A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP14784.

Calibration of Toenail Metal Concentrations for Sample Mass Heterogeneity and Between-Batch Variability: The COMET Approach

Roberto Pastor-Barriuso,[1,2] Enrique Gutiérrez-González,[3] Elena Varea-Jiménez,[1,4] José Luis Gómez-Ariza,[5,6] Gemma Castaño-Vinyals,[2,7,8,9] Nuria Aragonés,[2,10] Antonio José Molina,[2,11] Trinidad Dierssen-Sotos,[2,12,13] Guillermo Fernández-Tardón,[2,14] Pilar Amiano,[2,15,16] María Ederra-Sanz,[2,17,18] Victor Moreno,[2,19,20,21] José Juan Jiménez-Moleón,[2,22,23] Ana Molina-Barceló,[24] Rafael Marcos-Gragera,[2,25,26] Delphine Casabonne,[2,19,20] Juan Alguacil,[2,6] Jesús Humberto Gómez-Gómez,[2,27] Tamara García-Barrera,[5,6] Manolis Kogevinas,[2,7,8,9] Marina Pollán,[1,2] and Beatriz Pérez-Gómez[1,2]

[1]National Center for Epidemiology, Carlos III Institute of Health (ISCIII), Madrid, Spain
[2]Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain
[3]Public Health Directorate, Ministry of Health, Madrid, Spain
[4]National Center for Environmental Health, Carlos III Institute of Health (ISCIII), Madrid, Spain
[5]Department of Chemistry, Faculty of Experimental Sciences, University of Huelva, Huelva, Spain
[6]Centro de Investigación en Recursos Naturales, Salud y Medio Ambiente (RENSMA), Universidad de Huelva, Huelva, Spain
[7]Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain
[8]Universitat Pompeu Fabra (UPF), Barcelona, Spain
[9]Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain
[10]Public Health Division, Department of Health of the Community of Madrid, Madrid, Spain
[11]Research Group in Gene-Environment and Health Interactions (GIIGAS), Biomedicine Institute (IBIOMED), Universidad de León, León, Spain
[12]Department of Preventive Medicine and Public Health, University of Cantabria, Santander, Spain
[13]Instituto de Investigación Sanitaria Valdecilla (IDIVAL), Santander, Spain
[14]Health Research Institute of Asturias (ISPA), University of Oviedo, Oviedo, Spain
[15]Epidemiology of Chronic and Communicable Diseases Group, Biodonostia Health Research Institute, San Sebastián, Spain
[16]Sub Directorate for Public Health and Addictions of Gipuzkoa, Ministry of Health of the Basque Government, San Sebastián, Spain
[17]Institute of Public and Occupational Health of Navarre (ISPLN), Pamplona, Spain
[18]Navarra Institute for Health Research (IdiSNA), Pamplona, Spain
[19]Cancer Epidemiology Research Program, Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet de Llobregat, Barcelona, Spain
[20]Catalan Institute of Oncology (ICO), Hospitalet de Llobregat, Barcelona, Spain
[21]Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, Spain
[22]Departamento de Medicina Preventiva y Salud Pública, Universidad de Granada, Granada, Spain
[23]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain
[24]Cancer and Public Health Research Unit, Foundation for the Promotion of Health and Biomedical Research (FISABIO-Public Health), Valencia, Spain
[25]Epidemiology Unit and Girona Cancer Registry, Oncology Coordination Plan, Department of Health, Autonomous Government of Catalonia, Catalan Institute of Oncology, Girona, Spain
[26]Descriptive Epidemiology, Genetics and Cancer Prevention Group, Biomedical Research Institute of Girona (IDIBGI), Girona, Spain
[27]Department of Epidemiology, Regional Health Council, IMIB-Arrixaca, Murcia, Spain

Background: Toenails are promising biomarkers of long-term metal exposure in epidemiological studies, but their accuracy may be compromised by systematic and random errors associated with heterogeneous toenail sample masses, as well as by substantial variability across laboratory batches.

Objectives: We propose a novel modeling approach to calibrate toenail metal concentrations for the heterogeneity in sample masses and the variability between batches.

Methods: We developed a heteroscedastic spline mixed model relating sample mass and laboratory batch with measured concentrations, allowing for an average bias in measurements over all batches as a smooth function of sample mass, random variation in mass-related biases across batches, and mass-related heterogeneity in within-batch error variance. The model allowed partitioning the total variance of measured concentrations into the extraneous variances (due to different sample masses and laboratory batches) and the intrinsic variance (resulting from distinct metal exposures). We derived calibrated metal concentrations from the model by removing both sources of extraneous variation and estimating the predicted concentrations had all toenail samples been analyzed in a single batch and of the same mass. We provide the R script COMET (COrrected METals) to fit the proposed model, extract variance components, and calibrate metal concentrations.

Results: In a multicase–control study in Spain (MCC-Spain) with toenail determinations for 16 metals in 4,473 incident cases of five common cancers and 3,450 population controls, sample mass and batch accounted for 26%–60% of the total variance of measured concentrations for most metals. In comparison with calibrated concentrations, odds ratios for measured concentrations were biased by >10% toward or away from the null in one-quarter of the estimated metal–cancer associations.

Discussion: The proposed model allows correcting toenail metal concentrations for sample mass heterogeneity and between-batch variability and could be applied to other biological specimens of heterogeneous size, distinct laboratory techniques, and different study designs. https://doi.org/10.1289/EHP14784

Address correspondence to Roberto Pastor-Barriuso, National Center for Epidemiology, Carlos III Institute of Health (ISCIII), Monforte de Lemos 5, 28029 Madrid, Spain. Email: rpastor@isciii.es

Supplemental Material is available online (https://doi.org/10.1289/EHP14784).

The authors declare they have no conflicts of interest related to this work to disclose.

Conclusions and opinions are those of the individual authors and do not necessarily reflect the policies or views of EHP Publishing or the National Institute of Environmental Health Sciences.

Received 7 February 2024; Revised 30 January 2025; Accepted 31 January 2025; Published 18 April 2025.

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehpsubmissions@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Introduction

Biomarkers are commonly used in epidemiology to measure exposure to essential and nonessential metals due to their ability to integrate all dietary and environmental sources of exposure and not be affected by recall bias.[1] Toenails are suitable biomarkers of metal exposure in large epidemiological studies because they are easy to collect and store, may reflect longer-term exposures than standard biomarkers such as blood and urine,[2–4] and are considered less susceptible to external metal contamination than fingernails and hair, because they are exposed less extensively to outdoor air or water.[5]

However, two relevant methodological issues may compromise the accuracy of toenail metal concentrations as exposure biomarkers, namely, the heterogeneity in sample masses and the variability between laboratory batches. Collected toenail samples typically range from 1 mg to over 100 mg, and there is growing evidence of inverse associations of toenail sample mass with the measured concentration and the limit of detection for several metals and laboratory techniques.[2–4] Although inductively coupled plasma mass spectrometry and instrumental neutron activation analysis allow detecting low metal contents in small toenail samples, the measured concentrations in such small samples are prone to upward bias[6–9] and increased variance.[10] In addition, the variability in toenail metal determinations across batches may be a relevant source of uncertainty due to the lack of certified toenail reference materials and standardized analysis protocols.[2–4]

A widespread practice to control for mass-related bias is to exclude toenail samples below a certain mass threshold (usually 10 mg),[2] but this approach is inefficient with substantial proportion of small samples and may leave residual bias above this threshold. Some authors adjust directly for sample mass in multivariate risk models,[8,11–13] whereas others use residual methods to correct metal concentrations for sample mass.[7,9,14–16] These approaches, however, may produce incomplete control for nonlinear mass-related biases[6] and do not account for the effect of sample mass on the variance of the measurements. Moreover, with few exceptions,[15,16] studies overlook the variability in mass-related measurement errors across batches.

To address these issues, we developed a heteroscedastic spline mixed model to analyze toenail metal concentrations in a large population-based multicase–control study in Spain (MCC-Spain). The model enables us to extract variance components and calibrate metal concentrations for both the heterogeneity in toenail sample masses and the variability between laboratory batches.

Methods

Study Design and Population

The MCC-Spain study (www.mccspain.org) was conducted between September 2008 and December 2013 to identify environmental and genetic factors related to five common cancers. The study design has been described elsewhere.[17] In brief, this report included 7,923 of all 10,183 study participants (77.8%) 22–84 y of age with available toenail samples of at least 1 mg for metal determinations. Participants were recruited from 12 Spanish provinces (Asturias, Barcelona, Cantabria, Gipuzkoa, Girona, Granada, Huelva, León, Madrid, Murcia, Navarra, and Valencia) and included 1,422 histologically confirmed incident cases of colorectal cancer, 1,438 of breast cancer, 915 of prostate cancer, 343 of stomach cancer, 355 of chronic lymphocytic leukemia, and a random sample of 3,450 population controls frequency-matched to cases by province, sex, and 5-y age group. The overall response rate was 57%–87% among cases and 53% among controls. The study protocol was approved by the ethics committees of the participating institutions, and written informed consent was obtained at enrollment from all participants.

Laboratory Analysis

At recruitment, trained staff collected nail samples from big toes of both feet with stainless steel nail clippers and stored them in paper envelopes at room temperature. Whenever possible, participants were asked in advance not to apply nail polish or other lotions in the days prior to sampling. The median mass (first–99th percentile range) of toenail samples was 20.9 (1.5–114.8) mg. Before analysis, toenail samples were cleaned twice for 5 min in an ultrasonic bath with Triton solution, acetone, and deionized water, digested in a nitric acid and hydrogen peroxide mixture solution at 4:1 ratio through microwave irradiation, and diluted to 5 mL with deionized water.

Multi-element analysis of toenail samples was performed by inductively coupled plasma mass spectrometry (XSeries 2; Thermo Scientific) at the Environmental Bioanalytical Chemistry Unit of the University of Huelva, Spain, following standard quality-control procedures.[18] The 16 metals determined in this study were aluminum, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, cadmium, thallium, lead, and uranium. Analysts were blinded to case–control status of toenail samples, which were combined into 122 laboratory batches between June 2015 and October 2018, with an average batch size of 65 nail specimens and a geometric mean sample mass ranging from 3.6 to 45.4 mg between batches. The toenail metal concentration was calculated as the measured metal concentration in the dilution multiplied by the dilution volume and divided by the toenail sample mass and was expressed as parts per billion (ppb) or nanograms of metal per gram of toenail. There were few samples with metal concentrations below the limits of detection (Table S1), ranging from no samples for vanadium to 57 samples (0.7%) for uranium.

We performed a small reproducibility study with a pooled sample of discarded toenails from a foot care clinic. Toenails were cleaned, dried, and cryohomogenized and the resulting powder was divided into 10 samples of 5 and 10 mg and three samples of 30, 50, and 100 mg. All metals showed an upward bias in the measured concentrations with decreasing the toenail sample mass (Figure S1).

Heteroscedastic Spline Mixed Model for Measured Metal Concentrations

In the next two sections, we present a formal description of the heteroscedastic spline mixed model used to calibrate the measured metal concentrations for the heterogeneity in toenail sample masses and the variability between laboratory batches. The model was implemented in the R script COMET (COrrected METals) (see Supplemental Material section "R Script COMET").

In brief, the model included fixed effects for the average bias in log-transformed metal concentrations as a spline function of log-transformed sample mass, random effects for variation in this mass-related bias across batches, and heterogeneous within-batch error variance as a spline function of log-transformed mass, while accounting for factors used in the study design. On one side, this model decomposed the total variance of measured concentrations into the extraneous variances just due to different sample masses (average bias, bias variation between batches, heterogeneity in within-batch variance) and laboratory batches (between-batch variance at mean mass), and the intrinsic variance arising from distinct metal exposures (within-batch variance at mean mass). On the other side, by removing the effects of both extraneous factors, this model provided the calibrated metal concentrations that would have been observed if all toenail specimens had been of the same mass and analyzed in a single laboratory batch, conditional on study design factors.

Apart from standard quality control and blinding procedures, it is not necessary that toenail samples be randomly distributed between batches nor that batches be of homogeneous size for the calibration method to work, provided that each laboratory batch contains sufficient (at least 20–25) toenail specimens of varying mass and that the number of batches is not too small (at least 5–10). We developed the model to calibrate metal determinations in case–control studies, thus adjusting for case–control status and other stratifying or matching factors used in study design. The same model can be applied to cross-sectional studies and to baseline metal measurements in cohort studies, but with the term for disease status removed. Model extensions to deal with repeated measurements over follow-up in cohort studies are outlined in the discussion.

We developed the spline mixed model with heteroscedastic errors through a two-level hierarchical approach,[19,20] with a first level modeling metal measurements within each batch and a second level allowing for variation in model coefficients between batches (see Supplemental Material section "Development of the Heteroscedastic Spline Mixed Model"). Specifically, the log-transformed metal concentration Y_{ij} for toenail specimen j in batch i was related to the log-transformed mass m_{ij} of that specimen, the case–control status z_{1ij}, and the sociodemographic factors z_{2ij} of the corresponding participant through the spline mixed model

$$\begin{aligned} Y_{ij} = {} & \alpha_0 + a_i + (\delta + d_i)'[s(m_{ij}) - s(\overline{m})] \\ & + \eta'[s(\overline{m}_i) - s(\overline{m})] + \alpha_1' z_{1ij} + \alpha_2' z_{2ij} + \varepsilon_{ij}, \end{aligned} \quad (1)$$

where $s(m_{ij})$ and $s(\overline{m}_i)$ were natural cubic splines for the individual m_{ij} and the mean log-transformed mass of each batch $\overline{m}_i$ with internal knots at the 35th and 65th percentiles and boundary knots at the fifth and 95th percentiles of the overall log-transformed mass distribution (3.0, 13.9, 28.9, and 63.2 mg in the original scale). These splines required only three terms and allowed different cubic trends on either side of the 35th and 65th percentiles that were restricted to be linear beyond the fifth and 95th percentiles, so they could reproduce a large variety of smooth curves while avoiding implausible shapes at extreme log-transformed masses.[21]

In Model 1, the fixed intercept α_0 was the average log-transformed metal concentration at the overall mean log-transformed mass $\overline{m}$ for controls with reference levels of sociodemographic factors; the fixed effects α_1 were the average shifts between cases of each tumor type (colorectal, breast, prostate, stomach, and leukemia) and controls; the fixed effects α_2 were the average differences comparing each level of sociodemographic factors with the reference one, including geographical region (12 Spanish provinces), sex (male, female), age (<35 y and 5-y intervals from 35 to 84 y), and educational level (primary or less, high school, and college); and the random intercept a_i represented the between-batch variation in log-transformed metal concentrations at the overall mean log-transformed mass. The fixed spline parameters $\delta = (\delta_1, \delta_2, \delta_3)'$ and $\eta = (\eta_1, \eta_2, \eta_3)'$ jointly determined the average mass-related bias in the measured metal concentrations over all laboratory batches at fixed values of case–control status and sociodemographic factors, which can be decomposed into the average bias associated with toenail samples of different mass from the same batch (average sample-level effects δ) and the bias related to samples of the same mass, analyzed in batches with different geometric mean masses (batch compositional effects η).[19] The random spline parameters $d_i = (d_{1i}, d_{2i}, d_{3i})'$ represented the between-batch variation in the systematic biases associated with toenail samples of different mass.

Because the toenail sample mass may also affect the variance of the measured metal concentrations (Figure S1), we allowed the within-batch errors ε_{ij} to have heterogeneous variance σ_{ij}^2 of the form

$$\log(\sigma_{ij}) = \phi + \gamma'[s(m_{ij}) - s(\overline{m})],$$

which was related to the log-transformed mass m_{ij} through a natural cubic spline with the same knots described above. The scale $\exp(2\phi)$ was the variance of log-transformed metal concentrations at the overall mean log-transformed mass $\overline{m}$ and the variance parameters $\gamma = (\gamma_1, \gamma_2, \gamma_3)'$ determined the random within-batch variability in log-transformed metal concentrations as a smooth function of the log-transformed mass.[20]

In Model 1, the systematic bias and random error in the measured metal concentrations depended on the toenail sample mass, but given this nuisance factor, they were assumed to be nondifferential with respect to case–control status or sociodemographic factors,[22] as expected from blinded laboratory analyses. Note, however, that failure to account for the effect of sample mass on the mean and variance of metal determinations may result in measurement error being differential with respect to disease status if the sample mass distribution differs between cases and controls. The fixed effects α_0, δ, η, α_1, and α_2, the unstructured variance–covariance matrix Σ of the random effects a_i and d_i, and the variance parameters ϕ and γ were estimated using restricted maximum likelihood methods, as implemented in the lme function from the nlme package in R (version 4.0.5; R Development Core Team).We also estimated the best linear unbiased predictions of a_i and d_i for each laboratory batch.

Variance Components and Calibrated Metal Concentrations

Based on Model 1, we partitioned the total variance of log-transformed measured metal concentrations into the variance explained by toenail sample mass, the between-batch variance, and the within-batch variance (see Supplemental Material section "Variance Components in Heteroscedastic Linear Mixed Models"). Given case–control status and sociodemographic factors $z_{ij} = (z_{1ij}', z_{2ij}')'$ at their sample proportions $\overline{z} = (\overline{z}_1', \overline{z}_2')'$, the conditional variance of Y_{ij} was estimated as

$$\begin{aligned} \widehat{\mathrm{var}}(Y_{ij}|\overline{z}) \approx {} & \widehat{\beta}' \mathrm{S}_{x|z} \widehat{\beta} + \mathrm{tr}\left(\widehat{\Sigma} \mathrm{S}_{x_1|z}\right) + \overline{x}_1' \widehat{\Sigma} \overline{x}_1 \\ & + \exp\left(2\widehat{\theta}' \overline{x}_1\right) + 2\exp\left(2\widehat{\theta}' \overline{x}_1\right) \widehat{\theta}' \mathrm{S}_{x_1|z} \widehat{\theta}, \end{aligned}$$

where $\widehat{\beta}$, $\widehat{\Sigma}$, and $\widehat{\theta}$ were the estimates of the fixed spline parameters $\beta = (\alpha_0, \delta', \eta')'$, the variance-covariance matrix Σ of random spline parameters a_i and d_i, and the variance parameters $\theta = (\phi, \gamma')'$ obtained from Model 1. The variance–covariance matrices of mass-related covariates $x_{ij} = [1, s(m_{ij})' - s(\overline{m})', s(\overline{m}_i)' - s(\overline{m})']'$ and $x_{1ij} = [1, s(m_{ij})' - s(\overline{m})']'$ conditional on z_{ij} were estimated as

$$\mathrm{S}_{x|z} = \mathrm{S}_x - \mathrm{S}_{xz} \mathrm{S}_z^{-1} \mathrm{S}_{xz}',$$

$$\mathrm{S}_{x_1|z} = \mathrm{S}_{x_1} - \mathrm{S}_{x_1 z} \mathrm{S}_z^{-1} \mathrm{S}_{x_1 z}',$$

where S_x, S_{x_1}, and S_z were the sample variance-covariance matrices of x_{ij}, x_{1ij}, and z_{ij}, S_{xz} and $\mathrm{S}_{x_1 z}$ were the sample covariance matrices of x_{ij} and x_{1ij} with z_{ij}, and $\overline{x}_1$ was the sample mean of x_{1ij}. Thus, the conditional variance of log-transformed measured metal concentrations was partitioned into the variance explained by the average mass-related bias over all batches $\widehat{\beta}' \mathrm{S}_{x|z} \widehat{\beta}$, the between-batch variance in mass-related biases $\mathrm{tr}(\widehat{\Sigma} \mathrm{S}_{x_1|z})$, the between-batch variance at the mean log-transformed mass $\overline{x}_1' \widehat{\Sigma} \overline{x}_1$, the within-batch variance explained by toenail sample mass $2\exp(2\widehat{\theta}' \overline{x}_1) \widehat{\theta}' \mathrm{S}_{x_1|z} \widehat{\theta}$, and the within-batch residual variance at the mean log-transformed mass $\exp(2\widehat{\theta}' \overline{x}_1)$.

Table 1. Toenail sample mass and measured metal concentrations by sociodemographic characteristics among controls in a population-based multicase–control study in Spain, 2008–2013 ($n = 3{,}450$).

Characteristic	Toenail mass (mg)	Al (ppm)	V (ppb)	Cr (ppm)	Mn (ppb)	Fe (ppm)	Co (ppb)	Ni (ppm)	Cu (ppm)	Zn (ppm)	As (ppb)	Se (ppm)	Mo (ppb)	Cd (ppb)	Tl (ppb)	Pb (ppb)	U (ppb)
Province																	
Asturias	29.3	39.3	47.9	1.33	444	28.4	21.8	1.10	4.20	94	169	0.68	52.2	18.8	2.35	504	5.41
Barcelona	28.0	29.2	60.5	1.14	405	25.9	20.4	1.36	4.88	106	224	0.76	31.1	11.4	2.48	584	9.96
Cantabria	13.2	43.5	77.7	1.74	1,077	42.9	34.8	3.32	4.42	89	129	1.14	57.7	19.3	3.14	744	6.50
Gipuzkoa	20.6	45.4	64.8	1.85	540	42.5	42.1	2.35	4.29	98	185	0.68	49.8	18.3	3.50	712	5.50
Girona	24.3	47.1	55.8	1.63	574	39.1	31.3	2.02	5.14	91	144	0.67	88.5	21.6	3.75	595	9.52
Granada	18.4	36.9	57.7	1.38	578	34.4	26.0	1.65	3.86	93	281	0.80	59.2	17.4	5.73	671	6.04
Huelva	29.6	41.5	54.4	1.08	532	30.0	27.9	1.45	4.44	85	160	0.94	41.1	10.4	2.73	583	4.13
León	9.5	67.4	98.4	1.65	884	49.2	46.9	3.39	5.13	115	306	0.91	57.7	21.7	5.82	915	8.66
Madrid	22.8	38.0	49.1	1.16	305	17.1	15.3	2.30	4.51	105	188	0.92	46.6	15.9	4.52	416	5.62
Murcia	17.8	58.4	82.2	1.53	617	42.1	26.8	1.19	4.56	132	311	0.52	81.6	28.6	3.42	546	11.30
Navarra	3.9	115.6	175.4	2.15	1,160	78.1	63.9	4.88	7.37	151	351	1.41	115.7	61.9	16.22	1,618	17.17
Valencia	19.1	35.0	46.0	1.54	512	31.9	20.6	1.81	3.72	87	220	0.81	58.1	15.4	4.31	739	7.80
p-Value[a]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sex																	
Men	19.9	32.0	51.7	0.98	476	24.4	19.9	1.28	4.49	109	345	0.82	35.7	13.3	3.81	598	7.14
Women	16.1	58.4	85.3	2.05	613	42.5	37.0	3.54	4.93	99	130	0.91	70.1	23.6	4.20	709	7.87
p-Value[a]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	0.002
Age (y)																	
<35	16.3	52.0	78.5	1.52	609	40.3	36.2	3.39	5.93	102	155	1.00	74.2	24.6	5.65	751	9.21
35–39	14.5	55.7	81.2	1.60	656	38.4	36.0	2.93	5.26	102	171	0.99	71.2	23.9	4.48	702	8.73
40–44	17.8	53.2	78.7	1.94	640	39.0	37.0	2.97	4.83	102	153	0.85	61.1	20.1	4.03	606	7.55
45–49	16.1	51.1	78.2	1.76	589	38.4	32.8	2.73	4.84	104	157	0.90	62.9	22.4	4.13	683	7.50
50–54	16.9	51.2	78.2	1.93	645	37.1	30.8	2.72	5.20	102	169	0.87	58.6	19.8	3.96	725	7.93
55–59	19.0	42.0	65.5	1.61	545	32.1	27.3	2.28	4.75	103	206	0.88	47.8	17.1	3.96	651	7.56
60–64	20.8	40.8	60.4	1.25	529	29.6	24.3	1.91	4.68	101	228	0.83	45.8	16.4	3.79	615	6.98
65–69	18.5	39.1	63.1	1.31	517	30.6	24.3	1.80	4.58	107	250	0.89	45.3	16.0	4.01	649	8.21
70–74	17.6	41.9	64.9	1.36	492	29.7	26.1	2.12	4.64	109	244	0.82	47.0	17.4	4.18	650	7.71
75–79	17.4	39.5	62.7	1.20	522	31.3	25.7	1.80	4.43	102	223	0.84	46.1	16.5	3.58	636	6.60
80–84	15.1	43.1	60.0	1.11	460	29.8	26.3	1.90	4.35	101	215	0.88	50.9	17.1	4.52	645	6.67
p-Value[a]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.49	<0.001	0.10	<0.001	<0.001	0.15	0.43	0.002
Educational level																	
Primary or less	18.3	44.0	67.3	1.34	579	33.3	27.9	2.10	4.55	102	207	0.84	49.8	17.4	3.97	674	7.17
High school	18.1	43.0	65.8	1.48	523	31.7	26.5	2.11	4.64	103	202	0.86	50.8	17.6	3.85	620	7.52
College	16.7	42.6	66.1	1.54	486	30.8	26.9	2.28	5.19	109	233	0.93	50.3	18.6	4.29	648	8.26
p-Value[a]	0.08	0.72	0.79	0.01	<0.001	0.13	0.43	0.35	<0.001	0.01	0.004	0.001	0.85	0.28	0.12	0.06	0.002

Note: Data are geometric means. Of all 3,450 population controls, 2 (0.1%) had missing toenail concentrations for chromium, 2 (0.1%) for manganese, 4 (0.1%) for iron, 8 (0.2%) for cobalt, 1 (0.0%) for nickel, 9 (0.3%) for copper, 1 (0.0%) for zinc, 12 (0.3%) for arsenic, 2 (0.1%) for selenium, 3 (0.1%) for molybdenum, 16 (0.5%) for cadmium, 12 (0.3%) for thallium, 9 (0.3%) for lead, and 25 (0.7%) for uranium. Al, aluminum; As, arsenic; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Mn, manganese; Mo, molybdenum; Ni, nickel; Pb, lead; ppb, parts per billion; ppm, parts per million; Se, selenium; Tl, thallium; U, uranium; V, vanadium; Zn, zinc.

[a]*p*-Values for homogeneity of geometric means by sociodemographic characteristic were obtained from analyses of variance of log-transformed toenail sample mass and metal concentrations.

Table 2. Sociodemographic characteristics, toenail sample mass, and measured metal concentrations by cancer status in a population-based multicase–control study in Spain, 2008–2013 ($n = 7{,}923$).

	Colorectal			Breast			Prostate			Stomach			Leukemia		
Characteristic	Cases	Controls	p-Value[a]	Cases	Controls	p-Value[a]	Cases	Controls	p-Value[a]	Cases	Controls	p-Value[a]	Cases	Controls	p-Value[a]
No. of participants[b]	1,422	3,373	—	1,438	1,679	—	915	1,273	—	343	2,728	—	355	1,524	—
Province															
Asturias	70 (4.9)	227 (6.7)	—	70 (4.9)	123 (7.3)	—	16 (1.7)	104 (8.2)	—	13 (3.8)	227 (8.3)	—	47 (13.2)	227 (14.9)	—
Barcelona	230 (16.2)	730 (21.6)	—	224 (15.6)	275 (16.4)	—	329 (36.0)	455 (35.7)	—	61 (17.8)	730 (26.8)	—	227 (63.9)	730 (47.9)	—
Cantabria	126 (8.9)	329 (9.8)	—	129 (9.0)	163 (9.7)	—	152 (16.6)	166 (13.0)	—	23 (6.7)	329 (12.1)	—	19 (5.4)	329 (21.6)	—
Gipuzkoa	114 (8.0)	348 (10.3)	—	215 (15.0)	258 (15.4)	—	—	—	—	—	—	—	—	—	—
Girona	—	—	—	42 (2.9)	53 (3.2)	—	—	—	—	—	—	—	27 (7.6)	77 (5.1)	—
Granada	124 (8.7)	161 (4.8)	—	—	—	—	55 (6.0)	109 (8.6)	—	—	—	—	35 (9.9)	161 (10.6)	—
Huelva	32 (2.3)	101 (3.0)	—	42 (2.9)	43 (2.6)	—	22 (2.4)	58 (4.6)	—	—	—	—	—	—	—
León	359 (25.2)	420 (12.5)	—	213 (14.8)	197 (11.7)	—	—	—	—	118 (34.4)	420 (15.4)	—	—	—	—
Madrid	171 (12.0)	655 (19.4)	—	246 (17.1)	342 (20.4)	—	271 (29.6)	313 (24.6)	—	75 (21.9)	655 (24.0)	—	—	—	—
Murcia	23 (1.6)	35 (1.0)	—	—	—	—	—	—	—	—	—	—	—	—	—
Navarra	106 (7.5)	243 (7.2)	—	211 (14.7)	169 (10.1)	—	—	—	—	45 (13.1)	243 (8.9)	—	—	—	—
Valencia	67 (4.7)	124 (3.7)	—	46 (3.2)	56 (3.3)	—	70 (7.7)	68 (5.3)	—	8 (2.3)	124 (4.5)	—	—	—	—
Sex															
Men	904 (63.6)	1,684 (49.9)	—	—	—	—	915 (100.0)	1,273 (100.0)	—	237 (69.1)	1,403 (51.4)	—	214 (60.3)	858 (56.3)	—
Women	518 (36.4)	1,689 (50.1)	—	1,438 (100.0)	1,679 (100.0)	—	—	—	—	106 (30.9)	1,325 (48.6)	—	141 (39.7)	666 (43.7)	—
Age (y)	66.6 ± 10.9	62.5 ± 12.1	—	56.3 ± 12.3	58.7 ± 13.1	—	65.9 ± 7.3	65.8 ± 9.5	—	66.2 ± 12.3	62.6 ± 12.2	—	65.7 ± 10.2	62.7 ± 11.5	—
Educational level	—	—	<0.001	—	—	0.34	—	—	<0.001	—	—	<0.001	—	—	0.07
Primary or less	960 (67.5)	1,643 (48.7)	—	679 (47.2)	776 (46.2)	—	577 (63.1)	607 (47.7)	—	231 (67.3)	1,319 (48.4)	—	199 (56.1)	756 (49.6)	—
High school	294 (20.7)	1,002 (29.7)	—	476 (33.1)	537 (32.0)	—	205 (22.4)	377 (29.6)	—	73 (21.3)	814 (29.8)	—	101 (28.5)	474 (31.1)	—
College	168 (11.8)	728 (21.6)	—	283 (19.7)	366 (21.8)	—	133 (14.5)	289 (22.7)	—	39 (11.4)	595 (21.8)	—	55 (15.5)	294 (19.3)	—
Toenail mass (mg)	14.5 (2.6)	17.7 (2.6)	<0.001	16.0 (2.9)	16.0 (2.8)	0.98	22.6 (2.0)	24.3 (2.0)	0.02	15.5 (2.5)	17.0 (2.6)	0.08	28.2 (2.1)	22.8 (2.2)	<0.001
Aluminum (ppm)	43.3 (2.8)	43.3 (2.6)	0.97	62.1 (2.2)	58.0 (2.2)	0.02	26.5 (2.4)	25.1 (2.5)	0.18	39.3 (2.7)	43.4 (2.7)	0.08	31.1 (2.7)	34.9 (2.6)	0.04
Vanadium (ppb)	66.8 (2.5)	66.8 (2.5)	0.99	82.8 (2.2)	85.8 (2.2)	0.21	43.1 (2.4)	42.9 (2.3)	0.91	62.0 (2.5)	68.0 (2.6)	0.08	63.2 (3.2)	61.1 (2.4)	0.53
Chromium (ppm)	1.15 (3.1)	1.42 (3.0)	<0.001	1.95 (2.7)	2.04 (2.7)	0.21	0.95 (2.9)	0.88 (2.9)	0.09	0.89 (2.9)	1.39 (3.1)	<0.001	1.21 (4.1)	1.33 (3.0)	0.18
Manganese (ppb)	553 (2.7)	540 (2.7)	0.44	650 (2.3)	610 (2.4)	0.04	424 (2.6)	410 (2.8)	0.41	501 (2.6)	537 (2.8)	0.23	551 (3.3)	536 (2.6)	0.63
Iron (ppm)	33.3 (2.7)	32.1 (2.5)	0.22	42.7 (2.3)	42.2 (2.4)	0.70	21.5 (2.5)	21.5 (2.4)	0.92	29.9 (2.3)	30.9 (2.6)	0.55	32.7 (3.1)	30.8 (2.4)	0.30
Cobalt (ppb)	29.5 (3.0)	27.2 (2.8)	0.01	39.8 (2.6)	36.9 (2.5)	0.03	17.6 (2.7)	15.7 (2.5)	0.004	20.6 (2.9)	25.7 (2.9)	<0.001	23.9 (3.6)	24.2 (2.6)	0.82
Nickel (ppm)	2.39 (4.0)	2.15 (3.7)	0.01	3.46 (3.2)	3.59 (2.9)	0.34	1.39 (4.1)	1.18 (3.8)	0.01	2.20 (4.0)	2.20 (3.9)	0.98	1.20 (3.9)	1.66 (3.8)	<0.001
Copper (ppm)	4.77 (1.9)	4.70 (1.8)	0.45	4.89 (1.7)	4.97 (1.7)	0.41	4.15 (1.8)	4.12 (1.8)	0.78	4.14 (1.7)	4.82 (1.8)	<0.001	5.10 (2.2)	4.57 (1.7)	0.002
Zinc (ppm)	109 (1.7)	104 (1.7)	0.01	98 (1.7)	100 (1.7)	0.39	96 (1.8)	104 (1.7)	0.001	103 (1.6)	106 (1.7)	0.34	110 (1.8)	98 (1.6)	<0.001
Arsenic (ppb)	253 (2.8)	213 (2.6)	<0.001	139 (1.9)	131 (1.9)	0.01	275 (2.4)	289 (2.4)	0.19	274 (2.8)	214 (2.6)	<0.001	218 (2.8)	191 (2.4)	0.01
Selenium (ppm)	0.91 (2.1)	0.87 (1.8)	0.03	0.91 (1.7)	0.91 (1.7)	0.79	0.84 (1.8)	0.82 (1.9)	0.29	0.80 (1.8)	0.90 (1.8)	<0.001	0.81 (2.7)	0.81 (1.9)	0.99
Molybdenum (ppb)	52.1 (2.8)	49.6 (2.5)	0.10	73.1 (2.2)	69.7 (2.1)	0.08	33.2 (2.3)	32.1 (2.3)	0.36	40.7 (2.9)	49.0 (2.6)	0.001	36.0 (2.9)	43.3 (2.5)	0.001
Cadmium (ppb)	17.6 (2.8)	17.7 (2.6)	0.86	24.7 (2.5)	23.7 (2.4)	0.16	11.2 (2.6)	11.8 (2.5)	0.21	15.1 (2.7)	17.8 (2.7)	0.003	13.4 (2.7)	14.9 (2.4)	0.06
Thallium (ppb)	4.55 (3.1)	4.01 (3.0)	<0.001	5.54 (3.3)	4.19 (3.2)	<0.001	3.92 (2.7)	3.47 (2.8)	0.01	4.88 (2.9)	4.06 (3.0)	0.004	2.47 (3.0)	2.89 (2.7)	0.01
Lead (ppb)	700 (2.6)	653 (2.5)	0.02	709 (2.7)	705 (2.5)	0.89	556 (2.4)	511 (2.3)	0.02	518 (2.6)	649 (2.6)	<0.001	576 (2.6)	612 (2.3)	0.23
Uranium (ppb)	7.87 (2.6)	7.46 (2.5)	0.06	8.67 (2.5)	7.87 (2.4)	0.003	7.09 (2.5)	6.78 (2.4)	0.25	7.09 (2.4)	7.97 (2.4)	0.02	9.19 (2.4)	7.87 (2.2)	0.001

Note: Data are number (%) for province, sex, and educational level, mean ± standard deviation for age, and geometric mean (geometric standard deviation) for toenail sample mass and metal concentrations. Of all 7,923 participants, 1 (0.0%) had missing toenail concentrations for aluminum, 4 (0.1%) for chromium, 7 (0.1%) for manganese, 8 (0.1%) for iron, 32 (0.4%) for cobalt, 1 (0.0%) for nickel, 19 (0.2%) for copper, 2 (0.0%) for zinc, 39 (0.5%) for arsenic, 6 (0.1%) for selenium, 8 (0.1%) for molybdenum, 42 (0.5%) for cadmium, 26 (0.3%) for thallium, 25 (0.3%) for lead, and 57 (0.7%) for uranium. —, no data; ppb, parts per billion; ppm, parts per million.

[a]p-Values for homogeneity of percentages by cancer status were obtained from Pearson chi-squared tests and p-values for homogeneity of geometric means by cancer status were obtained from analyses of variance of log-transformed toenail sample mass and metal concentrations.

[b]Number of cases and controls from the same provinces (and sex for breast and prostate cancer).

The log-transformed metal concentrations corrected for toenail sample mass and laboratory batch $\widehat{Y}_{ij}^{c}$ were calculated from Model 1 as the estimated marginal mean over all batches at the mean log-transformed mass plus the within-batch residuals rescaled to their estimated variance at the mean log-transformed mass,

$$\widehat{Y}_{ij}^{c} = \widehat{E}(Y_{ij}|\overline{m}) + \widehat{\mathrm{var}}(Y_{ij}|a_i, d_i, \overline{m})^{1/2} \frac{Y_{ij} - \widehat{E}(Y_{ij}|a_i, d_i)}{\widehat{\mathrm{var}}(Y_{ij}|a_i, d_i)^{1/2}}$$
$$= \widehat{\alpha}_0 + \widehat{\alpha}_1{}' z_{1ij} + \widehat{\alpha}_2{}' z_{2ij} + \exp\{-\widehat{\gamma}'[s(m_{ij}) - s(\overline{m})]\} e_{ij}.$$

Provided that Model 1 was properly specified, these calibrated values represented the log-transformed metal concentrations that would have been observed had all toenail samples been analyzed in the same average laboratory batch and sample masses been set to the geometric mean for all participants (17.6 mg), conditional on their case–control status and sociodemographic factors. The conditional variance of log-transformed calibrated metal concentrations corresponded to the within-batch residual variance of the measurements at the mean log-transformed mass. The R script COMET to fit Model 1, extract variance components, and calibrate metal concentrations is provided in Supplemental Material section "R Script COMET."

Bias and Precision of Effect Estimates

To compare the effect of exposure measurement calibration on modeled effect estimates, we estimated the association of toenail metal concentrations with the odds of each common cancer using three different logistic regression models. The first model used log-transformed measured metal concentrations as exposure and adjusted for sociodemographic factors (geographical region, sex, age group, and educational level), the second model retained the measured concentrations and further adjusted for toenail sample mass (natural cubic spline of log-transformed values), and the third model used log-transformed calibrated

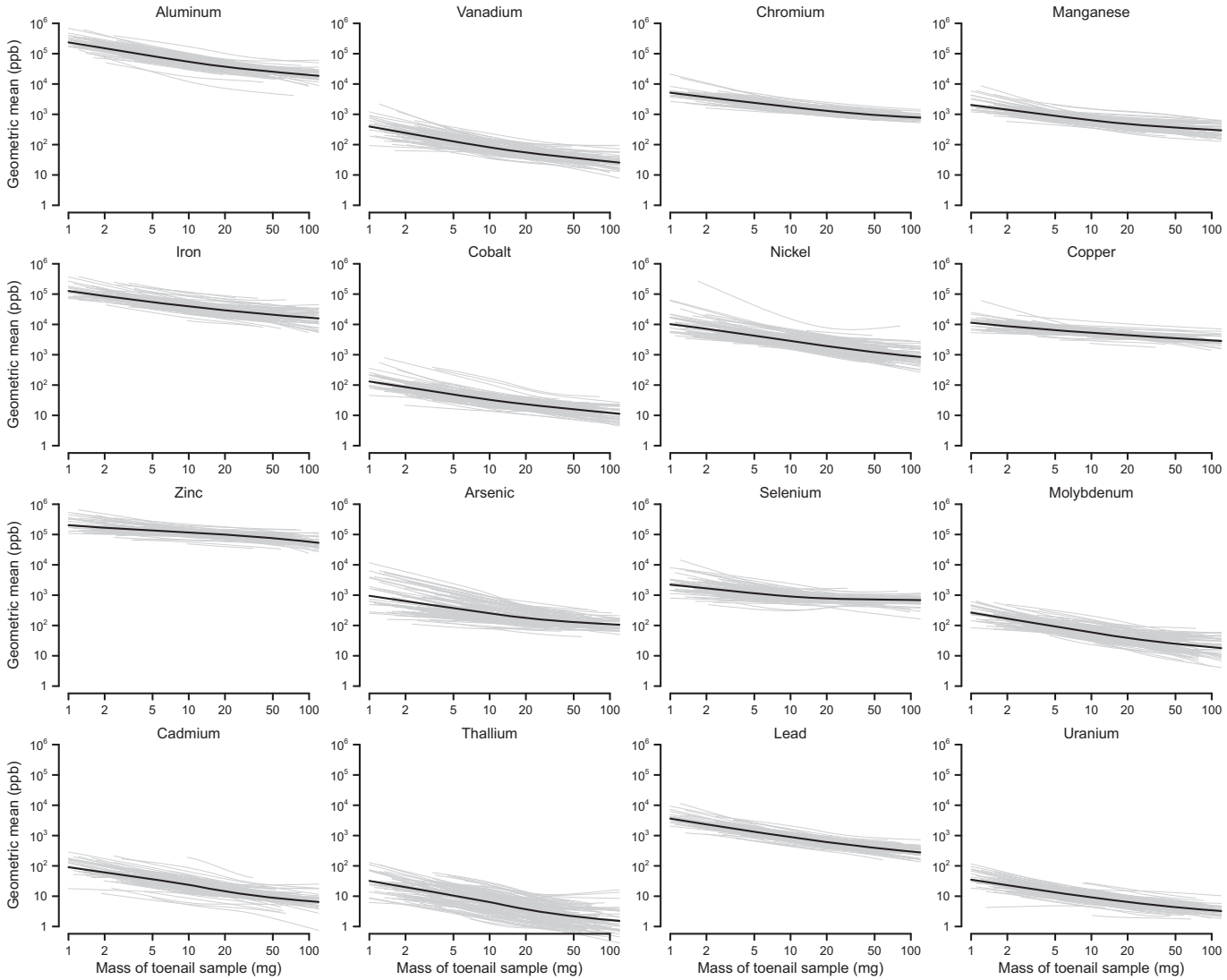

Figure 1. Average and batch-specific sample-level biases in measured metal concentrations associated with toenail samples of different mass in a population-based multicase–control study in Spain, 2008–2013 ($n = 7{,}923$). The average sample-level bias in measured metal concentrations over all laboratory batches (thick black curve) was determined by the estimates of the fixed spline parameters α_0, δ_1, δ_2, and δ_3 associated with individual log-transformed toenail sample masses in heteroscedastic spline mixed models for log-transformed metal concentrations, whereas the batch-specific sample-level biases (gray curves) were determined by these fixed-effects estimates plus the best linear unbiased predictions of the random spline parameters a_i, d_{1i}, d_{2i}, and d_{3i} for each laboratory batch. The intercept α_0 was adjusted to an average batch with the overall geometric mean mass and the overall percentages of disease status and sociodemographic factors. Note: ppb, parts per billion.

Table 3. Systematic bias and random error in measured metal concentrations associated with toenail sample mass in a population-based multicase–control study in Spain, 2008–2013 ($n = 7{,}923$).

Parameter	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Tl	Pb	U
Average sample-level bias[a]																
α_0	10.59 ± 0.05	4.08 ± 0.05	7.21 ± 0.04	6.22 ± 0.04	10.33 ± 0.05	3.20 ± 0.05	7.64 ± 0.06	8.41 ± 0.03	11.54 ± 0.03	5.24 ± 0.06	6.68 ± 0.04	3.73 ± 0.06	2.77 ± 0.06	1.41 ± 0.09	6.47 ± 0.04	1.92 ± 0.05
δ_1	−1.09 ± 0.05	−1.13 ± 0.05	−0.84 ± 0.06	−0.83 ± 0.05	−0.84 ± 0.05	−1.00 ± 0.06	−1.12 ± 0.07	−0.51 ± 0.04	−0.40 ± 0.04	−1.01 ± 0.05	−0.50 ± 0.05	−1.20 ± 0.06	−1.29 ± 0.05	−1.46 ± 0.05	−1.07 ± 0.05	−1.01 ± 0.04
δ_2	−2.23 ± 0.09	−2.37 ± 0.10	−1.67 ± 0.10	−1.71 ± 0.09	−1.78 ± 0.09	−2.10 ± 0.11	−2.13 ± 0.13	−1.16 ± 0.08	−0.97 ± 0.08	−1.99 ± 0.11	−1.13 ± 0.11	−2.35 ± 0.11	−2.31 ± 0.10	−2.68 ± 0.11	−2.19 ± 0.09	−2.06 ± 0.09
δ_3	−1.13 ± 0.05	−1.20 ± 0.05	−0.86 ± 0.05	−0.84 ± 0.05	−0.91 ± 0.05	−1.08 ± 0.06	−1.25 ± 0.07	−0.58 ± 0.03	−0.62 ± 0.04	−0.98 ± 0.06	−0.39 ± 0.05	−1.26 ± 0.06	−1.37 ± 0.05	−1.53 ± 0.06	−1.17 ± 0.04	−1.07 ± 0.05
p-Value[b]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Batch compositional bias																
η_1	0.31 ± 0.19	0.16 ± 0.20	0.28 ± 0.18	0.08 ± 0.18	−0.16 ± 0.21	−0.19 ± 0.22	−0.19 ± 0.26	0.26 ± 0.13	0.05 ± 0.13	0.40 ± 0.24	−0.22 ± 0.15	0.22 ± 0.21	0.00 ± 0.23	−0.47 ± 0.34	0.14 ± 0.16	−0.12 ± 0.20
η_2	0.76 ± 0.59	−0.04 ± 0.60	0.01 ± 0.51	0.08 ± 0.52	0.33 ± 0.63	0.20 ± 0.66	1.01 ± 0.77	0.14 ± 0.39	0.18 ± 0.40	1.05 ± 0.75	−0.87 ± 0.48	0.17 ± 0.65	−0.44 ± 0.71	−0.67 ± 1.08	1.16 ± 0.48	−1.22 ± 0.60
η_3	0.35 ± 0.39	0.19 ± 0.40	−0.35 ± 0.33	0.26 ± 0.40	0.25 ± 0.44	0.79 ± 0.43	0.95 ± 0.56	0.13 ± 0.27	0.07 ± 0.28	0.61 ± 0.46	−0.34 ± 0.29	1.08 ± 0.48	0.04 ± 0.45	−0.50 ± 0.75	0.24 ± 0.32	−0.16 ± 0.38
p-Value[b]	0.17	0.43	0.39	0.61	0.81	0.16	0.41	0.06	0.95	0.09	0.18	<0.001	0.74	0.26	0.07	0.11
Between-batch variation in sample-level biases[c]																
SD(a_i)	0.34	0.35	0.26	0.32	0.37	0.37	0.44	0.22	0.23	0.52	0.27	0.44	0.44	0.70	0.27	0.35
SD(d_{1i})	0.33	0.38	0.35	0.31	0.36	0.44	0.47	0.31	0.32	0.48	0.47	0.50	0.38	0.44	0.29	0.30
SD(d_{2i})	0.60	0.81	0.58	0.73	0.68	0.83	0.84	0.58	0.66	0.95	0.81	1.00	0.71	0.92	0.61	0.58
SD(d_{3i})	0.35	0.41	0.26	0.36	0.37	0.44	0.47	0.25	0.31	0.54	0.41	0.61	0.45	0.54	0.31	0.37
p-Value[d]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Within-batch error variance																
ϕ	−0.43	−0.51	−0.03	−0.24	−0.40	−0.30	0.02	−0.84	−0.96	−0.67	−0.77	−0.61	−0.52	−0.61	−0.38	−0.46
γ_1	0.05	0.12	0.17	0.17	0.14	0.11	0.18	−0.22	−0.35	−0.10	−0.53	−0.21	0.07	0.13	0.08	0.10
γ_2	−0.03	0.22	0.33	0.36	0.24	0.02	0.20	−0.48	−0.30	−0.22	−0.63	−0.36	−0.04	0.11	0.11	0.08
γ_3	0.11	0.16	0.18	0.10	0.14	0.09	0.20	0.10	−0.06	0.00	−0.21	−0.22	0.09	0.14	0.23	0.24
p-Value[d]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Parameter estimates ± SEs were obtained from mixed models of log-transformed metal concentrations on natural cubic splines of log-transformed toenail sample mass allowing for average bias at both sample and batch levels, random variation in sample-level biases across batches, and heterogeneous variance of within-batch errors. See "Methods" section "Heteroscedastic Spline Mixed Model for Measured Metal Concentrations" for details on model parameters. Al, aluminum; As, arsenic; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Mn, manganese; Mo, molybdenum; Ni, nickel; Pb, lead; SD, standard deviation; SE, standard error; Se, selenium; Tl, thallium; U, uranium; V, vanadium; Zn, zinc.

[a]The intercept α_0 was adjusted to the overall percentages of disease status and sociodemographic factors.

[b]Wald test p-values for null average sample-level bias ($\delta_1 = \delta_2 = \delta_3 = 0$) and null batch compositional bias ($\eta_1 = \eta_2 = \eta_3 = 0$).

[c]The random between-batch variations a_i, d_{1i}, d_{2i}, and d_{3i} were correlated but, for simplicity, their estimated correlations were omitted and only the standard deviations were reported.

[d]Likelihood ratio test p-values for null variation in sample-level biases across batches [$\mathrm{var}(d_{1i}) = \mathrm{var}(d_{2i}) = \mathrm{var}(d_{3i}) = 0$] and homogeneous variance of within-batch errors ($\gamma_1 = \gamma_2 = \gamma_3 = 0$).

concentrations and adjusted for the same sociodemographic factors as in the first model. The estimated odds ratios (ORs) per 2-fold increase in metal concentrations were compared across models in terms of relative bias (ratio of estimated ORs) and precision (ratio of standard errors (SEs) of log-transformed ORs).

Results

Measured Metal Concentrations

Among controls, nearly all metals showed higher geometric mean concentrations in women, younger subjects, participants with higher educational levels, and those from Navarra and León, which corresponded to the groups with toenail samples of less mass (Table 1). Adjusting for sociodemographic factors and taking controls from the same province and sex as reference, breast cancer cases had geometric mean concentrations at least 20% higher for thallium and leukemia patients for manganese, whereas stomach cancer cases had geometric mean concentrations at least 20% lower for chromium, cobalt, and lead (Table 2). The geometric mean mass of toenail samples was 6%–9% lower in colorectal and prostate cancer cases and 9%–12% higher in breast, stomach, and leukemia cases than in the corresponding controls after accounting for sociodemographic characteristics.

Adjusting for sociodemographic factors, the odds of stomach cancer decreased by at least 20% for each 2-fold increase in measured concentrations of cobalt, copper, zinc, selenium, and lead, whereas the odds of leukemia increased over 20% per 2-fold increase in copper and zinc concentrations (Table S2). However, because toenail samples had less mass in colorectal and prostate cancer cases and more mass in breast, stomach, and leukemia cases than in the corresponding controls, further adjustment for sample mass resulted in consistently lower ORs for colorectal and prostate cancer and higher ORs for breast, stomach cancer, and leukemia for all metals (Table S2).

Assessment of Heteroscedastic Spline Mixed Model

Information criteria were used to assess the relative contribution of fixed effects, random effects, and variance parameters to the maximum likelihood fit of Model 1 (Table S3). The progressive decrease in Akaike information criterion indicated model fit improvements with the inclusion of each component related to sample mass and laboratory batch, consistent with results from likelihood ratio tests. Indeed, the Bayesian information criterion with higher penalty for model complexity still assigned the lowest values to the full model for most metals, suggesting that Model 1 was unlikely to overcorrect metal concentrations.

Systematic Bias Associated with Toenail Sample Mass

In preliminary analyses within each laboratory batch, all metals displayed consistently increasing geometric mean concentrations with decreasing the mass of toenail sample, albeit the mean concentrations at a given mass and the mass-related changes varied substantially across batches (Figure S2). The average smooth trends in geometric mean metal concentrations over all laboratory batches are shown in Figure 1 (thick black curves), as estimated by the fixed spline parameters δ_1, δ_2, and δ_3 in Model 1 (Table 3). Although the increase in geometric mean concentrations with decreasing toenail sample mass was evident for all metals, it was somewhat stronger for vanadium, molybdenum, and thallium. With the exception of zinc, the upward bias in measured metal concentrations was particularly marked for toenail samples below 20 mg. There was no evidence of batch compositional effects for most metals, as determined by the fixed spline parameters η_1, η_2, and η_3 (Table 3), indicating that the toenail mass composition of the batches did not affect the bias in measured metal concentrations for their individual samples. The only exception was molybdenum, which showed greater upward bias for toenail samples of the same mass analyzed in batches with geometric mean mass higher than 20 mg (Figure S3). The average mass-related bias over all laboratory batches accounted for a large proportion of the total variance of measured metal concentrations given case–control status and sociodemographic factors, ranging from nearly 10% for chromium

Table 4. Variance components of measured metal concentrations in toenail samples in a population-based multicase–control study in Spain, 2008–2013 ($n = 7{,}923$).

		Variance component (%)				
Metal	Total variance[a]	Average mass-related bias[b]	Between-batch variance in mass-related bias	Between-batch variance in intercept[c]	Within-batch mass-related variance	Within-batch residual variance[c]
Aluminum	0.730	0.153 (20.9)	0.018 (2.5)	0.109 (14.9)	0.001 (0.2)	0.449 (61.4)
Vanadium	0.684	0.175 (25.6)	0.026 (3.8)	0.120 (17.5)	0.002 (0.3)	0.362 (52.8)
Chromium	1.104	0.096 (8.7)	0.012 (1.0)	0.062 (5.6)	0.007 (0.7)	0.927 (84.0)
Manganese	0.790	0.088 (11.2)	0.019 (2.5)	0.094 (11.9)	0.004 (0.5)	0.584 (73.9)
Iron	0.722	0.107 (14.9)	0.023 (3.2)	0.143 (19.8)	0.002 (0.3)	0.447 (61.9)
Cobalt	0.866	0.143 (16.5)	0.029 (3.4)	0.136 (15.7)	0.001 (0.1)	0.557 (64.4)
Nickel	1.499	0.172 (11.5)	0.038 (2.5)	0.217 (14.4)	0.008 (0.5)	1.064 (71.0)
Copper	0.340	0.039 (11.5)	0.013 (3.9)	0.049 (14.4)	0.006 (1.7)	0.233 (68.5)
Zinc	0.286	0.040 (14.0)	0.020 (6.9)	0.058 (20.2)	0.003 (1.1)	0.165 (57.8)
Arsenic	0.706	0.116 (16.5)	0.037 (5.3)	0.270 (38.2)	0.001 (0.1)	0.282 (39.9)
Selenium	0.400	0.039 (9.8)	0.029 (7.1)	0.073 (18.2)	0.010 (2.5)	0.249 (62.3)
Molybdenum	0.698	0.178 (25.5)	0.047 (6.8)	0.174 (25.0)	0.003 (0.5)	0.295 (42.3)
Cadmium	0.789	0.209 (26.5)	0.033 (4.1)	0.176 (22.3)	0.001 (0.1)	0.370 (46.9)
Thallium	1.116	0.316 (28.3)	0.042 (3.7)	0.454 (40.7)	0.001 (0.1)	0.303 (27.2)
Lead	0.768	0.171 (22.2)	0.013 (1.6)	0.068 (8.8)	0.005 (0.7)	0.512 (66.6)
Uranium	0.727	0.148 (20.3)	0.019 (2.7)	0.113 (15.5)	0.005 (0.7)	0.442 (60.8)

Note: Variance components (%) were obtained by combining sample means and variances of mass-related covariates with parameter estimates from mixed models of log-transformed metal concentrations on natural cubic splines of log-transformed toenail sample mass allowing for average mass-related bias over all batches, random variation in mass-related biases across batches, and heterogeneous mass-related variance of within-batch errors. See "Methods" section "Variance Components and Calibrated Metal Concentrations" for details on variance decomposition.

[a]Conditional variance of log-transformed measured metal concentrations given disease status and sociodemographic factors at their overall percentages.

[b]Average mass-related bias over all batches including average sample-level bias and batch compositional bias.

[c]Between-batch and within-batch variances at the overall mean log-transformed mass.

and selenium to more than 25% for vanadium, molybdenum, cadmium, and thallium (Table 4).

The bias associated with toenail sample mass varied substantially across laboratory batches, as indicated by the standard deviations of random spline parameters d_{1i}, d_{2i}, and d_{3i} (Table 3). The batch-specific smooth trends in geometric mean metal concentrations as a function of toenail mass are displayed in Figure 1 (gray curves), with larger variation in slopes for arsenic and selenium. The between-batch variance in mass-related biases explained more than 5% of the total variance of measured concentrations for zinc, arsenic, selenium, and molybdenum (Table 4).

Random Error Associated with Toenail Sample Mass

The mass of toenail sample affected not only the mean, but also the variance of measured metal concentrations. The smooth trends in the within-batch error variance, as estimated by the variance spline parameters γ_1, γ_2, and γ_3 (Table 3), closely resembled the heterogeneous variances observed by percentile of toenail sample mass (Figure 2). As expected, most metals showed higher variance of log-transformed metal concentrations at large sample masses, which corresponded to higher geometric standard deviations around lower geometric mean concentrations at large masses. For copper, zinc, and selenium, however, there was a marked J-shaped pattern with an upturn in within-batch error variance at small sample masses, which explained between 1% and 2.5% of their total variance. Combining the bias in mean concentrations and the heterogeneity in error variances, the mass of toenail sample accounted for 10%–15% of the total variance of measured concentrations for chromium, manganese, and nickel, 15%–20% for iron, cobalt, copper, and selenium, 20%–25% for aluminum, zinc, arsenic, lead, and uranium, and 30%–35% for vanadium, molybdenum, cadmium, and thallium (Table 4).

Calibrated Metal Concentrations

In addition to mass-related changes, the measured metal concentrations varied across laboratory batches. For toenail samples fixed at the overall geometric mean mass (17.6 mg), the intra-batch correlation coefficient ranged from $0.062/(0.062+0.927)=0.06$ for

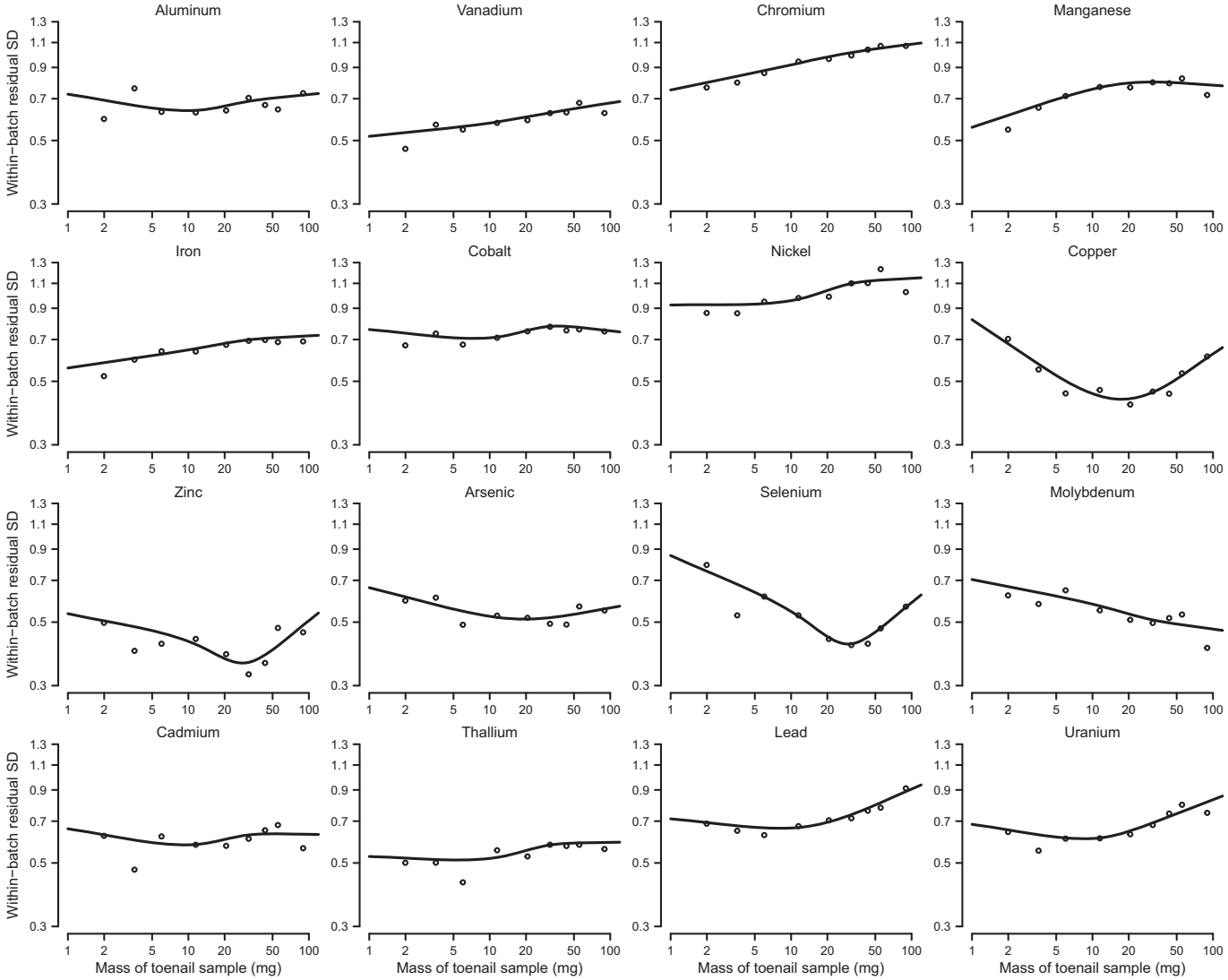

Figure 2. Within-batch error variance in measured metal concentrations associated with toenail sample mass in a population-based multicase–control study in Spain, 2008–2013 ($n=7{,}923$). The smooth curve for the within-batch residual SD in log-transformed measured metal concentrations was determined by the estimates of the variance spline parameters ϕ, γ_1, γ_2, and γ_3 associated with log-transformed toenail sample masses in heteroscedastic spline mixed models. The circles corresponded to the within-batch residual SDs by <fifth, fifth–ninth, 10th–19th, 20th–39th, 40th–59th, 60th–79th, 80th–89th, 90th–94th, and ≥95th percentile of the overall toenail sample mass distribution. Note: SD, standard deviation.

chromium to $0.454/(0.454+0.303)=0.60$ for thallium (Table 4). The calibrated metal concentrations corrected the measurements for both the heterogeneity in sample masses and the variability between batches. Conditional on case–control status and sociodemographic factors, the variance of calibrated concentrations represented between 40% and 74% of the total variance of the measurements for most metals, with extreme values of 27% for thallium and 84% for chromium (Table 4, last column). This variance reduction and the removed association of calibrated metal concentrations with toenail sample mass are shown in Figure 3. Given case–control status and sociodemographic factors, partial correlations between log-transformed calibrated concentrations and log-transformed sample mass were below 0.002 in absolute value for all metals, showing that the calibration method was effective in removing the effect of sample mass on toenail metal determinations. Partial correlations between log-transformed measured and calibrated concentrations ranged from 0.55 to 0.93.

Adjusting for sociodemographic factors, the odds of prostate cancer increased by 26% and 20% per 2-fold increase in calibrated concentrations of arsenic and uranium, respectively; the odds of stomach cancer decreased by 34%, 22%, and 23% for each 2-fold increase in calibrated concentrations of copper, zinc, and lead, respectively; and the odds of leukemia increased by 43% per 2-fold increase in calibrated copper concentrations (Table S4). These ORs for calibrated metal concentrations differed substantially from those obtained for measured concentrations (Figure 4). Even though standard adjustment for toenail sample mass was able to control for the average bias in measured metal concentrations, the variation in mass-related biases across laboratory batches and the mass-related heterogeneity in error variances were not taken into account. Thus, in comparison with ORs for calibrated concentrations, the mass-adjusted ORs for measured concentrations remained biased either toward or away from the null, with 42 and 18 of the 80 metal-cancer associations (52.5% and 22.5%) exceeding the 5% and 10% bias thresholds in either direction, respectively (Figure 4). In addition, due to the reduced variability of calibrated concentrations, the SEs of log-transformed ORs were larger for calibrated than for measured concentrations [median (interquartile range) increase of 18.3% (12.1%–28.0%)], exceeding 50% only for thallium.

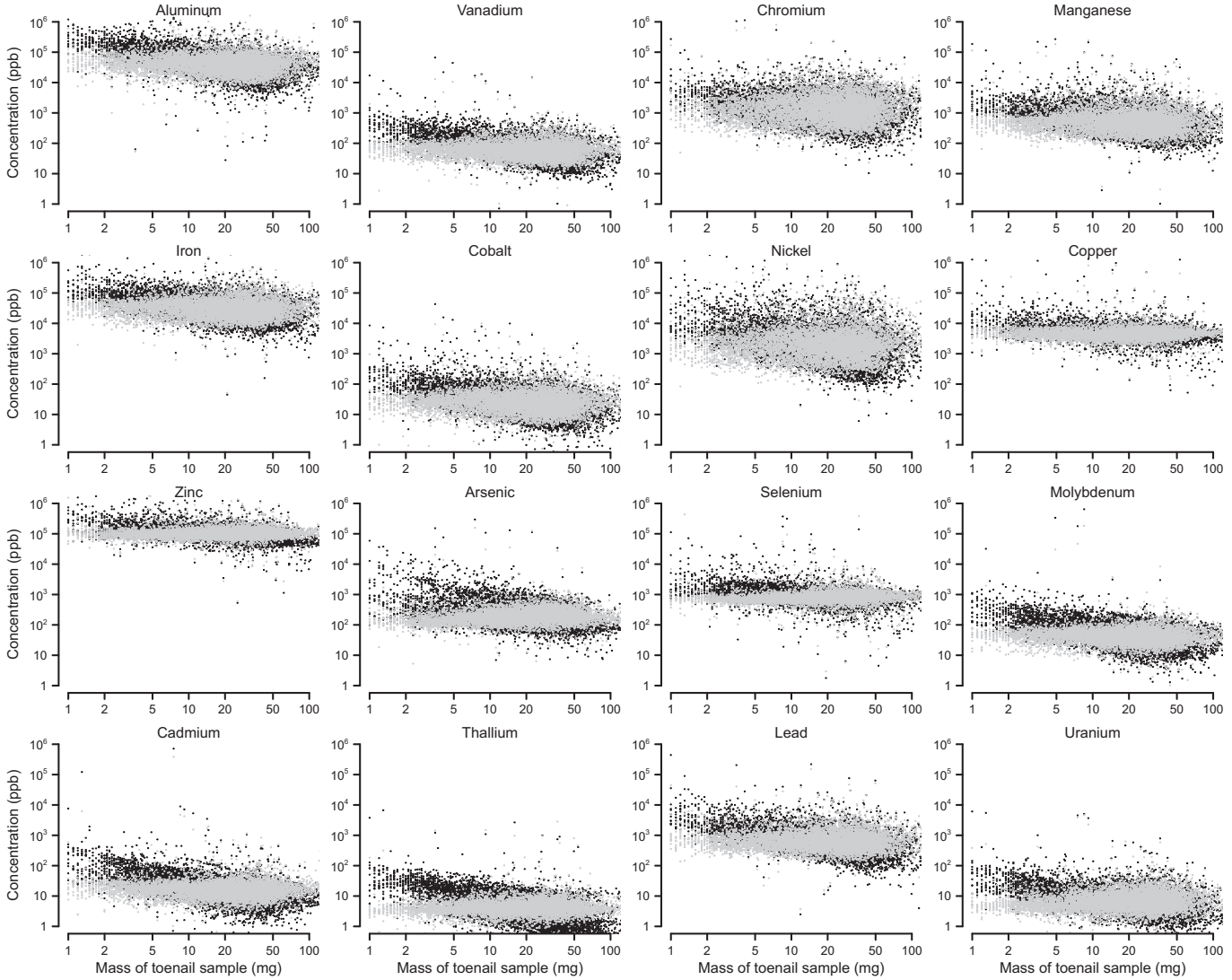

Figure 3. Measured and calibrated metal concentrations by toenail sample mass in a population-based multicase–control study in Spain, 2008–2013 ($n=7{,}923$). Measured metal concentrations (black dots) were obtained by inductively coupled plasma mass spectrometry in toenail samples of different mass in 122 laboratory batches. Calibrated metal concentrations (gray dots) were calculated from heteroscedastic spline mixed models and represented the concentrations that would have been observed had all toenail specimens been analyzed in the same average batch and toenail sample masses been set to the geometric mean for all participants (17.6 mg). Note: ppb, parts per billion.

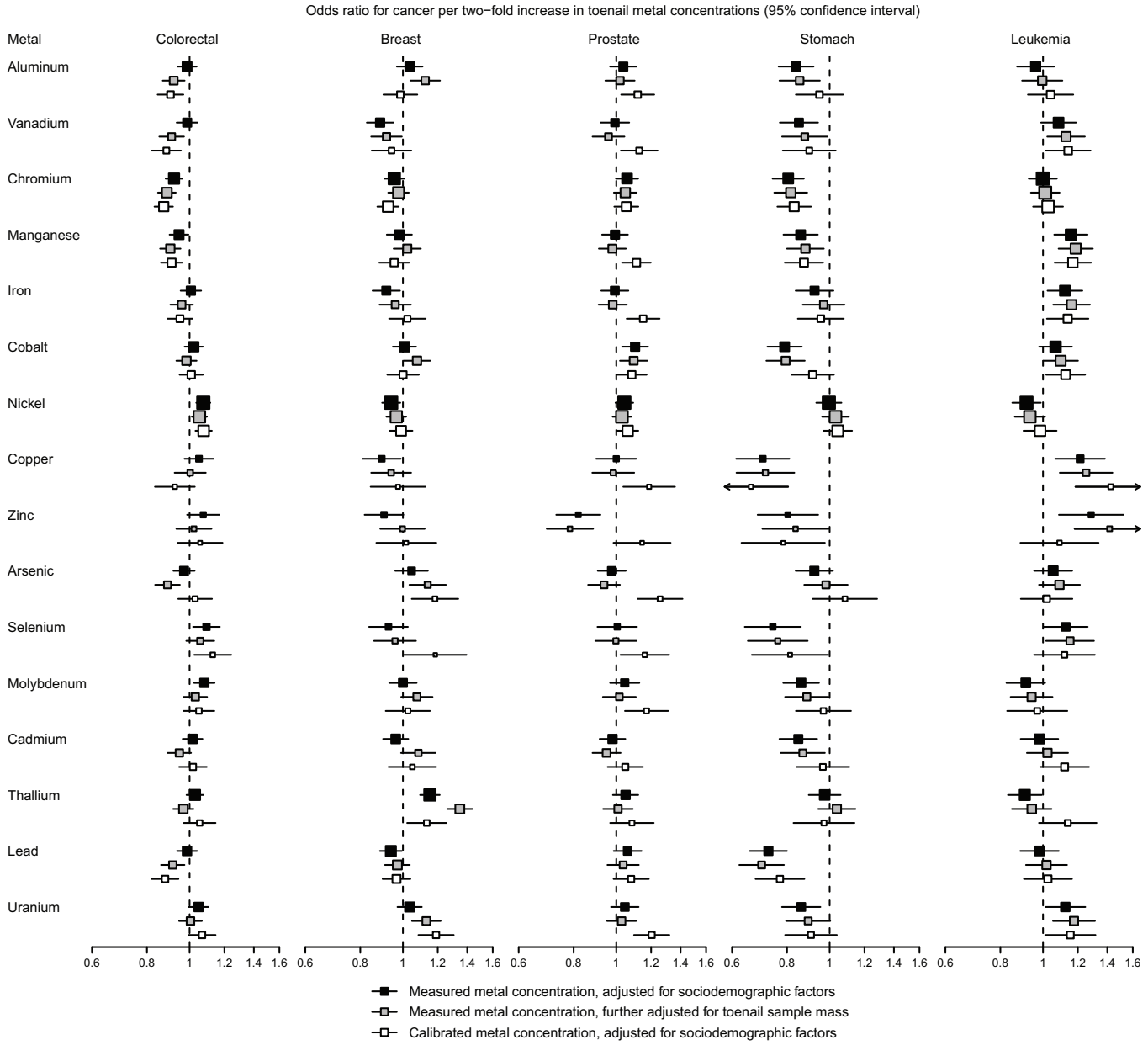

Figure 4. ORs for common cancers per 2-fold increase in toenail metal concentrations in a population-based multicase–control study in Spain, 2008–2013 ($n = 7{,}923$). The ORs (black squares) were obtained from logistic regression models on log-transformed measured metal concentrations adjusted for geographical region (12 Spanish provinces), sex (male, female), age (<35 y and 5-y intervals from 35 to 84 y), and educational level (primary or less, high school, and college). The ORs (gray squares) were obtained from the same models on log-transformed measured metal concentrations further adjusted for toenail sample mass (natural cubic spline of log-transformed values with internal knots at the overall 35th and 65th percentiles and boundary knots at the fifth and 95th percentiles). The ORs (white squares) were obtained from logistic regression models on log-transformed calibrated metal concentrations adjusted for geographical region, sex, age, and educational level. The area of each square is inversely proportional to the variance of the log-transformed OR. Horizontal lines represent 95% confidence intervals. Values are available in Tables S2 and S4. Note: OR, odds ratio.

Discussion

In this paper, we propose a heteroscedastic spline mixed model to fully account for the heterogeneity in sample masses and the variability between batches, which may pose a threat to validity and precision of toenail metal determinations in epidemiological studies. The model facilitates decomposition of the total variance of measured metal concentrations into the extraneous variances merely due to differing toenail sample masses and laboratory batches and the intrinsic variance resulting from heterogeneous metal exposures. The calibrated metal concentrations derived from the model remove these extraneous variations and represent the predicted concentrations if all toenail samples had been of the same mass and analyzed in a single batch.

Since the mid-2000s, there has been growing interest in the use of toenails as biomarkers of metal exposure.[2–4] Toenail samples collected in epidemiological studies vary greatly in size, with substantial proportion of small samples, and their metal contents are analyzed in multiple laboratory batches. Most studies do not address the impact of sample mass and batch on toenail metal determinations, but our results from MCC-Spain highlight that both factors may account for a quarter to half the variability of measured concentrations for most metals. Thus, although the

development of standardized collection and analysis protocols could partly reduce such extraneous variations,[2–4] metal concentrations should be calibrated for sample mass heterogeneity and between-batch variability to obtain unbiased estimates of their health effects.

The toenail sample mass introduced both systematic and random errors in the measured metal concentrations. As already reported for selenium and cadmium,[6–9] we observed an increase in geometric mean concentrations with decreasing sample mass for all analyzed metals, which was exacerbated for samples below 20 mg. At a minimum, standard adjustment and residual methods,[7–9,11–16] should expand the usual linear term for sample mass with splines or other flexible specifications[21,23] to allow close control for nonlinear mass-related biases. Moreover, the upward bias in small toenail samples varied substantially across laboratory batches, which may further require correcting for batch-specific mass-related biases. This correction has been accomplished by including fixed interaction terms between sample mass and batch indicators,[15,16] but random between-batch variations provide more robust and efficient estimates of batch-specific mass-related biases with many batches of limited size.[19,20]The proposed model also tested for batch compositional effects[19] and found no association between the toenail mass composition of batches and the bias in individual samples for most metals.

Aside from the systematic bias in mean concentrations, the toenail sample mass also affected the random error of the measurements. Most log-transformed metal concentrations showed higher error variances at large sample masses, which corresponded in the original scale to higher error percentages relative (not absolute) to the lower geometric mean concentrations at large masses. However, commonly studied essential metals such as copper, zinc, and selenium also exhibited severe decreases in relative precision at small sample masses. This lower precision has already been documented for selenium[10] and could be due to small toenail samples producing low experimental signals out of the optimal prediction range of the calibration line.[24] To our knowledge, the proposed heteroscedastic model is the first attempt to correct for the nonnegligible heterogeneity in error variances related to sample mass.

Results from MCC-Spain further show that, even for toenail samples of the same mass, there may be considerable variation in metal determinations between laboratory batches. Using the estimated between-batch and within-batch variances at the geometric mean mass, the intra-batch correlation coefficient of measured concentrations was above 0.10 for most metals and exceeded 0.30 for arsenic, molybdenum, cadmium, and thallium. Factors accounting for this between-batch variation may include different storage periods[25] and sample digestion processes, distinct recovery rates,[26] and uncontrolled instrument or operator variability. As implemented in a previous study,[27] our model includes a random intercept to correct metal concentrations for between-batch variability at fixed sample masses.

We use a heteroscedastic linear mixed model for log-transformed concentrations, but other modeling approaches are possible to correct toenail metal determinations. A generalized linear model with log link and gamma errors for untransformed concentrations would provide nearly identical control for the average mass-related bias,[28] but would not readily extend to both random between-batch variations and heteroscedastic relative errors, with no simple and general derivation of variance components.[29,30] The proposed model can be applied to population-based or nested case–control studies, as well as to baseline metal determinations in cohort studies, upon conditioning on stratifying and matching factors used in study design and on disease status in case–control studies. For repeated measurements over follow-up, however, their correlation should be accounted for by extending the model with a random intercept for subjects, either nested within batch if toenail samples from the same participant are included in a single batch or cross-classified by batch if analyzed in different batches.[19] In addition, the model can be used to correct metal concentrations in toenails and other biological samples of heterogeneous and usually reduced size, such as fingernail, hair, and tumor tissue, analyzed with different laboratory techniques.

As illustrated in this MCC-Spain multicase–control study, ignoring the impact of sample mass and laboratory batch on toenail metal determinations results in biased estimates of their ORs for cancer. We found that calibrated metal concentrations are effective in correcting this bias but yield somewhat larger SEs and wider confidence intervals. The loss of precision was most marked for metals strongly affected by sample mass and batch (e.g., thallium determinations in MCC-Spain with almost three-quarters of extraneous variation), suggesting that toenails might not be reliable exposure biomarkers for certain metals in current practice. Regardless of the statistical correction method, the development of standardized protocols for sample collection and analysis would enhance the use and interpretation of toenail metal concentrations in epidemiological studies.[2–4]

In conclusion, we provide a unified modeling approach to correct for major sources of systematic and random errors in measured metal concentrations, namely, sample mass heterogeneity and between-batch variability. The proposed model could be applied to toenail samples or other biological specimens of heterogeneous size, distinct laboratory techniques, and different study designs. The annotated R script COMET intends to support the implementation of this approach in future research on health effects of metal exposure.

Acknowledgments

This work was supported by the Spanish Health Research Fund of the Carlos III Institute of Health (ISCIII) (grant FIS PI12-00150). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the Carlos III Institute of Health (ISCIII). V.M. received grants from the Carlos III Institute of Health (ISCIII) (grant PI17-00092), the Spanish Association Against Cancer (AECC) Scientific Foundation (grant GCTRA18022MORE), and the Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP) (action Genrisk).

The authors do not have permission to share data from the MCC-Spain study (www.mccspain.org), but the R code used to conduct the analysis is provided in the Supplemental Material (see section "R Script COMET").

References

1. Santonen T, Aitio A, Fowler BA, Nordberg M. 2015. Biological monitoring and biomarkers. In: *Handbook on the Toxicology of Metals*. Nordberg GF, Fowler BA, Nordberg M, eds. 4th ed. San Diego, CA: Academic Press, 155–171.
2. Gutiérrez-González E, García-Esquinas E, de Larrea-Baz NF, Salcedo-Bellido I, Navas-Acien A, Lope V, et al. 2019. Toenails as biomarker of exposure to essential trace metals: a review. Environ Res 179(pt A):108787, PMID: 31610392, https://doi.org/10.1016/j.envres.2019.108787.
3. Signes-Pastor AJ, Gutiérrez-González E, García-Villarino M, Rodríguez-Cabrera FD, López-Moreno JJ, Varea-Jiménez E, et al. 2021. Toenails as a biomarker of exposure to arsenic: a review. Environ Res 195:110286, PMID: 33075355, https://doi.org/10.1016/j.envres.2020.110286.
4. Salcedo-Bellido I, Gutiérrez-González E, García-Esquinas E, Fernández de Larrea-Baz N, Navas-Acien A, Téllez-Plaza M, et al. 2021. Toxic metals in toenails as biomarkers of exposure: a review. Environ Res 197:111028, PMID: 33753073, https://doi.org/10.1016/j.envres.2021.111028.
5. Karagas MR, Tosteson TD, Blum J, Klaue B, Weiss JE, Stannard V, et al. 2000. Measurement of low levels of arsenic exposure: a comparison of water and toenail concentrations. Am J Epidemiol 152(1):84–90, PMID: 10901333, https://doi.org/10.1093/aje/152.1.84.

6. Baskett CK, Spate VL, Morris JS, Anderson HD, Mason MM, Reams CL, et al. 1995. Investigation of the appearance of supplemental enriched Se-76 using the human nail as a dietary monitor. J Radioanal Nucl Chem, Articles 195(1):97–108, https://doi.org/10.1007/BF02036478.
7. Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB, et al. 1998. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J Natl Cancer Inst 90(16):1219–1224, PMID: 9719083, https://doi.org/10.1093/jnci/90.16.1219.
8. Yoshizawa K, Rimm EB, Morris JS, Spate VL, Hsieh CC, Spiegelman D, et al. 2002. Mercury and the risk of coronary heart disease in men. N Engl J Med 347(22):1755–1760, PMID: 12456851, https://doi.org/10.1056/NEJMoa021437.
9. Platz EA, Helzlsouer KJ, Hoffman SC, Morris JS, Baskett CK, Comstock GW. 2002. Prediagnostic toenail cadmium and zinc and subsequent prostate cancer risk. Prostate 52(4):288–296, PMID: 12210489, https://doi.org/10.1002/pros.10115.
10. Kardinaal AF, Kok FJ, Kohlmeier L, Martin-Moreno JM, Ringstad J, Gómez-Aracena J, et al. 1997. Association between toenail selenium and risk of acute myocardial infarction in European men: the EURAMIC study. Am J Epidemiol 145(4):373–379, PMID: 9054242, https://doi.org/10.1093/oxfordjournals.aje.a009115.
11. Guallar E, Sanz-Gallardo MI, van't Veer P, Bode P, Aro A, Gómez-Aracena J, et al. 2002. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 347(22):1747–1754, PMID: 12456850, https://doi.org/10.1056/NEJMoa020157.
12. Colangelo LA, He K, Whooley MA, Daviglus ML, Morris S, Liu K. 2014. Selenium exposure and depressive symptoms: the coronary artery risk development in young adults trace element study. Neurotoxicology 41:167–174, PMID: 24560993, https://doi.org/10.1016/j.neuro.2014.02.003.
13. Gutierrez-Bedmar M, Martínez-González MA, Muñoz-Bravo C, Ruiz-Canela M, Mariscal A, Salas-Salvadó J, et al. 2017. Chromium exposure and risk of cardiovascular disease in high cardiovascular risk subjects: nested case-control study in the Prevention with Mediterranean diet (PREDIMED) study. Circ J 81(8):1183–1190, PMID: 28392547, https://doi.org/10.1253/circj.CJ-17-0032.
14. Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Baskett CJ, et al. 1993. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev 2(5):493–497, PMID: 8220096, http://cebp.aacrjournals.org/content/2/5/493 [accessed 14 April 2025].
15. Garland M, Morris JS, Stampfer MJ, Colditz GA, Spate VL, Baskett CK, et al. 1995. Prospective study of toenail selenium levels and cancer among women. J Natl Cancer Inst 87(7):497–505, PMID: 7707436, https://doi.org/10.1093/jnci/87.7.497.
16. Garland M, Morris JS, Colditz GA, Stampfer MJ, Spate VL, Baskett CK, et al. 1996. Toenail trace element levels and breast cancer: a prospective study. Am J Epidemiol 144(7):653–660, PMID: 8823061, https://doi.org/10.1093/oxfordjournals.aje.a008977.
17. Castaño-Vinyals G, Aragonés N, Pérez-Gómez B, Martín V, Llorca J, Moreno V, et al. 2015. Population-based multicase-control study in common tumors in Spain (MCC-Spain): rationale and study design. Gac Sanit 29(4):308–315, PMID: 25613680, https://doi.org/10.1016/j.gaceta.2014.12.003.
18. Gutiérrez-González E, Fernández-Navarro P, Pastor-Barriuso R, García-Pérez J, Castaño-Vinyals G, Martín-Sánchez V, et al. 2022. Toenail zinc as a biomarker: relationship with sources of environmental exposure and with genetic variability in MCC-Spain study. Environ Int 169:107525, PMID: 36150295, https://doi.org/10.1016/j.envint.2022.107525.
19. Raudenbush SW, Bryk AS. 2002. *Hierarchical Linear Models: Applications and Data Analysis Methods*. 2nd ed. Thousand Oaks, CA: Sage Publications.
20. Pinheiro JC, Bates DM. 2000. Extending the basic linear mixed-effects model. In: *Mixed-Effects Models in S and S-PLUS*. Pinheiro JC, Bates DM, eds. New York, NY: Springer, 201–270.
21. Durrleman S, Simon R. 1989. Flexible regression models with cubic splines. Stat Med 8(5):551–561, PMID: 2657958, https://doi.org/10.1002/sim.4780080504.
22. Yland JJ, Wesselink AK, Lash TL, Fox MP. 2022. Misconceptions about the direction of bias from nondifferential misclassification. Am J Epidemiol 191(8):1485–1495, PMID: 35231925, https://doi.org/10.1093/aje/kwac035.
23. Greenland S. 1995. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. Epidemiology 6(4):356–365, PMID: 7548341, https://doi.org/10.1097/00001648-199507000-00005.
24. Skoog DA, Holler FJ, Crouch SR. 2018. *Principles of Instrumental Analysis*. 7th ed. Boston, MA: Cengage Learning.
25. St-Pierre J, Kada R, Kennedy G, Zayed J, Ghadirian P. 2006. Variation of selenium concentration in toenails following long-term storage, washing and reactor irradiation. J Radioanal Nucl Chem 269(2):347–349, https://doi.org/10.1007/s10967-006-0390-4.
26. Rodrigues EG, Kile M, Dobson C, Amarasiriwardena C, Quamruzzaman Q, Rahman M, et al. 2015. Maternal-infant biomarkers of prenatal exposure to arsenic and manganese. J Expo Sci Environ Epidemiol 25(6):639–648, PMID: 26306926, https://doi.org/10.1038/jes.2015.45.
27. O'Brien KM, White AJ, Jackson BP, Karagas MR, Sandler DP, Weinberg CR. 2019. Toenail-based metal concentrations and young-onset breast cancer. Am J Epidemiol 188(4):34–43, PMID: 32242893, https://doi.org/10.1093/aje/kwaa035.
28. McCullagh P, Nelder JA. 1989. Models for data with constant coefficient of variation. In: *Generalized Linear Models*. McCullagh P, Nelder JA, eds. 2nd ed. Boca Raton, FL: Chapman & Hall, 285–322.
29. McCulloch CE, Searle SR, Neuhaus JM. 2008. Generalized linear mixed models. In: *Generalized, Linear, and Mixed Models*. McCulloch CE, Searle SR, Neuhaus JM, eds. 2nd ed. New York, NY: John Wiley & Sons, 188–211.
30. Nakagawa S, Johnson PCD, Schielzeth H. 2017. The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. J R Soc Interface 14(134):20170213, PMID: 28904005, https://doi.org/10.1098/rsif.2017.0213.