1.a. Full Title: Heavy metal exposure and carotid atherosclerosis in the CLUE II / ARIC study

b. Abbreviated Title (Length 26 characters): Heavy metals and atherosclerosis

2. Writing Group (list individual with lead responsibility first):

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3. Timeline:

   This project will be submitted for funding to the Johns Hopkins Center for a Livable Future and other funding organizations. The timeline will be 2 years from the start of funding.

4. Rationale:

   Heavy metals are highly reactive compounds typically associated with important toxic effects at high doses of exposure. The effect of chronic low exposure to many heavy metals, especially in relation to cardiovascular disease, has seldom been studies. We are particularly interested in the cardiovascular effects of mercury and arsenic, for which there is some evidence as well as a mechanistic basis to suspect that they may be related to cardiovascular risk.

   Mercury is a persistent pollutant that comes from natural and anthropogenic sources [1]. Exposure to elemental mercury occurs mainly through inhalation in occupational settings and from mercury-containing dental fillings [1]. Exposure to inorganic mercury compounds comes from diet but is limited by a low absorption [1]. Also, fish and marine product consumption is an important source of methylmercury exposure population-wide. Mercury may increase oxidative stress and influence the development of atherosclerosis in several ways. Since mercury is a transition metal, it may promote the production of free radicals, and several experimental models have shown mercury-related generation of free radicals. Mercury binds selenium forming mercury selenide, an insoluble complex that cannot serve
as a cofactor for glutathione-peroxidase (an important scavenger of hydrogen and lipid peroxides) [2,3]. In addition, methylmercury has very high affinity for thiol-containing proteins, peptides and amino-acids, and it inactivates the anti-oxidant properties of glutathione, catalase, and superoxide dismutase.

Mercury intake from fish has been associated with an excess risk of cardiovascular disease in Eastern Finland, and this finding has been related to the promotion of lipid peroxidation [4,5] and to an accelerated progression of carotid atherosclerosis [6]. A similar association was found in the EURAMIC study, a large case-control study realized in several European countries and Israel [7]. Finally, a small nested-case control study in Sweden did not find an increased risk of cardiovascular disease with increased levels of erythrocyte methylmercury, but the high levels of plasma phospholipids ω3 fatty acids found in this population limits the generalizability of their findings [8]. These studies also indicate the need to assess ω3 fatty acid levels in addition to mercury levels to fully understand the effect of mercury derived from fish intake on cardiovascular risk.

Arsenic is found naturally in the environment as elemental, inorganic or organic compounds [9]. Occupational exposure to arsenic dust is an important source of exposure, with an estimated 1.5 millions workers potentially exposed to arsenic in the USA [10]. In the general population, the main sources of arsenic exposure are drinking water and food [9]. In most populations, characterized by low arsenic levels in drinking water, food is the main source of exposure, particularly organic arsenic compounds from fish and seafood [9]. Both organic and inorganic arsenic are classified as carcinogenic substances by the International Agency for Research on Cancer [10,11]. Animal and in vitro studies support the possibility of a causal association between arsenic and cardiovascular diseases. Arsenic induces arterial thrombosis and platelet aggregation [12]. Other possible mechanisms involve a persistent oxidative stress [13] and the development of hypertension associated to chronic arsenic exposure [14,15].

The association of arsenic with cardiovascular diseases has been clearly established in areas with relatively high levels of arsenic in drinking water [16-18], as well as in occupational settings [19,20]. In the United States, an increased risk of mortality for diseases of the arteries, arterioles and capillaries was observed for counties exceeding 20 µg/l of arsenic in public drinking water supplies in an ecological study involving 30 US counties [21]. Less information is available on the effect of low chronic exposure or with sources of exposure different from drinking water or occupational dusts.

Long-term exposure to arsenic and mercury can be assessed measuring the levels of these metals in toenails. This method has shown a good correlation with global exposure, independently of the source, and with other sites of assessment, such as hair or urine measurements [22]. Consequently, the CLUE II and ARIC cohorts represent a unique opportunity to study the effect of mercury and arsenic with arteriosclerosis in a general population not characterized by high exposure levels.
5. Main Hypothesis/Study Questions:

- Carotid intima media thickness (IMT) and its progression over follow-up are associated with:
  - Higher arsenic exposure, measured as toenail arsenic.
  - Higher mercury exposure, measured as toenail mercury.
- The associations of mercury and arsenic with IMT and its progression are independent of established coronary risk factors and of dietary factors.

6. Data (variables, time window, source, inclusions/exclusions):

All necessary ARIC data are already available. As part of the study, toenail heavy metals and plasma fatty acids will be analyzed in samples from the CLUE II specimen bank. All the analyses will be restricted to study subjects in Washington County who participated both in ARIC and CLUE II. Data analysis will be performed by Ana Navas and Eliseo Guallar at Johns Hopkins.

The variables of interest in the proposed study include:
- Main outcome: progression of IMT
- Main exposures: mercury and arsenic levels in toenails
- Other covariates: sociodemographic and cardiovascular risk factors, and dietary intake data, plasma fatty acids (from CLUE II samples).

7.a. Will the data be used for non-CVD analysis in this manuscript? _____ Yes  ____x__ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? _____ Yes  ____ No
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? _____ Yes  ____x__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”? _____ Yes  ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: [http://bios.unc.edu/units/cscc/ARIC/stdy/studymem.html](http://bios.unc.edu/units/cscc/ARIC/stdy/studymem.html)

_____x_____ Yes  _______ No
References