METALS AND CARDIOVASCULAR DISEASE: POTENTIAL MECHANISMS AND DESIGNS TO ASSESS CAUSALITY

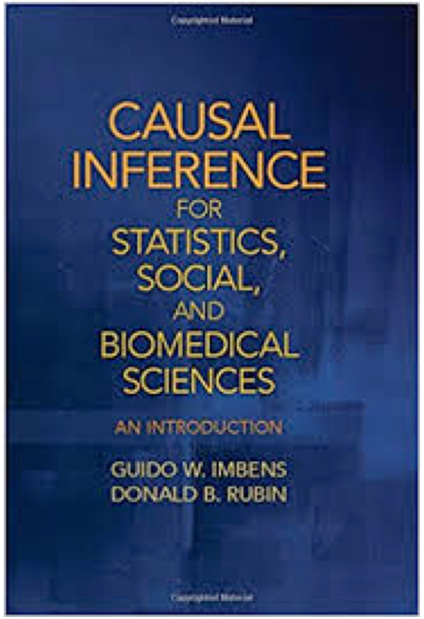
María Téllez Plaza, MD PhD
Científico Titular de OPIS,

Departamento de Epidemiología de las Enfermedades Crónicas
Centro Nacional de Epidemiología (CNE), ISCIII

Seminario, 8 de Noviembre del 2018

- *"… statistical theory has been relatively silent on questions of causality. Many, especially older, textbooks avoid any mention of the term other than in settings of randomized experiments. Some mention it mainly to stress that correlation or association is not the same as causation, and some even caution their readers to avoid using causal language in statistics. Nevertheless, for many users of statistical methods, causal statements are exactly what they seek."*

- Imbens, Guido W.; Rubin, Donald B.. Causal Inference in Statistics, Social, and Biomedical Sciences: An Introduction (Kindle Locations 417-420). Cambridge University Press.



OUTLINE

- **LEARNING OBJECTIVE:** to review conducted and ongoing work that examines the role of metals as (causal) cardiovascular risk factors
  - Introduction: the challenge of cardiovascular disease prevention and control
  - Metals, cardiovascular risk and potential mechanisms
  - Designs to support the causality of metals in ongoing studies

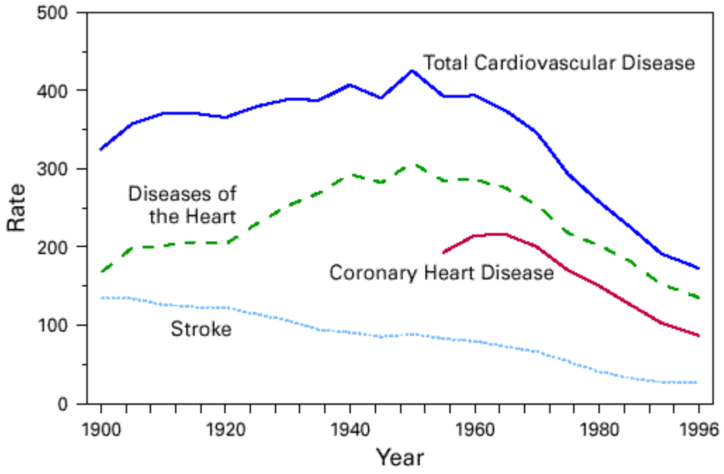
Introduction

The Challenge of Cardiovascular Disease

Prevention and Control

Major public health achievement: Decline in CVD

FIGURE 1. Age-adjusted death rates* for total cardiovascular disease, diseases of the heart, coronary heart disease, and stroke,† by year — United States, 1900–1996



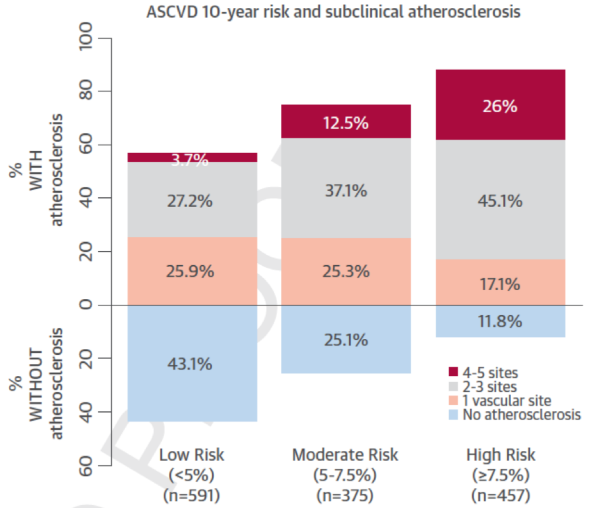
*Per 100,000 population, standardized to the 1940 U.S. population.
†Diseases are classified according to *International Classification of Diseases* (ICD) codes in use when the deaths were reported. ICD classification revisions occurred in 1910, 1921, 1930, 1939, 1949, 1958, 1968, and 1979. Death rates before 1933 do not include all states. Comparability ratios were applied to rates for 1970 and 1975.

- " conventional risk factors **only partly explain** the observed gradient in coronary heart disease" Marmot M et al, Am J Epidemiol 1975

- "**Measured risk factor** and treatment variables, while important, **neither explain why the decline** [in cardiovascular disease] began when it did, **nor** much of **the similarities and differences** in the start time and rate of the decline across countries or between men and women." Ezzati M et al, Nat Rev Cardiol, 2015

57% of participants without cardiovascular risk factors showed subclinical atherosclerosis

FIGURE 3 **Presence of Subclinical Atherosclerosis According to Traditional Risk Equations**

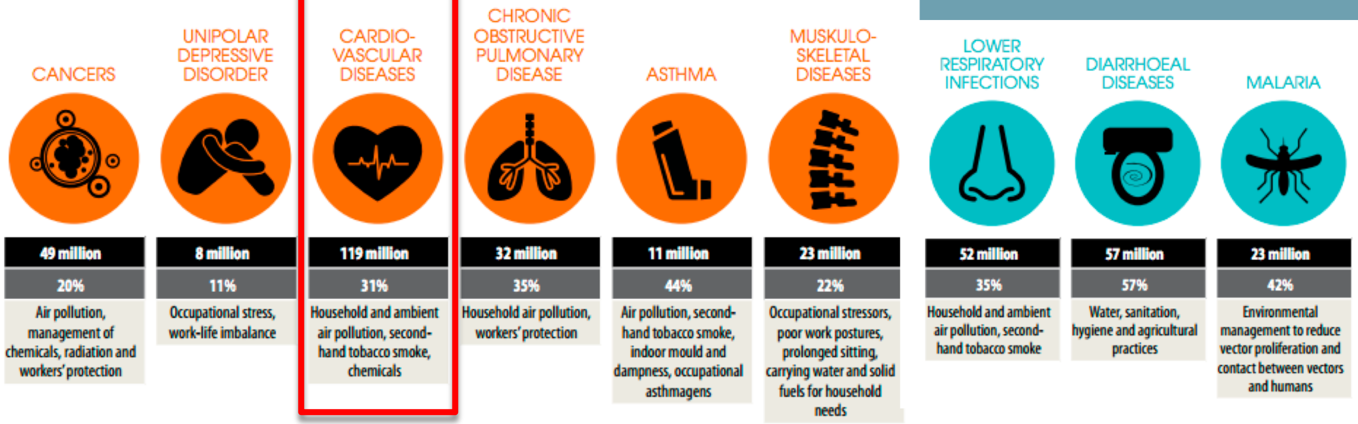


Presence and extent of subclinical atherosclerosis according to the 10-year risk for atherosclerotic cardiovascular disease (ASCVD) calculated using the Pooled Cohort Equations, classified as low (<5%), moderate (5% to 7.5%), or high (≥7.5%) risk.

AWHS ARAGON WORKERS' HEALTH STUDY

Laclaustra et al. Journal of the American College of Cardiology · March 2016
DOI: 10.1016/j.jacc.2015.12.056

Environment and burden of disease



31% of the burden of disease from fatal CVD globally could be avoided if environmental risks were removed (*World Health Organization*, 2016)

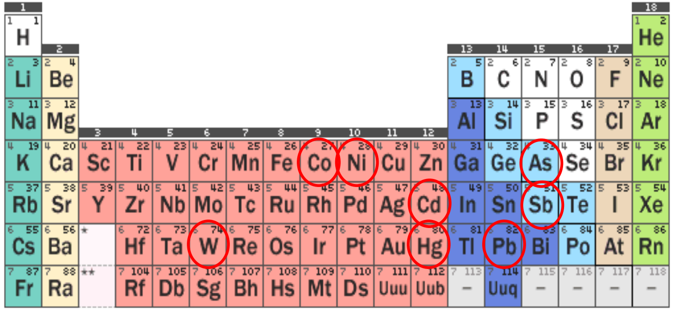
WHO, 2016. Preventing Disease Through Healthy Environments.

Metals, Cardiovascular Risk and Mechanisms

Comprehensive Review of Accumulated Evidence

Established Expertise in Evaluating Chronic Cardiovascular Health Effects of Arsenic, Cadmium, Lead and Other Metals

Metals with evidence in support of potential cardiovascular effects



Nigra et al. Current Environ Health Reports, 2016

Evidence at low-moderate levels is increasing

Arsenic and CVD – high exposure levels

1930s–1980s — **Case series / Ecological studies**
- German vintners (As in pesticides, PAD)
- Taiwan & Chile (water As, PAD & other CVD)

1990s — **Cohort studies in Taiwan**
- Ecological water As assessment
- CVD mortality (all, CHD, stroke)

2007 — **Ecological study in Chile**
- Natural experiment before & after water As
- Myocardial infarction mortality

2011–2013 — **HEALS cohort in Bangladesh**
- Water and urine As
- CVD incidence & mortality (all, CHD, stroke)

As levels:	> 500 µg/L	100 µg/L	10-100 µg/L	< 10 µg/L

Children and young adults exposed to arsenic in drinking water at 900 µg/L in Chile showed thickening of the arterial intima and myocardial infarction

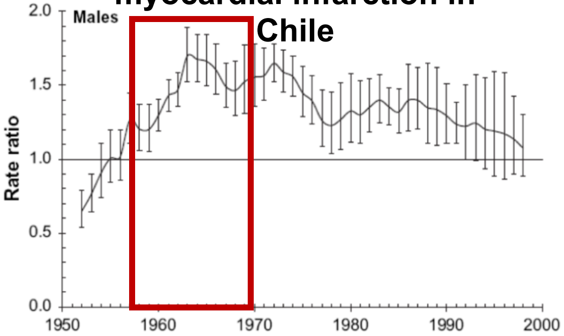
Rosenberg HG. Arch Pathol 1974;97:360-365

Fig 1.—Cross section of epicardial branch of left coronary artery. Note fibrous intimal thickening, replication of elastic fibers internal to lamina elastica. Medial coat and adventitia show slight changes (case 1) (Verhoff-van Giesson,

Black Foot Disease Taiwan

Tromboangeitis obliterans + arteriosclerosis

Ecological study of myocardial infarction in Chile



Yuan Y et al. Am J Epidemiol 2007

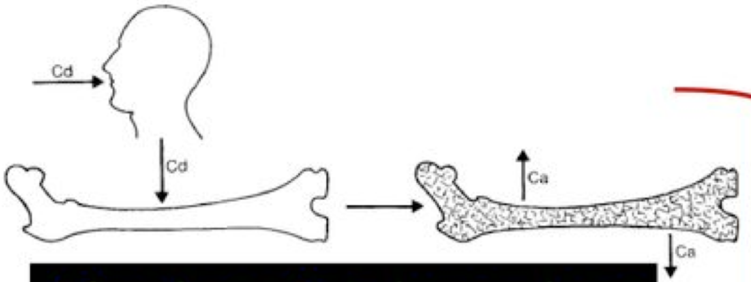
SATURNINE GOUT, AND ITS DISTINGUISHING MARKS.

By G. LORIMER, M.A., M.D.Edin., Buxton.

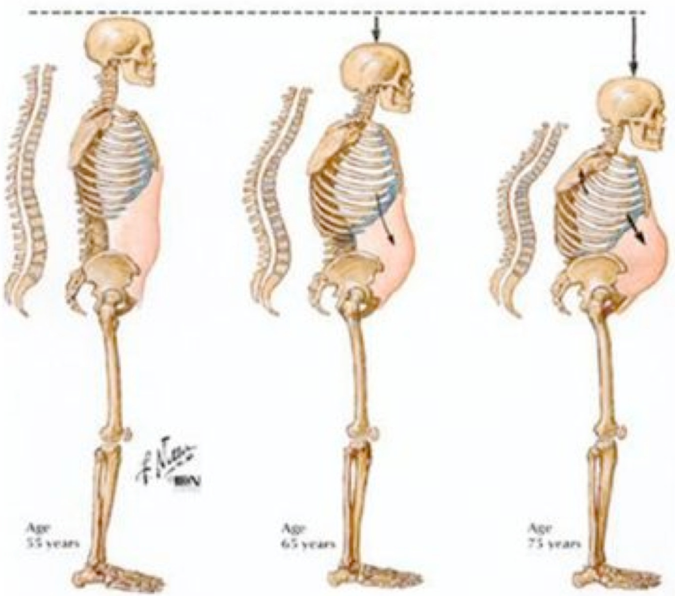
The conclusions arrived at are based upon an analysis of 107 cases of gout due to plumbism, which have occurred in the writer's experience, and the subsequent remarks constitute a record of facts so observed.

6. *Arterial Thickening and Degeneration.*—This condition, noted in sixty-nine cases, consists of a sclerosis of the arterial coats, along with atheromatous changes. It is, in fact, a premature ageing of the arterial system. *a*. It may be due to the action of lead, which causes contraction of the muscular walls of the arteries, and raises arterial tension. *b*. It may be connected with the renal changes which arise in saturnine arthritis. *c*. It may depend on the condition of the blood in gout, which gives rise to increased arterial tension, and predisposes to atheroma. Cardiac hypertrophy is observed in saturnine gout, especially at the advanced period of the disease. The arterial changes, however, may occur independently of the cardiac. Pericarditis has been noted by Charcot and Gumbolt. One instance only was noted by the writer in the cases referred to.

1912 in Japan

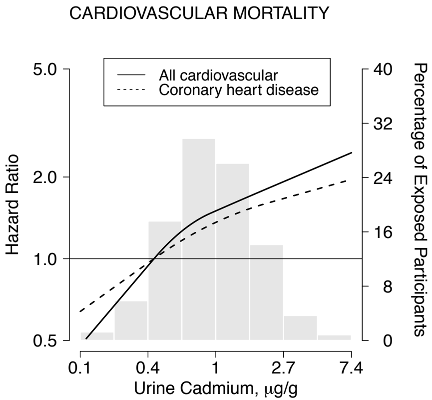


***Cd* replaces *Ca* in human bone**



Itai – Itai disease

Low chronic exposure : Dose-response evaluation to inform the risk assessment



EPIDEMIOLOGY

Cadmium exposure and incident cardiovascular disease. Tellez-Plaza M, Guallar E, Howard BV, Umans JG, Francesconi KA, Goessler W, Silbergeld EK, Devereux RB, Navas-Acien A. Epidemiology. 2013 May;24(3):421-9. doi: 10.1097/EDE.0b013e31828b0631.

STRONG HEART STUDY

Original Strong Heart Study

4,549 adults 45-74 y

Visit 1 1989-91 — **Visit 2** 1993-95 — **Visit 3** 1998-99

64% baseline response rate

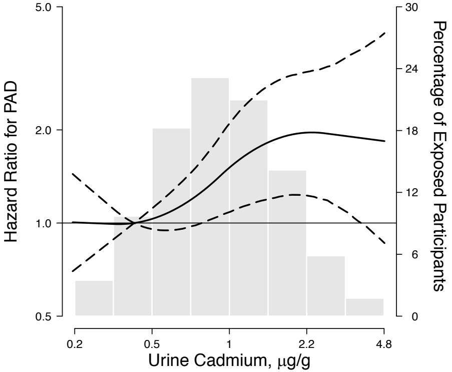
89% **88%** **retention rate**

Ongoing Surveillance: Morbidity & Mortality

Circulation
Cardiovascular Quality and Outcomes

American Heart Association

Cadmium Exposure and Incident Peripheral Arterial Disease
Maria Tellez-Plaza, Eliseo Guallar, Richard R. Fabsitz, Barbara V. Howard, Jason G. Umans, Kevin A. Francesconi, Walter Goessler, Richard B. Devereux and Ana Navas-Acien



Ana Navas-Acien

ENVIRONMENTAL HEALTH PERSPECTIVES
OPEN ACCESS

Cadmium Exposure and All-Cause and Cardiovascular Mortality in the U.S. General Population

Maria Tellez-Plaza,[1,2,3] Ana Navas-Acien,[1,3,4] Andy Menke,[1] Ciprian M. Crainiceanu,[5] Roberto Pastor-Barriuso,[6,7] and Eliseo Guallar[1,2,4,8]

Hazard ratio (95%CI) for cardiovascular mortality endpoints comparing the 80th to the 20th percentile of cadmium distributions

		Blood Cadmium	*Urine Cadmium*
	Deaths	**HR (95% CI)**	**HR (95% CI)**
All-Cardiovascular	191	1.69 (1.03, 2.77)	1.74 (1.07, 2.83)
Heart disease	113	1.98 (1.11, 3.54)	2.53 (1.54, 4.16)
Coronary heart disease	88	1.73 (0.88, 3.40)	2.09 (1.06, 4.13)

Models were adjusted for sex, race/ethnicity, education, post-menopausal status, body mass index, blood lead, total cholesterol, HDL cholesterol, cholesterol lowering medication, hypertension, diabetes, estimated GFR, smoking status, cumulative smoking dose, serum cotinine.

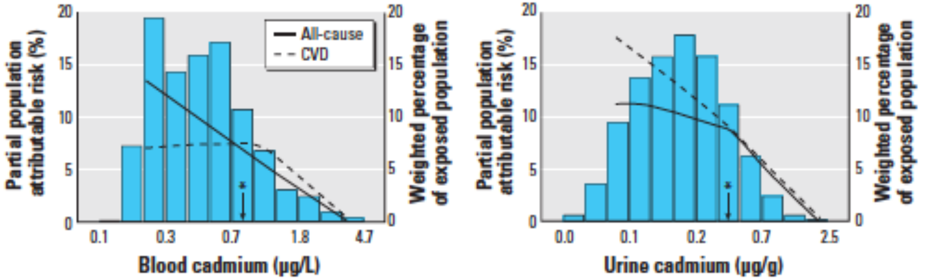
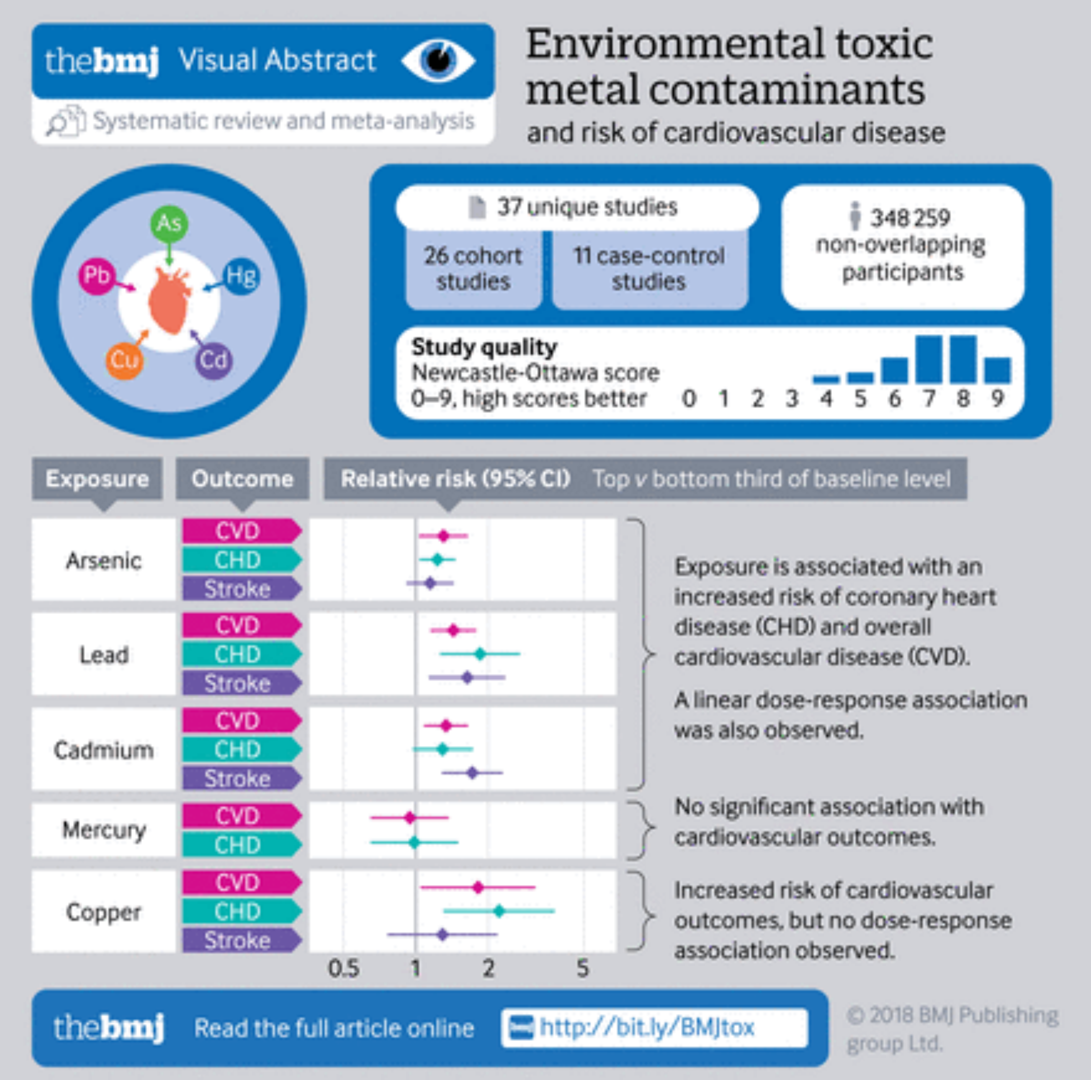
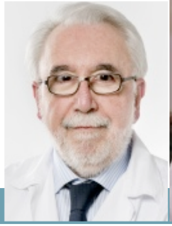


Figure 1. Partial PAR associated with cadmium exposure. Relative risks for calculating the partial PAR were obtained from fully adjusted Cox proportional hazards models (model 3). The partial PAR represents the estimated fraction of deaths that would be avoided in the population had cadmium exposure in participants with levels above a given percentile of the cadmium distribution been similar to cadmium exposure in participants with levels below that concentration, assuming that the effects of cadmium are causal and that other risk factors remained unchanged. The asterisk indicates the 80th percentiles of blood and urine cadmium distributions (0.80 µg/L and 0.57 µg/g creatinine, respectively). Bars indicate the weighted percent of the exposure in the population and lines indicate estimated PARs according to blood or urine cadmium concentrations. For example, model estimates suggest that if cadmium exposure in participants with urine cadmium concentrations above the 80th percentile of the distribution was reduced to that of participants below the 80th percentile, 9.2% of cardiovascular deaths in the U.S. population would be avoided.



Chowdhury et al. BMJ 2018

Metals and CVD in Cohort Studies from Spain: The Hortega Follow-Up Study



J. Redon

J.C. Martin-Escudero

Arsenic (comparing the 80th and the 20th percentiles)	All-CVD	p-value
Cases/Non Cases	165/997	
iAs	1.33 (1.00-1.76)	0.05
MMA	1.25 (0.95-1.65)	0.12
DMA	**1.47 (1.13-1.91)**	**0.004**
iAS + MMA + DMA	**1.42 (1.08-1.86)**	**0.01**

2002-03 → Prospective follow up, $N_{\text{CVD-free}} = 1{,}502$ → November 2015

Incidence:
177 CVD
137 Cancer

Área de Salud de Valladolid Oeste

Cardiovascular risk factors
1500 Candidate SNPS: metals metabolisms and transport, and cardiometabolic pathways
Oxidative stress: GSSG/GSH, MDA, 8-oxo-dG
Metabolomics: TMAO, Lipid particles (Biosfer Teslab)

Metals (Dr. J.L. Gomez-Ariza, U. Huelva):
Plasma: Hg, Cd, Pb, Se, As, Ti, Cr, Zn ;
Urine: Cd, Ba, V, Zn, W, As total, Ti, U, Sb,
Subsample (AsIII, AsV, MMA, DMA)

Source: Lopez-Zumel et al. Arsenic Exposure, Arsenic Metabolism, and Incident Cardiovascular Disease in the Hortega Follow-up Study. Unpublished data.

Metals and CVD in Cohort Studies from Spain: The Hortega Follow-Up Study

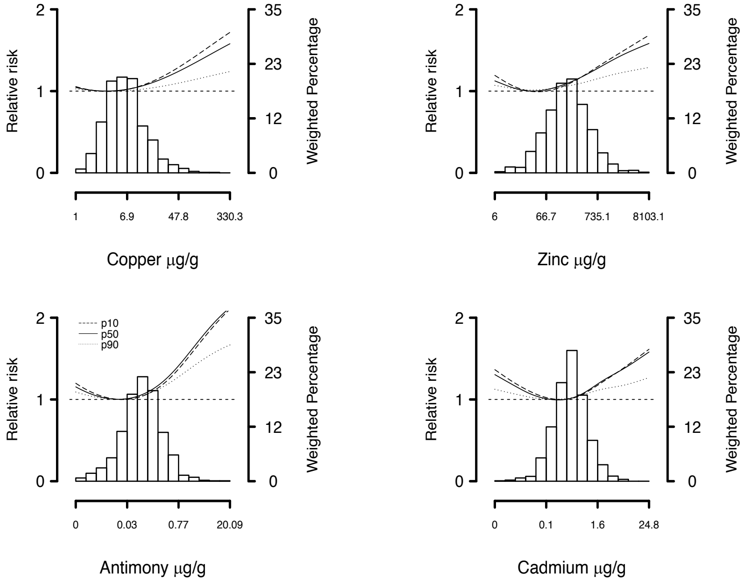
	Tertile 1, Non-cases/Cases	Tertile 2, Non-cases/Cases	Tertile 3, Non-cases/Cases	p80 vs. p20	p-trend
Essential metals					
Co	338/60	328/61	339/45		
	1.00 (Referent)	1.22 (0.82, 1.81)	1.29 (0.83, 1.99)	1.15 (0.91, 1.46)	0.25
Cu	351/30	322/69	332/67		
	1.00 (Referent)	**2.40 (1.48, 3.88)**	**1.87 (1.17, 3.00)**	**1.35 (1.06, 1.72)**	**0.02**
Mo	335/52	338/54	332/60		
	1.00 (Referent)	0.98 (0.65, 1.47)	1.12 (0.73, 1.71)	1.18 (0.88, 1.58)	0.28
Zn	352/30	331/56	322/80		
	1.00 (Referent)	1.47 (0.91, 2.40)	**1.93 (1.21, 3.08)**	**1.43 (1.07, 1.90)**	**0.01**
Non-essential metals					
Sb	350/51	331/55	324/60		
	1.00 (Referent)	1.41 (0.93, 2.13)	**1.57 (1.04, 2.36)**	**1.51 (1.13, 2.03)**	**0.006**
Ba	341/35	334/54	330/77		
	1.00 (Referent)	1.17 (0.74, 1.85)	1.25 (0.80, 1.94)	1.32 (0.96, 1.82)	0.08
Cd	356/36	327/63	322/67		
	1.00 (Referent)	**2.27 (1.44, 3.57)**	**2.31 (1.47, 3.65)**	**1.46 (1.13, 1.88)**	**0.003**
Cr	345/51	330/62	330/53		
	1.00 (Referent)	1.37 (0.90, 2.09)	**1.63 (1.08, 2.46)**	**1.64 (1.05, 2.58)***	**0.03***
V	346/53	327/64	332/49		
	1.00 (Referent)	1.45 (0.97, 2.18)	**1.51 (1.00, 2.29)**	**1.31 (1.01, 1.71)**	**0.04**

Urine Cu, Zn, Sb, Cd, Cr and V were individually associated with increased cardiovascular risk.

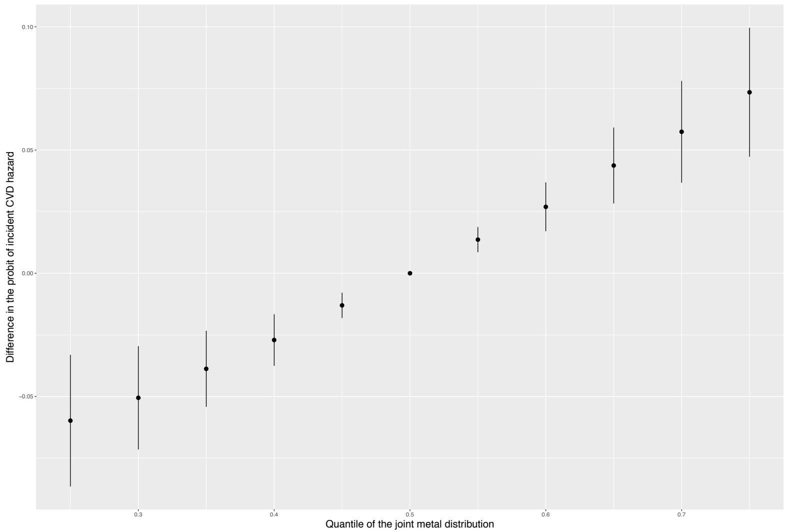
Source: Domingo-Relloso et al. The association of urine metals and metal mixtures with cardiovascular incidence in an adult population from Spain: the Hortega Follow-Up Study. Submitted.

Metals and CVD in Cohort Studies from Spain: The Hortega Follow-Up Study

Relative risk (RR) of incident CVD by concentrations of selected individual metals when all the other metals are fixed at their 10th, 50th and 90th percentiles from BKMR



Overall association of the metal mixture with CVD incidence (Difference in the probit of incident CVD hazard and 95% credibility intervals) when all predictors are at a particular percentile compared to the value when all of them are at their 50th percentile.



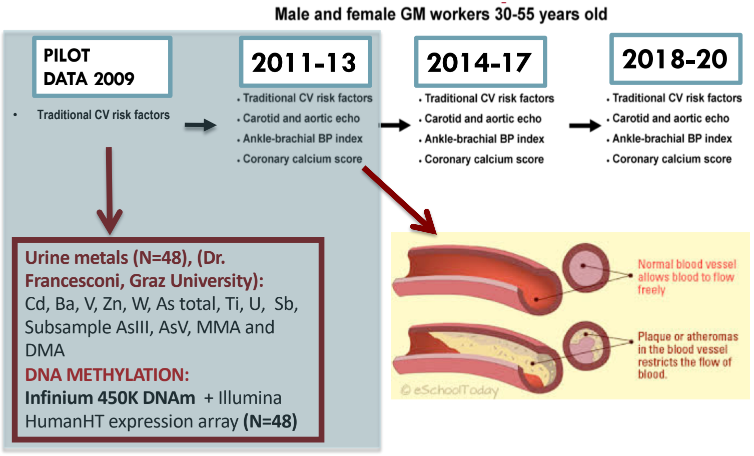
Source: Domingo-Relloso et al. The association of urine metals and metal mixtures with cardiovascular incidence in an adult population from Spain: the Hortega Follow-Up Study. Submitted.

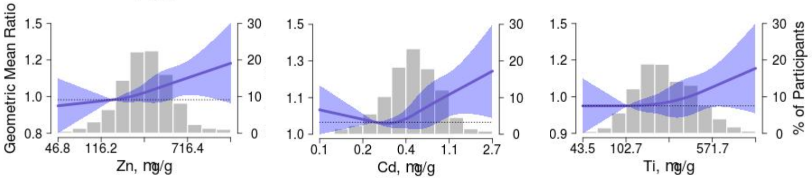
Metals and CVD in Cohort Studis from Spain: The Aragon Workers Health Study (AWHS)

ARAGON WORKERS' HEALTH STUDY

Geometric mean ratio of general plaque score by urine metal concentrations

	Tertile 1	Tertile 2	Tertile 3	p80 vs. p20	p-trend
Essential metals					
Co	1.00 (Ref)	1.08 (0.97, 1.20)	0.97 (0.45, 2.06)	0.98 (0.93, 1.03)	0.36
Cu	1.00 (Ref)	1.03 (0.41, 2.61)	1.04 (0.49, 2.20)	1.01 (0.96, 1.06)	0.61
Mo	1.00 (Ref)	1.01 (0.23, 4.51)	1.00 (0.15, 6.63)	1.00 (0.95, 1.06)	0.91
Zn	1.00 (Ref)	1.07 (0.92, 1.25)	1.11 (1.10, 1.13)	1.06 (1.01, 1.12)	0.02
Non-essential metals					
Sb	1.00 (Ref)	1.02 (0.27, 3.83)	0.99 (0.21, 4.55)	1.01 (0.96, 1.07)	0.64
Ba	1.00 (Ref)	1.00 (0.16, 6.43)	0.97 (0.35, 2.69)	0.99 (0.94, 1.03)	0.53
Cd	1.00 (Ref)	0.99 (0.19, 5.32)	1.11 (1.08, 1.14)	1.09 (1.00, 1.19)	0.05
Cr	1.00 (Ref)	1.02 (0.31, 3.33)	0.98 (0.30, 3.23)	1.00 (0.95, 1.05)	0.950
V	1.00 (Ref)	0.94 (0.72, 1.24)	1.07 (0.86, 1.33)	1.01 (0.97, 1.06)	0.556
Ti	1.00 (Ref)	1.02 (0.28, 3.67)	1.09 (1.03, 1.16)	1.04 (0.99, 1.10)	0.10





Source: Caballero-Mateos et al. A Panel of Metals and Subclinical Atherosclerosis: The AWHS-Metal Study Unpublished Data

Metals and CVD: potential mechanisms

- Increase oxidative stress and inflammation
- Affect endothelial function (interference with calcium signaling pathways)
- Endocrine disruption
- Epigenetics…

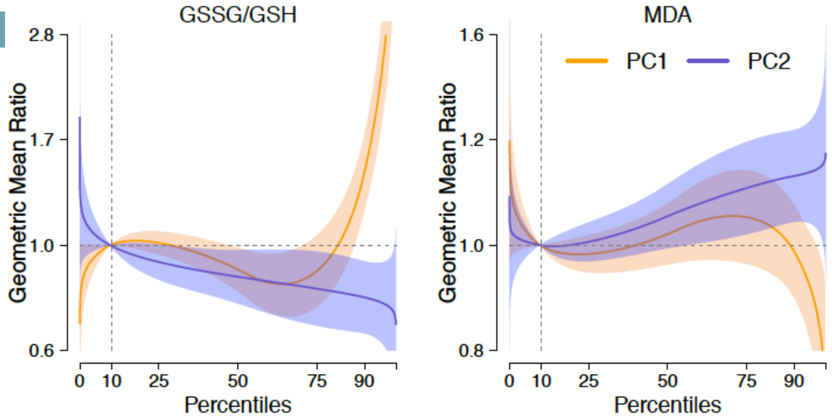
Metal biomarkers and oxidative stress

Individual metal levels and oxidative stress association

		GSSG/GSH		MDA		8-Oxo-dG	
		GMR (95%CI)	P-trend	GMR (95%CI)	P-trend	GMR (95%CI)	P-trend
Essential	Co	0.98 (0.89, 1.08)	0.70	0.99 (0.94, 1.03)	0.543	1.03 (0.98, 1.09)	0.195
	Cu	1.06 (0.95, 1.18)	0.30	1.04 (0.98, 1.09)	0.188	1.04 (0.99, 1.10)	0.142
	Mo	**1.14 (1.03, 1.27)**	**0.01**	1.01 (0.96, 1.07)	0.611	1.04 (0.94, 1.15)	0.201
	Zn	0.99 (0.88, 1.11)	0.85	**1.07 (1.01, 1.14)**	**0.019**	**1.07 (1.01, 1.13)**	**0.02**
Non-essential	Sb	0.99 (0.90, 1.09)	0.84	1.00 (0.95, 1.05)	0.883	1.05 (0.96, 1.15)*	0.001*
	Ba	**1.17 (1.05, 1.31)**	**0.006**	1.02 (0.96, 1.08)	0.561	1.00 (0.95, 1.06)	0.962
	Cd	1.07 (0.97, 1.19)	0.19	**1.12 (1.02, 1.23)***	**0.021***	**1.10 (1.00, 1.21)***	**0.05***
	Cr	**1.23 (1.04, 1.46)***	**0.002***	0.98 (0.92, 1.03)	0.413	1.04 (0.99, 1.10)	0.131
	V	**1.18 (1.00, 1.40)***	**<0.001***	0.97 (0.92, 1.03)	0.288	1.03 (0.98, 1.09)	0.256

Source: Unpublished data

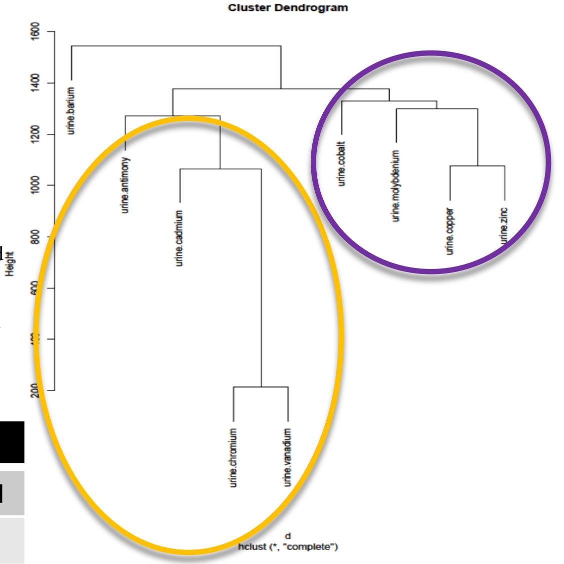
Metal mixtures and oxidative stress



Lines represent adjusted Geometric Mean Ratios for each oxidative stress biomarker based on restricted quadratic splines for the first two metal principal components with knots at 10th, 50th and 90th percentiles. The reference value was set at 10th percentile of each principal component. Geometric Mean Ratios were adjusted for age (years, splines), sex, education (< secondary education, ≥ secondary education), smoking status (never, former, current), urine cotinine (<34, 34-500, ≥500 ng/mL), cumulative smoking (0, <12, ≥12 packages year), alcohol consumption (g/day), diabetes status (no, yes) and estimated glomerular filtration rate (mL/minute per $1.73m^2$

PC1: Non-essential metals

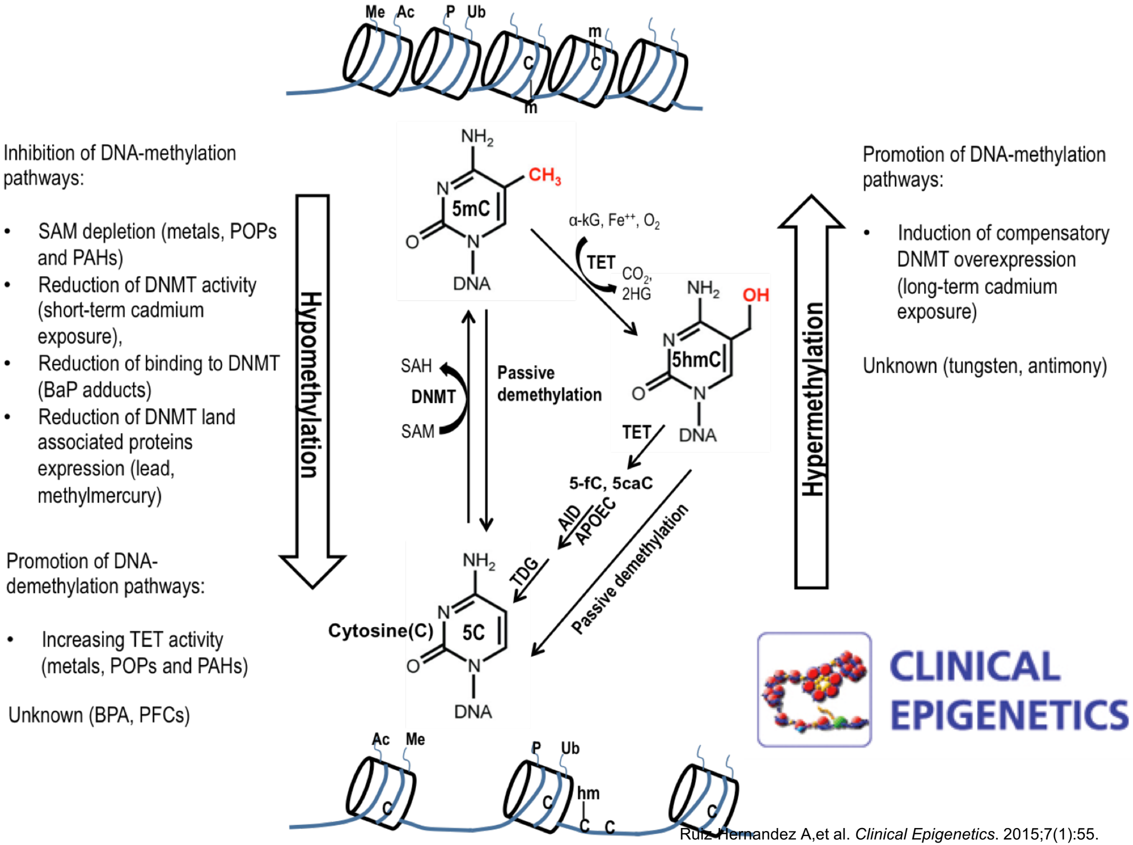
PC2: Essential metals



	GSSG/GSH		MDA	
	GMR (95%CI)	p-trend	GMR (95%CI)	p- trend
PC1	**1.35 (1.20 1.52)**	**<0.001**	1.07 (0.96, 1.18)*	0.21*
PC2	**0.84 (0.75 0.94)**	**0.002**	**1.08 (1.02, 1.15)**	**0.001**

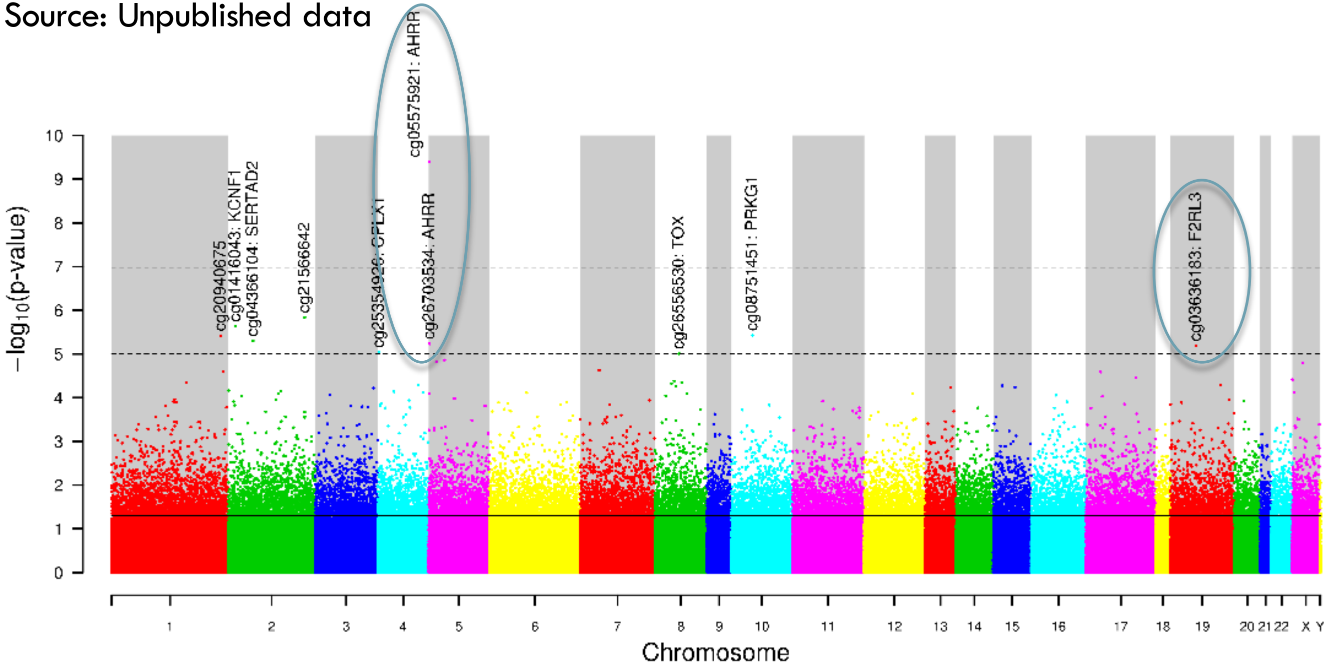
Source: Unpublished data

In vivo an in vitro evidence supporting a role of metals as determinants de 5mC and 5hmC



Ruiz-Hernandez A,et al. *Clinical Epigenetics*. 2015;7(1):55. doi:10.1186/s13148-015-0055-7.

Source: Unpublished data



Gao *et al. Clinical Epigenetics* (2015) 7:113
DOI 10.1186/s13148-015-0148-3

CLINICAL EPIGENETICS

REVIEW **Open Access**

DNA methylation changes of whole blood cells in response to active smoking exposure in adults: a systematic review of DNA methylation studies

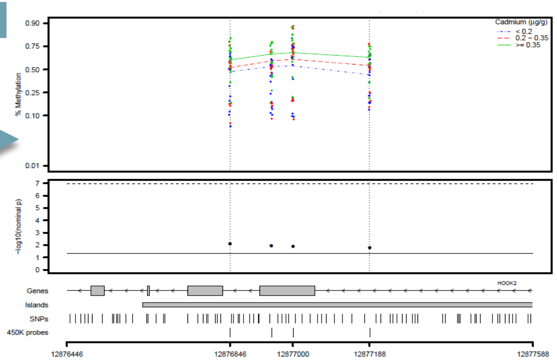
Xu Gao[1], Min Jia[1], Yan Zhang[1], Lutz Philipp Breitling[1] and Hermann Brenner[1,2,3*]

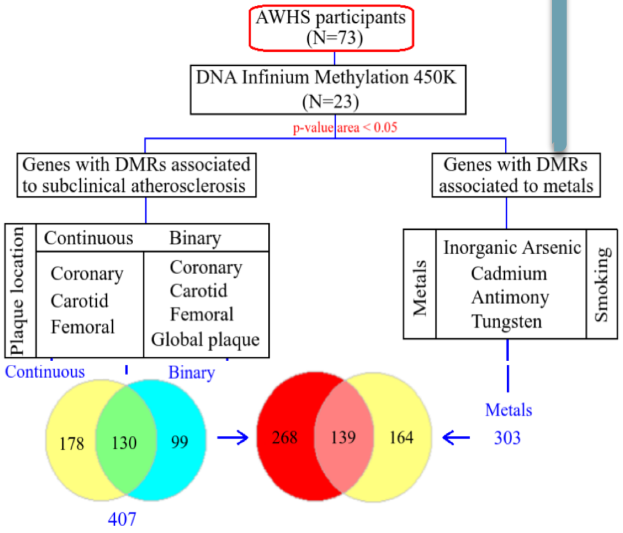
Abstract

Active smoking is a major preventable public health problem and an established critical factor for epigenetic modification. In this systematic review, we identified 17 studies addressing the association of active smoking exposure with methylation modifications in blood DNA, including 14 recent epigenome-wide association studies (EWASs) and 3 gene-specific methylation studies (GSMSs) on the gene regions identified by EWASs. Overall, 1460 smoking-associated CpG sites were identified in the EWASs, of which 62 sites were detected in multiple (≥3) studies. The three most frequently reported CpG sites (genes) in whole blood samples were cg05575921 (*AHRR*), cg03636183 (*F2RL3*), and cg19859270 (*GPR15*), followed by other loci within intergenic regions *2q37.1* and *6p21.33*.

Active smoking and DNA methylation in AHRR and F2RL3 in Infinium 450K

Estimation of differentially methylated regions (DMR) by metals and atherosclerosis





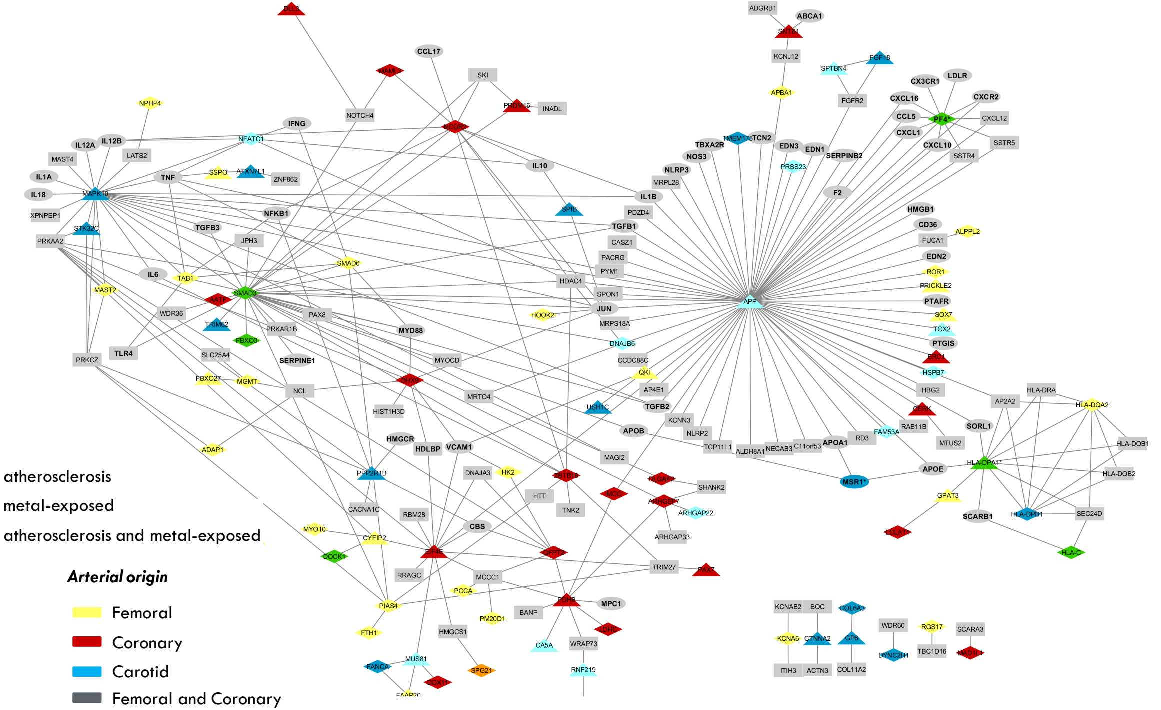
Enrichment analysis of atherosclerosis-DMRs using KEGG, Gene Ontology, PHARMGKB, SMPDB, TRRUST

- Pathways with False Discovery Rate (FDR) adjusted p-value < 0.05.

SOURCE	SET_NAME	SIZE	p-value	FDR(adj p-value)	
BEDPATHWAYS	HLA class II histocompatibility antigen	4	0.000119598	0.008075465	IMMUNE RESPONSE AND INFLAMMATION
BEDPATHWAYS	HLA class I histocompatibility antigen	3	0.000190809	0.008075465	IMMUNE RESPONSE AND INFLAMMATION
BEDPATHWAYS	Antigen processing and presentation	5	0.000210664	0.008075465	IMMUNE RESPONSE AND INFLAMMATION
KEGG	04612_Antigen processing and presentation	7	6.20523E-05	0.008625276	IMMUNE RESPONSE AND INFLAMMATION
BEDPATHWAYS	Notch signaling	5	0.00150387	0.024530078	
BEDPATHWAYS	Notch signaling inhibited	5	0.00150387	0.024530078	
BEDPATHWAYS	Notch signaling pathway	5	0.00150387	0.024530078	
BEDPATHWAYS	Hepatocyte glycolisis	4	0.00170644	0.024530078	ALTERED METABOLISM
BEDPATHWAYS	Prostate basal cell glycolysis (hsa1)	5	0.00303472	0.037766713	ALTERED METABOLISM
BEDPATHWAYS	Melanocyte Glycolysis (hsa1)	5	0.003284062	0.037766713	ALTERED METABOLISM
GOLOCATION	transport vesicle membrane	6	0.000209515	0.02459925	TRANSPORT
GOLOCATION	lysosomal membrane	14	0.000254475	0.02459925	
GOLOCATION	integral component of lumenal side of endoplasmic reticulum membrane	8	0.000656801	0.045217214	
GOLOCATION	trans-Golgi network membrane	6	0.000779607	0.045217214	

Source: Riffo-Campos et al. Philosophical Transactions B

Protein interaction networks of identified DMRs and atherosclerosis BED



Gene identification

- △ Differentially methylated in atherosclerosis
- ▭ Differentially methylated in metal-exposed
- ◇ Differentially methylated in atherosclerosis and metal-exposed

Type of variable (Outline)

- Binary
- Continuous
- Both

∗ Significantly atherosclerosis related protein

Bold Effectors

Arterial origin

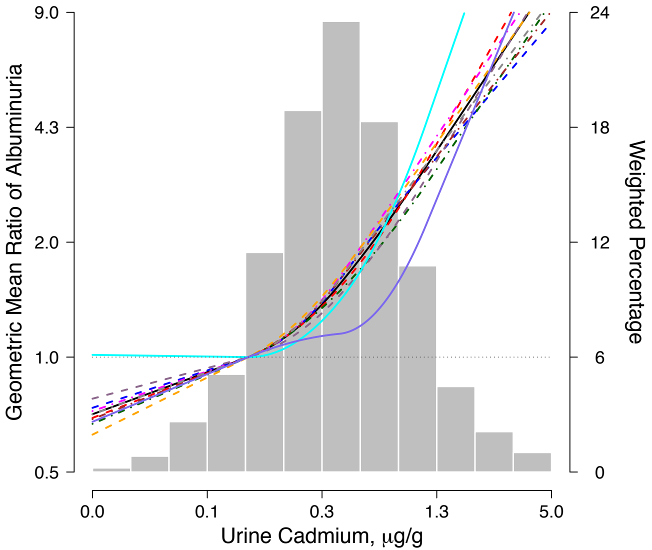
- Femoral
- Coronary
- Carotid
- Femoral and Coronary
- Femoral and Carotid
- Coronary and Carotid
- Femoral, Coronary and Carotid

- Hub proteins related to both atherosclerosis and metals-DMRs (e.g. SMAD3, NOP56...)
- Hub proteins related to metals-DMRs (e.g. SSTR5, HDAC4, AP2A2, CXCL12, SSTR4 ...)

Source: Riffo-Campos et al. Philosophical Transactions B

GENE-ENVIRONMENT INTERACTIONS: CADMIUM AND ALBUMINURIA

Objective: To test the hypothesis that carriers of specific genotypes are at increased metal-related effects. Gene-by-environment interactions can point to key pathways and provide biological insight



SLC30A4: Endosomal zinc transporter. No known role in albuminuria.

RAC1:Rho-family small GTP-ase, with a role in the maintenance of podocytes and proximal tubules integrity. Involved in ROS overproduction in endothelium and endothelial dysfunction

GMR of albuminuria levels comparing 80th to 20th percentile of cadmium distribution, by genotypes of SNPs with significant interactions at the Bonferroni level

SLC30A4	RAC1	N	GMR (95% CI)	P - int
rs3087816	rs4720672			<0.001
T/T + T/C (ref)	T/T (ref)	892	1.82 (1.65, 2.01)	
T/T + T/C (ref)	T/C + C/C	344	3.02 (1.85, 4.94)	
C/C	T/T (ref)	34	2.43 (2.07, 2.85)	
C/C	T/C + C/C	14	19.1 (8.02, 45.49)	

Source: Grau-Perez et al. Environment International 2017.

International Journal of Epidemiology, 2017, 1903–1912
doi: 10.1093/ije/dyx176
Advance Access Publication Date: 30 August 2017
Original article

Hazardous Substances

Declining exposures to lead and cadmium contribute to explaining the reduction of cardiovascular mortality in the US population, 1988–2004

Adrian Ruiz-Hernandez,[1,2] Ana Navas-Acien,[3–5] Roberto Pastor-Barriuso,[6,7] Ciprian M Crainiceanu,[8] Josep Redon,[1,2,9] Eliseo Guallar[3,5,10] and Maria Tellez-Plaza[2,4]*

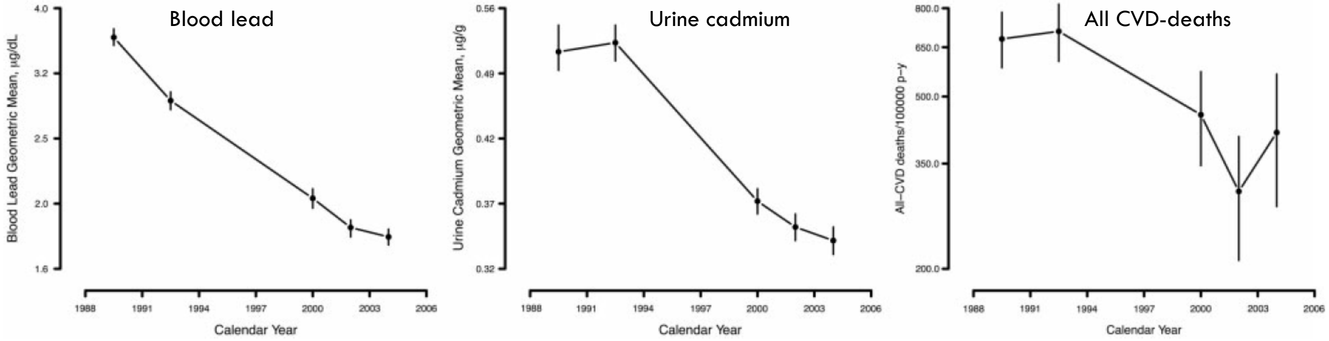
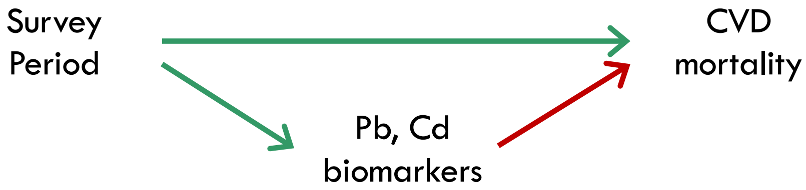


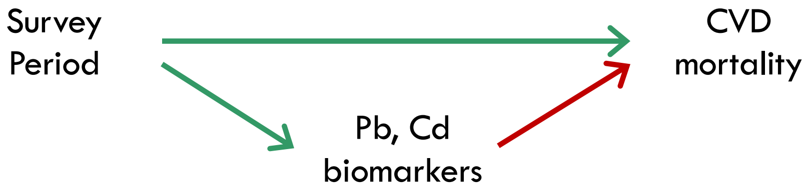
Figure 1. Age-, sex- and race-adjusted geometric mean blood lead and urine cadmium concentrations and cardiovascular disease (CVD) mortality rates across 1988–2004 National Health and Nutrition Examination Survey phases. Vertical bars show 95% confidence intervals based on 15 000 bootstrap re-samples.

Can the effect of period in CVD mortality be explained (i.e. mediated) by temporal changes in lead and cadmium exposure?

Can the effect of period in CVD mortality be explained (i.e. mediated) by temporal changes in lead and cadmium exposure?

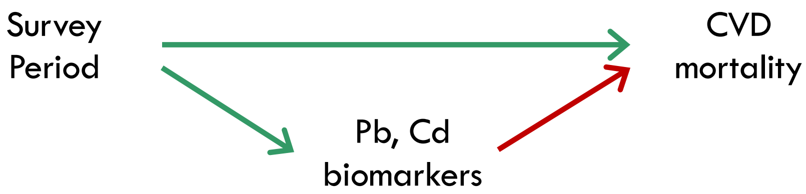


Can the effect of period in CVD mortality be explained (i.e. mediated) by temporal changes in lead and cadmium exposure?



- **Nested Aalen additive hazard models** for CVD deaths with the same set of confounders (age, sex, race, smoking status, physical activity, obesity, hypertension, diabetes, total cholesterol, low HDL cholesterol, lipid-lowering medication) **one adjusting for metals and one not** (Jiang and VanderWeele. AJE 2015;182:105-08; WanderWeele. Epidemiology 2011;22:582-85).

Can the effect of period in CVD mortality be explained (i.e. mediated) by temporal changes in lead and cadmium exposure?



- **Nested Aalen additive hazard models** for CVD deaths with the same set of confounders (age, sex, race, smoking status, physical activity, obesity, hypertension, diabetes, total cholesterol, low HDL cholesterol, lipid-lowering medication) **one adjusting for metals and one not** (Jiang and VanderWeele. AJE 2015;182:105-08; WanderWeele. Epidemiology 2011;22:582-85).

- Among **230.7 CVD deaths/100,000 person-year avoided** in the US comparing 1999-2004 to 1988-1994:
  - **52.0** deaths /**100,000 person-year (22.5% 95 CI [-96.7, -8.4 %])** were attributable to changes in lead and
  - **19.4** deaths /**100,000 person-year (8.4% 95 CI [-36.4, -4.3 %])** were attributable to cadmium
  - after adjustment for sociodemograhic, CVD risk factors and changes in medication use over the 2 periods

International Journal of Epidemiology, 2017, 1903–1912
doi: 10.1093/ije/dyx176
Advance Access Publication Date: 30 August 2017
Original article

Hazardous Substances

Declining exposures to lead and cadmium contribute to explaining the reduction of cardiovascular mortality in the US population, 1988–2004

Adrian Ruiz-Hernandez,[1,2] Ana Navas-Acien,[3–5]
Roberto Pastor-Barriuso,[6,7] Ciprian M Crainiceanu,[8] Josep Redon,[1,2,9]
Eliseo Guallar[3,5,10] and Maria Tellez-Plaza[2,4]*

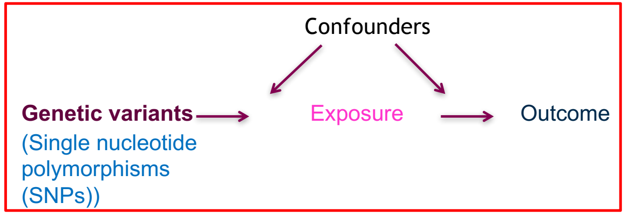
Key Messages

- Blood lead and urine cadmium have been associated with a broad range of cardiovascular endpoints in multiple epidemiologic studies. However, the contribution of lead and cadmium changes over time to cardiovascular mortality trends has not been formally investigated.
- Our findings suggest that reducing lead and cadmium exposures may be an overlooked public health achievement by preventing a substantial amount of cardiovascular deaths in the USA.
- Since both metals remain associated with cardiovascular disease at relatively low levels of exposure, primary prevention strategies minimizing avoidable lead and cadmium exposures could further contribute to the prevention and control of cardiovascular disease in general populations.

Study Designs to Further Support the Causality of Metals

Some ongoing work

Mendelian randomization studies: Instrumental variable analysis with genetic instruments



(Davies-Smith G, Ebrahim S., 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?, IJE, 2003)

Metals *associated* with cardiovascular disease, **do metals *cause* cardiovascular disease?**

Check whether people with genetically elevated metal biomarker levels have more cardiovascular disease

- Likelihood-based Bayesian methods: Discovery-Replication approach using Individual data from cardiovascular cohorts

- Meta-analysis-like MR methods: Increasingly popular using publically available genome wide association studies (GWAS) with separate samples or UK Biobank GWAS

STRONG HEART STUDY

N=4,549 (45 -74 years)

Illumina Metabochip (N=~3000)

ortega

ARAGON WORKERS' HEALTH STUDY

Next Gen. Sequencing (N=~3000)

Initial Report on Na_2EDTA Chelation for

TREATMENT OF ANGINA PECTORIS WITH DISODIUM ETHYLENE DIAMINE TETRAACETIC ACID*†

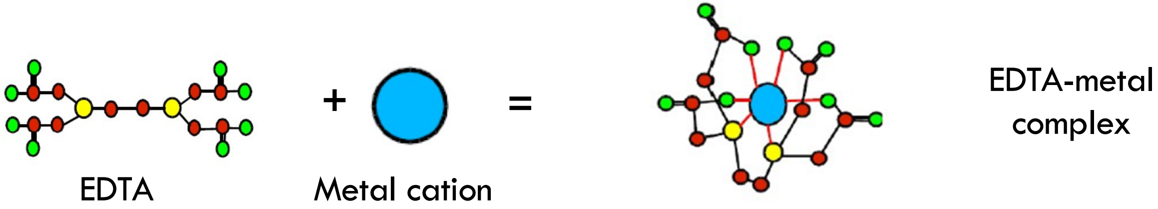
By Norman E. Clarke, M.D.

Charles N. Clarke, M.D.

and

Robert E. Mosher, Ph.D.

(From the Department of Research, Providence Hospital, Detroit, Michigan)



Clarke et al. *Am J Med Sci.* 1956;654

Effect of Disodium EDTA Chelation Regimen on Cardiovascular Events in Patients With Previous Myocardial Infarction

The TACT Randomized Trial

Gervasio A. Lamas, MD
Christine Goertz, DC, PhD
Robin Boineau, MD, MA
Daniel B. Mark, MD, MPH
Theodore Rozema, MD
Richard L. Nahin, PhD, MPH
Lauren Lindblad, MS
Eldrin F. Lewis, MD, MPH
Jeanne Drisko, MD
Kerry L. Lee, PhD
for the TACT Investigators

Importance Chelation therapy with disodium EDTA has been used for more than 50 years to treat atherosclerosis without proof of efficacy.

Objective To determine if an EDTA-based chelation regimen reduces cardiovascular events.

Design, Setting, and Participants Double-blind, placebo-controlled, 2×2 factorial randomized trial enrolling 1708 patients aged 50 years or older who had experienced a myocardial infarction (MI) at least 6 weeks prior and had serum creatinine levels of 2.0 mg/dL or less. Participants were recruited at 134 US and Canadian sites. Enrollment began in September 2003 and follow-up took place until October 2011 (median, 55 months). Two hundred eighty-nine patients (17% of total; n=115 in the EDTA group and n=174 in the placebo group) withdrew consent during the trial.

Interventions Patients were randomized to receive 40 infusions of a 500-mL chelation solution (3 g of disodium EDTA, 7 g of ascorbate, B vitamins, electrolytes, procaine, and heparin) (n=839) vs placebo (n=869) and an oral vitamin-mineral regimen vs an oral placebo. Infusions were administered weekly for 30 weeks, followed by 10 infusions 2 to 8 weeks apart. Fifteen percent discontinued infusions (n=38 [16%] in the chelation group and n=41 [15%] in the placebo group) because of adverse events.

Main Outcome Measures The prespecified primary end point was a composite of total mortality, recurrent MI, stroke, coronary revascularization, or hospitalization for angina. This report describes the intention-to-treat comparison of EDTA chelation vs placebo. To account for multiple interim analyses, the significance threshold required at the final analysis was *P*=.036.

Results Qualifying previous MIs occurred a median of 4.6 years before enrollment. Median age was 65 years, 18% were female, 9% were nonwhite, and 31% were diabetic. The primary end point occurred in 222 (26%) of the chelation group and 261 (30%) of the placebo group (hazard ratio [HR], 0.82 [95% CI, 0.69-0.99]; *P*=.035). There was no effect on total mortality (chelation: 87 deaths [10%]; placebo, 93 deaths [11%]; HR, 0.93 [95% CI, 0.70-1.25]; *P*=.64), but the study was not powered for this comparison. The effect of EDTA chelation on the components of the primary end point other than death was of similar magnitude as its overall effect (MI: chelation, 6%; placebo, 8%; HR, 0.77 [95% CI, 0.54-1.11]; stroke: chelation, 1.2%; placebo, 1.5%; HR, 0.77 [95% CI, 0.34-1.76]; coronary revascularization: chelation, 15%; placebo, 18%; HR, 0.81 [95% CI, 0.64-1.02]; hospitalization for angina: chelation, 1.6%; placebo, 2.1%; HR, 0.72 [95% CI, 0.35-1.47]). Sensitivity analyses examining the effect of patient dropout and treatment adherence did not alter the results.

Conclusions and Relevance Among stable patients with a history of MI, use of an intravenous chelation regimen with disodium EDTA, compared with placebo, modestly reduced the risk of adverse cardiovascular outcomes, many of which were revascularization procedures. These results provide evidence to guide further research but are not sufficient to support the routine use of chelation therapy for treatment of patients who have had an MI.

Trial Registration clinicaltrials.gov Identifier: NCT00044213

JAMA. 2013;309(12):1241-1250 www.jama.com

TREATMENT OF LEAD TOXICITY with chelation was first reported with EDTA in the early 1950s.[1] Apparent success in reducing metastatic calcium deposits[2] led Clarke et al[3] in 1956 to treat angina patients with EDTA, and others to use chelation for various forms of atherosclerotic disease.[4-6] Chelation therapy evolved to constitute infusions of vitamins and disodium EDTA, a drug that binds divalent and some trivalent cations, including calcium, magnesium, lead, cadmium, zinc, iron, aluminum, and copper, facilitating their urinary excretion.[7,8]

Over the next decades, based on favorable anecdotal and case report experience, chelation practitioners increased their use of EDTA for coronary and peripheral artery disease. The 2007 National Health Statistics Report compared chelation use since 2002 and noted

For editorial comment see pp 1291 and 1293.

Author Video Interview available at www.jama.com.

Author Affiliations are listed at the end of this article.
A complete list of the TACT Investigators appears in the eAppendix.

Corresponding Author: Gervasio A. Lamas, MD, Columbia University Division of Cardiology, Mount Sinai Medical Center, 4300 Alton Rd, Miami Beach, FL 33140 (gervasio.lamas@msmc.com).

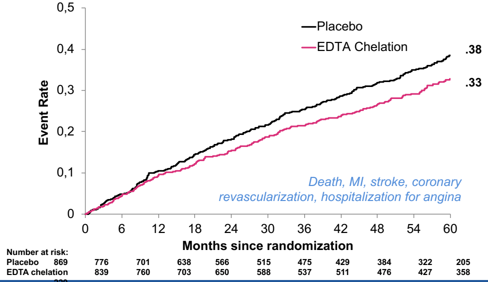
Gervasio (Tony) Lamas
Mount Sinai Medical Center
Miami, USA, TACT2 PI

EDTA: Placebo

HR (95% CI)
0.82 (0.69, 0.99)

P = 0.035

TACT Primary Endpoint Results



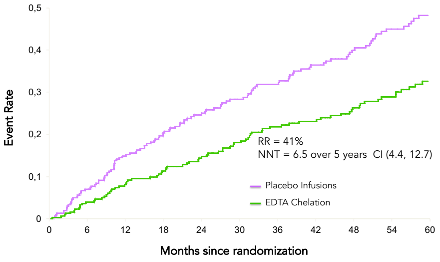
Lamas G, Goertz C, Boineau R et al. Effect of Disodium EDTA Chelation Regimen on Cardiovascular Events in Patients with Previous Myocardial Infarction. *JAMA*. 2013;309(12):1246.

With Diabetes:

HR (95% CI)
0.59 (0.44, 0.79)

P = 0.002
(Bonferroni adjusted)

TACT Results in Participants with Diabetes



OPPORTUNITIES FOR CAUSAL INFERENCE IN METAL RESEARCH IN THE CONTEXT OF CHELATION TRIALS

Ana Navas-Acien

TACT2

TRIAL TO ASSESS CHELATION THERAPY

- Replicative trial of EDTA chelation and high-dose oral vitamins in 1200 post-MI diabetic patients
- Storing biospecimens for measuring metals and testing future mechanistic hypotheses

CONCLUSION: IS IT TIME FOR RECOGNITION?

Environmental metals and cardiovascular disease

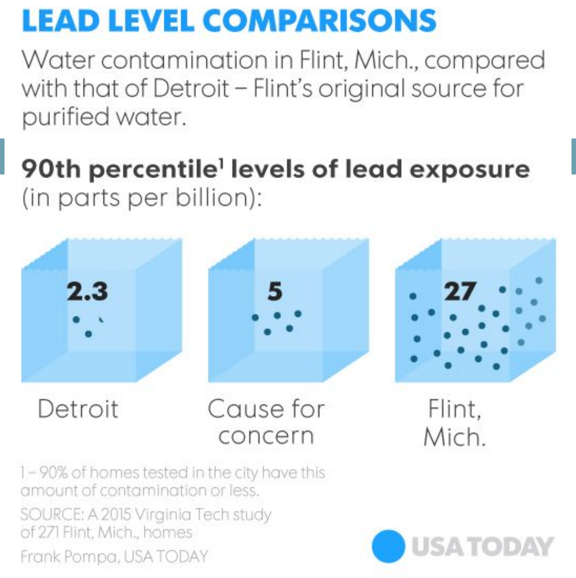
BMJ 2018 ; 362 doi: https://doi.org/10.1136/bmj.k3435 (Published 29 August 2018)
Cite this as: *BMJ* 2018;362:k3435

Maria Tellez-Plaza 1, scientist,Eliseo Guallar 2, professor,Ana Navas-Acien 3, professor

Exposure to environmental metals remains substantial because of widespread soil contamination; persistence of past uses (house paint and plumbing for lead); continuing industrial uses (plastics and batteries); and presence in tobacco and tobacco smoke, drinking water and ambient air, and dust near industrial sources and waste sites.[7] [8] Cadmium content in fertilizers provide an additional exposure pathway through diet and tobacco since vegetables and grains bioconcentrate cadmium. Emerging tobacco products such as electronic cigarettes also increase metal exposure.[11] The main source of metals in electronic cigarettes seems to be the heating coil, from where metals leach into the inhaled aerosol. [11] In low and middle income countries, including many countries in Africa and Asia, exposure to high levels of arsenic and lead is still a serious threat to public health that requires urgent action.[12] [13] [14]

Despite widespread distribution of toxic metal contaminants, technical reports from environmental and public health agencies often disregard the mounting evidence of associated cardiovascular risk.[15] [16] Similarly, metal exposures are neglected by the organizations that produce cardiovascular prevention guidelines. The evidence indicates a clear need to minimize unnecessary metal exposures.

Flint Water Crisis



CONCLUSION: IS IT TIME FOR RECOGNITION?

Environmental metals and cardiovascular disease

BMJ 2018 ; 362 doi: https://doi.org/10.1136/bmj.k3435 (Published 29 August 2018)
Cite this as: *BMJ* 2018;362:k3435

Maria Tellez-Plaza 1, scientist,Eliseo Guallar 2, professor,Ana Navas-Acien 3, professor

Exposure to environmental metals remains substantial because of widespread soil contamination; persistence of past uses (house paint and plumbing for lead); continuing industrial uses (plastics and batteries); and presence in tobacco and tobacco smoke, drinking water and ambient air, and dust near industrial sources and waste sites.[7] [8] Cadmium content in fertilizers provide an additional exposure pathway through diet and tobacco since vegetables and grains bioconcentrate cadmium. Emerging tobacco products such as electronic cigarettes also increase metal exposure.[11] The main source of metals in electronic cigarettes seems to be the heating coil, from where metals leach into the inhaled aerosol. [11] In low and middle income countries, including many countries in Africa and Asia, exposure to high levels of arsenic and lead is still a serious threat to public health that requires urgent action.[12] [13] [14]

Despite widespread distribution of toxic metal contaminants, technical reports from environmental and public health agencies often disregard the mounting evidence of associated cardiovascular risk.[15] [16] Similarly, metal exposures are neglected by the organizations that produce cardiovascular prevention guidelines. The evidence indicates a clear need to minimize unnecessary metal exposures.

- Technical reports disregard mounting evidence pointing to the potentially causal cardiovascular effects of metals

DE GRUYTER

Pure Appl. Chem. 2018; 90(4): 755–808

IUPAC Technical Report

Gunnar F. Nordberg[a,*], Alfred Bernard[a], Gary L. Diamond[a], John H. Duffus[a], Paul Illing[a], Monica Nordberg[b], Ingvar A. Bergdahl[b], Taiyi Jin[b] and Staffan Skerfving[b]

Risk assessment of effects of cadmium on human health (IUPAC Technical Report)

https://doi.org/10.1515/pac-2016-0910
Received September 12, 2016; accepted October 20, 2017

DIRECTORATE-GENERAL FOR INTERNAL POLICIES

POLICY DEPARTMENT A
ECONOMIC AND SCIENTIFIC POLICY

Economic and Monetary Affairs

Employment and Social Affairs

Environment, Public Health and Food Safety

Industry, Research and Energy

Internal Market and Consumer Protection

Scientific aspects underlying the regulatory framework in the area of fertilisers - state of play and future reforms

In-Depth Analysis for the IMCO Committee

EN

2017

CONCLUSION: IS IT TIME FOR RECOGNITION?

Environmental metals and cardiovascular disease

BMJ 2018 ; 362 doi: https://doi.org/10.1136/bmj.k3435 (Published 29 August 2018)
Cite this as: *BMJ* 2018;362:k3435

Maria Tellez-Plaza 1, scientist,Eliseo Guallar 2, professor,Ana Navas-Acien 3, professor

Exposure to environmental metals remains substantial because of widespread soil contamination; persistence of past uses (house paint and plumbing for lead); continuing industrial uses (plastics and batteries); and presence in tobacco and tobacco smoke, drinking water and ambient air, and dust near industrial sources and waste sites.[7 8] Cadmium content in fertilizers provide an additional exposure pathway through diet and tobacco since vegetables and grains bioconcentrate cadmium. Emerging tobacco products such as electronic cigarettes also increase metal exposure.[11] The main source of metals in electronic cigarettes seems to be the heating coil, from where metals leach into the inhaled aerosol.[11] In low and middle income countries, including many countries in Africa and Asia, exposure to high levels of arsenic and lead is still a serious threat to public health that requires urgent action.[12 13 14]

Despite widespread distribution of toxic metal contaminants, technical reports from environmental and public health agencies often disregard the mounting evidence of associated cardiovascular risk.[15 16] Similarly, metal exposures are neglected by the organizations that produce cardiovascular prevention guidelines. The evidence indicates a clear need to minimize unnecessary metal exposures.

ACKNOWLEDGEMENTS

Ana Navas-Acien, Columbia University

Eliseo Guallar, Johns Hopkins University

Josep Redón, IIS INCLIVA

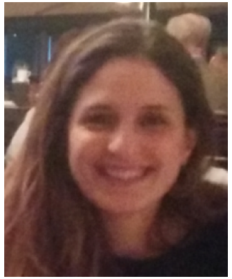
J. Carlos Martin Escudero, Hospital Rio Hortega

J. Antonio Casasnovas, IACS

Beatriz Pérez, Área CVD, CNE

Iñaki Galán, Área CVD, CNE

Roberto Pastor, CNE



Maria Grau-Perez, IIS INCLIVA

M. Jose Caballero, IIS INCLIVA

Cristina López, IIS INCLIVA

Arce Domingo, Columbia University

Ángela Riffo-Campos, IIS INCLIVA

Pablo Olmedo-Palma, Universidad de Granada

Miguel Herreros, IIS INCLIVA

Alex Dominguez, IIS INCLIVA