern about potential childhood exposure from ingestion and hand-to-mouth activity. However, it is not known whether this estrogenic metal may contribute to early menarche and puberty in girls.

Hypothesis/questions addressed: The primary hypothesis of this proposal is that urinary Cd concentration, a marker of lifetime body burden, is associated with an earlier age at menarche and early onset of pubertal development.

Objectives/aims: Our specific aims are as follows:

1. Determine the urinary concentrations of Cd, a measure of lifetime exposure and body burden, in a cohort of girls and whether concentrations differ by age, race/ethnicity, and among Chinese girls, nativity and generational status.
2. Evaluate whether urinary Cd concentration is associated with early age at menarche.
3. Evaluate whether urinary Cd concentration is associated with earlier estrogen-based or androgen-based pubertal development.

Methods and approaches: This proposed study will utilize existing data and urine specimens from the GRowth and LifeStyle Study (GRLS), a prospective cohort study of girls. A total of 214 girls, aged 10-13 years at baseline and primarily non-Hispanic White or Chinese, provided overnight urine specimens at baseline that will be used to measure urinary Cd concentrations, completed a baseline interview, provided a self-assessment of Tanner stage based on standard pictorial depictions and verbal descriptions of breast development and pubic hair growth, and had their height and weight measured. A total of 87 girls had their first menstrual period prior to baseline, while 134 girls were pre-menarcheal at baseline and followed for up to two years using monthly questionnaires to ascertain the onset of menarche and an annual interview that included self-assessed Tanner stage and the collection of an additional overnight urine specimen. We will evaluate the hypothesis that Cd body burden is associated with early menarche and pubertal development using regression-based longitudinal and cross-sectional approaches.

Impact on breast cancer: Early-life exposure to this estrogenic metal may contribute to earlier pubertal development and attainment of menarche and thus also play a role in the etiology of breast cancer. As Cd exposures are potentially modifiable, this proposed study offers tremendous potential to contribute to our knowledge about the etiology of early menarche, a known risk factor for breast cancer.

Advocacy involvement and sensitivity to advocacy concerns: This project has high potential for meaningful translation into the reduction of children's exposures to this estrogenic metal. If this study finds an association between early pubertal development and Cd exposure, it could provide a major impetus for further regulatory actions to reduce both the use of Cd in industrial processes and thus exposure in children and adults. To ensure our results are translated into actions aimed at mitigating the burden of exposure, we will disseminate our results to the scientific and lay communities, as well as to policy makers, in the form of a scientific manuscript and lay-friendly fact sheet. Breast cancer and environmental advocacy organizations will play a critical role in the translation of findings from our study into meaningful and measurable interventions.