ORIGINAL ARTICLE

Relationship between lead exposure and mild cognitive impairment

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Keywords

Neurobehavioral effects • Lead exposed workers blood lead level • Low lead exposure • ALAD • ZPP

Summary

Introduction. *Since it is still controversial whether-low-to moderate long-term lead below current threshold values causes neurobehavioural deficits in adults.*
Methods. *Forty lead-exposed workers subjects with a mean blood lead (PbB) level of 56.4 μg/dL and 40 non-lead-exposed aged matched subjects (PbB: 15.4 μg/dL) with the same socio-economic background were investigated. Participants were administered a neuropsychological tests consisting of BAMT (Branches Alternate Movements Task), FT (Finger Tapping Speed), DS (Digit Span) POMS (Profile of Mood States).*
Results. *Authors noted a significant relationship between the exposed and the referent groups in tests mainly involving executive functions, short time memory and psycho-emotional variables. In addition, Poisson regression test performed on single psycho-emotional factors (POMS), has allowed to evidence a significant influence of Pb e ZPP levels on tension, anxiety and depression.*
Conclusions. *The present study showed that lead exposure among adults at levels previously considered safe, results in impairment of certain cognitive abilities.*

Introduction

Lead (Pb) is a metal with many important industrial uses. Occupational exposure to lead can produce toxic effects on multiple organ systems including renal dysfunction, hematopoietic diseases, neurocognitive and reproductive disorders. Although occupational exposure to this neurotoxic agent has declined steadily over the past 20 years, the presence of lead in occupational settings continues to be a source of both acute and chronic exposure, resulting in blood levels ranging 40 to 120 μg/dL, as demonstrated by the Agency for Toxic Substances and Disease Registry [1].
Several studies showed an association between lead and cognitive abilities in children at blood levels even below 10 μg/dL without evidence of a safe lower threshold [2]. In 2015, United States National Institute for Occupational Safety and Health (NIOSH) indicated 5 μg/dL as the reference blood lead level (PbB) for adults. Nonetheless, the U.S. Occupational Safety and Health Administration (OSHA) recommends to remove workers from lead exposure when PbB is above 60 μg/dL and readmit them when it is below 40 μg/dL. Moreover, the American Conference of Governmental Industrial Hygienists (ACGIH) suggests a biological exposure index of 30 μg/dL for workers.
Workers exposed to lead often show impaired performance on neurobehavioral test involving attention, processing, speed, visuospatial abilities, working memory and motor function. It has also been suggested that lead can adversely affect general intellectual performance [3]. Exposure to inorganic lead in the environmental and occupational settings continues to be a serious public health problem. At high exposure levels, lead causes encephalopathy, kidney damage, anaemia and toxicity to the reproductive system. Even at lower doses, lead produces alterations in cognitive development in children and adults. A really safe level for lead exposure has not been defined, as health risks associated with this metal have been shown even at very low doses [4].
Recent meta–analyses reported worse neurobehavioral performances for exposed than unexposed workers with PbB levels lower than 50-60 μg/dL. The authors concluded that none of the individual studies were conclusive or adequate in providing information on the effects of lead on cognitive function [5-7]. Furthermore, mild cognitive impairment (MCI) is considered to be a high-risk state for developing dementia with about 50% of MCI patients progressing to dementia [8]. However, several lines of evidence have now suggested that environmental exposure to lead may play an important role. A recent study investigating cumulative lead exposure and cognitive function in adult men reported that the degree of performance impairment over time, particularly in visuospatial and visuomotor domains, increased with increasing bone lead concentration, a marker of cumu-

lative exposure [9]. Recent animal studies report that early developmental lead exposure in rodents resulted in an age-related elevation in amyloid precursor protein (APP) and its amyloidogenic Aβ product, markers of Alzheimer's Disease (AD) [10, 11] and over-expression of the β-amyloid protein precursor gene 20 months after exposure had ceased. Subsequent studies in non-human primates that were exposed to lead during development have shown similar effects [12]. Furthermore, lead may be indirectly linked to dementia through its demonstrated hypertensive effect [13-16], a risk factor considered to play an important role in the development of dementia [17]. In addition, lead could act on neurotransmission, such as the acetylcholine system which is known to be compromised in AD [18, 19]. One limitation of the current understanding of the potential risk posed by lead exposure for dementias or MCI is the lack of information on the specific behavioural profile with which lead may be associated. In particular, it is unknown whether lead exposure reproduces the specific behavioural deficits, many of which can also be directly evaluated in experimental animal models, that have proven to be predictive of dementias in human. The present study was conducted to evaluate the association between occupational lead exposure and MCI using biological markers and validated behavioural measures.

Materials and methods

The present study was carried out at the Occupational Health Institute, Medical School, University of Messina, Italy.

Forty male workers, employed in a battery recycling plant placed in Messina, Italy, responders to an invitation to participate in the health surveillance program, fulfilled the inclusion criteria for the present study.

Inclusion criteria were: living in Messina metropolitan area, working in the battery storage plant for at least 5 h/day, willing and able to attend required study visits, lack of any systemic disease. Workers under medication with both cerebro-active drugs and any other substances able to interfere with neuro-behavioural performances were excluded from the study.

A total of forty workers, with mean age of 37.15 years (SD ± 8.09), matched the inclusion criteria. The control group included forty healthy male subjects with no present or past exposure to lead, age-matched, chosen from people working in several offices located in the urban area of Messina. Informed consent was obtained from workers.

All participants were interviewed by well-trained occupational physicians, and information about socio-demographic characteristics, disease history, alcohol consumption, cigarette smoking, dietary patterns (ethnic products intake), residential area (presence of nearby industries or factories), occupational history (of the last 3 years for possible lead-exposing occupation) were gathered.

Cognitive and behaviour measures were administered to workers by a specialist in clinical psychology after the working shift, in a standardized environment and using uniform procedures.

The evaluation of both biomarkers of exposure and effect (blood lead, PbB; aminolevulinate dehydratase, ALAD; Zn protoporphirin, ZPP; haemoglobin, Hb) and psychological tests in the exposed workers with respect to non exposed subjects was performed.

Environmental assessment of workplace lead levels was given by factory management and was over the threshold limit value of 0.05 mg/m^3 set by the ACGIH [20].

Biological monitoring

Venous blood samples were taken for the determination of lead dose (PbB) and effect (Hb, ALAD and ZPP) biomarkers.

The whole blood specimens were collected using a lead-free heparinized evacuated tubes. Blood samples were stored at +4°C until the analysis, which was performed within 2 weeks.

Psycho-diagnostic protocol

BAMT (Branches Alternate Movements Task) was performed on all subjects to assess motor coordination [21]. Subjects alternatively touch their knees crossing arms and the sequence is repeated alternatively for 30 seconds.

FT (Finger Tapping) speed measures the maximum number of repetitive movements made beating as quickly as possible a button with the index finger, holding the arm supported in a fixed position and comfortable and alternating hands (dominant/non-dominant) for a total of 6 tests in 10 seconds [22].

DS (Digit Span), a simple traditional evaluation of short term memory: a series of numbers, each time increasing in length, is repeated forwards and in reverse order. Subtest is on the basis of correct answers [23].

Profile of Mood States (POMS), administrable to adults with compulsory education in a maximum range of time of 10 minutes, in the Italian version is made up of 58 items that define the six factors of mood [24]:

- Tension-Anxiety = T
- Depression-Dejection = D
- Anger-Hostility = A
- Vigor-Activity = V
- Fatigue-Inertia = F
- Confusion-Bewilderment = C

To get the score of each of the six factors, the scores of the single answers to each single item that define the score itself are added to every item. 0,1,2,3 or 4 points are given except for the two terms "relaxed" in the scale Confusion-Bewilderment that must be inverted in the assignment result (4,3,2,1 or 0). The factor Vigor-Activity is evaluated with a negative sign and referred to male sex. The rough scores are converted into standard ones.

Tab. I. Socio-demographic characteristics and biomarkers of lead exposure and effect in exposed and non-exposed workers.

	Exposed	Controls	p <
Population under study	40	40	NS
Age (years, mean ± SD)	37.15 ± 8.09	37.3 ± 8.1	NS
Education (years, mean ± SD)	15.3 ± 2.73	12.0 ± 1.7	NS
Seniority (years, mean ± SD)	4.3 ± 2.05	4.6 ± 1.8	NS
Alcohol consumption			
None	0 (0)	0 (0)	NS
At least 1 drink a day	40 (100%)	40 (100%)	
Smoking habits			
Never	10 (25%)	8 (20%)	NS
Current	24 (60%)	26 (70%)	NS
Former	6 (15%)	4 (10%)	NS
PbB (µg/dL, mean ± SD)	56.4 ± 14.4	15.4 ± 1.5	0.005
ZPP (µg/dL, mean ± SD)	53.8 ± 22.9	23.4 ± 1.4	0.005
ALAD (u/ml, mean ± SD)	28.2 ± 9.4	58.0 ± 1.9	0.005

Tab. II. Comparison of neuro-behavioural and psycho-emotional variables (mean ± SD of score) between exposed and non exposed workers.

	Exposed	Controls	p <
NEURO-BEHAVIORAL VARIABLES			
BAMT	3.8 ± 1.1	4.4 ± 0.5	0.005
Fingers	3.8 ± 1.0	4.7 ± 0.5	0.005
Direct Digitation	5.5 ± 0.8	5.5 ± 0.8	NS
Inverse Digitation	3.5 ± 0.7	4.9 ± 0.4	0.005
PSICO-EMOTIONAL VARIABLES			
Tension	58.3 ± 9.1	50.6 ± 6.7	0.005
Depression	57.0 ± 10.6	48.3 ± 7.2	0.005
Aggressiveness	54.2 ± 9.9	47.2 ± 7.9	0.005
Vigor	57.0 ± 6.1	68.2 ± 3.7	0.005
Tiredness	61.2 ± 11.0	50.4 ± 7.3	0.005
Confusion	62.6 ± 11.4	51.5 ± 7.4	0.005

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science the Methodological S.R.L. NPC Test [25, 26]; the Minitab Release 13.31 [27] and R 2.1.1 [28] for the estimation of Poisson regression.

Descriptive variables were evaluated for differences between means for continuous variables and with non parametric analysis for not continuous variables.

The differences of both lead exposure indices and psychological tests between the group under study and the control group were analysed by Student's unpaired *t* test. Furthermore, correlation between lead exposure biomarkers and seniority and Hb level were tested using Pearson's linear correlation test.

In order to analyze the influence of lead exposure on neurobehavioral test (BAMT, FT, DS with Direct and Inverse Digitations) a multivariate ordinal logistic regression was performed because the scores of the neurobehavioral tests constitute ordinal categorical variables.

The influence of exposure to lead on the performance levels of each psycho-emotional test (POMS) was tested through the estimation of a generalized linear model of Poisson because the scores of the POMS are discreet and not negative. The fixed level of significance for the whole statistical analysis was $p < 0.005$.

Results

The socio-demographic characteristics and biomarkers of exposed and non exposed workers are shown in Table I. PbB and ZPP mean levels were significantly higher in exposed than in non exposed workers. As expected, the mean value of ALAD was significantly lower in exposed than non exposed workers. A PbB level higher than the threshold limit value (60 g/dL) was found in18 (45%) of the exposed workers. Current blood lead level (PbB) of the exposed workers ranged from 24 to 76 µg/dL. PbB of the controls ranged from 13 to 18 µg/dL.

Regarding to considered whole psycho-emotional variables, the authors evidenced significant differences between the two groups. The results of neuroemotional variables are showed in Table II. The values of tension, depression, aggressiveness, tiredness and confusion, resulted higher in the exposed workers than the controls. An inverse direction was found for the vigour, that resulted higher for the controls with respect to the exposed workers (Tab. II).

Tab.III. Pearson correlation test between biomarkers of lead exposure and effect vs seniority and Hb.

	PbB	ALAD	ZPP
Hb	0.153	-0.459*	-0.264
Seniority	-0.354**	0.076	-0.320**

* p < 0.01 **p < 0.05

Tab. IV. Ordinal Logistic Regression between biomarkers of Pb exposure and effect vs neurobehavioral variables.

	PbB	ALAD	ZPP
BAMT	-0.223 *	0.025	-0.018
FINGER	-0.008	-0.149	-0.395
D Digitation	0.558*	0.068	0.401*
I Digitation	0.031	0.032	0.002

* p < 0.005

Tab. V. Poisson regression coefficients between biomarkers of Pb exposure and effect vs psycho-emotional variables (POMS test).

POMS variables	PbB	ALAD	ZPP
T	*0.011	0.004	*0.007
D	*0.012	*0.017	*0.008
A	0.001	0.010	0.004
V	0.006	0.002	0.003
S	-00.001	0.004	0.002
C	0.003	0.005	0.002

* p < 0.005

Table III shows correlation between biomarkers of lead exposure and effect and both seniority and Hb concentration in the groups under study. Seniority was inversely correlated to the PbB level while ZPP was negatively correlated to the working age.

The estimation of ordinal logistic regression model (gompit was the used link function) has allowed to evaluate the influence of some variables on the scores obtained by neurobehavioral tests. Table IV reports the estimation of two models of ordinal logistic regression: in the first model the answer variable is BAMT, that implies a score ranging from 1 to 5. In the exposed subjects it assumes the value 1 in 2 cases (5%), value 2 in 2 cases (5%), value 3 in 10 cases (25%), 4 in 14 cases (35%), 5 in 12 cases (30%), so that the presence of four constants was evidenced in the model.

In the second model the answer variable is FINGERS, that requires a score ranging from 1 to 6. In the exposed group it assumes the value 1 in any case (0%), value 2 in 4 cases (10%), value 3 in 14 cases (35%), value 4 in 10 cases (25%), value 5 in 12 cases (30%);no case with value 6 was found. Therefore the model includes three constants.

As showed in Table IV, the BAMT is significantly dependent from PbB level (the number of correct movements of exposed subjects decreases with the increase of lead levels); the variable FINGERS is dependent on working age: the number of correct movements performed with the fingers decreases as the number of years of exposure increases.

Finally, the Poisson regression performed on the levels of performance of the single psycho-emotional tests (POMS) has allowed to underline that the levels of PbB and ZPP significantly influence the tension and the depression (Tab. V).

Discussion and conclusions

The findings of the present study showed that occupational lead exposure results in impairment of certain cognitive abilities at levels considered safe by certain scientific committees. At a mean PbB of 56.4 μg/dL, we observed a significant relationship between the exposed and the referent groups in tests mainly involving executive functions, short time memory (BAMT test, FT test and DS Inverse) and for all the psychiatric symptoms measured by the POMS test.

These results are consistent with previous studies. A cross-sectional analysis of 107 occupationally exposed individuals showed increased rates of depression, confusion, anger, fatigue, and tension as measured by the POMS test among those with blood levels > 40 μg/dL [29]; authors found that cumulative measures of blood lead levels in currently exposed lead workers were associated with tension, anxiety, hostility and depression measured by the POMS questionnaire. Lindgren et al. [30] examined the POMS factor structure in retired lead smelter workers and showed that the resulting "general distress" factor was significantly related to cumulative exposure but not to current PbB level. Psychiatric symptoms (as measured by POMS), were positively associated with both the risk of Alzheimer diseases and a steeper rate of cognitive decline [31]. Because late life symptoms of depression are closely associated with dementia, investigators have put forth a number of hypotheses that suggesting that depression a) may be a risk factor for cognitive decline, b) has risk factors in common with dementia c) is an early reaction to declining cognition and d) influences the threshold at which dementia emerges. The exact temporal and mechanistic relation remains unclear. Regardless of the exact relation between depressive symptoms and cognitive function, however, the assessment of the impact of lead exposure on these outcomes is not compromised.

The mechanism with which lead exposure affects cognition in older adults has yet to be revealed, but several pathways have been proposed such as lead's impact on oxidative stress neural apoptosis, neurotransmitter storage and release, mitochondrial damage, and hippocampal changes [31-33]. Of particular relevance to MCI on dementia is oxidative stress, with higher levels of oxidative stress markers (e.g. isoprostanes, nitrotyrosine, 8-hydroxyguanosine, 8-hydroxyguanine) among patients with MCI and AD [34].

Although it is known to induce oxidative stress [35] the relationship of lead exposure with these specific markers of effect is not known; lead may also affect cognitive function indirectly through its effect on hypertension,

which is increasingly being recognized as a target for the prevention of dementias.
According to the recent scientific literature on this topic our results support the hypothesis that increased blood lead levels can also be associated with measurable neurocognitive abnormalities. From a neurobiological point of view, it is of great interest that neuropsychological effects may occur at concentrations several orders of magnitude below the clinical threshold for acute lead poisoning [36-38]. It could therefore be argued that there is no "safe" level for the adverse effects of lead on neuronal functioning and that these can only be measured using neuropsychological tests.
There are some limitations of our study that should be pointed out. For example a variety of factors can influence a person's susceptibility, such socioeconomic status, genetic factors and it cannot be determined from our data to which extent these factors influenced test results. Despite these limitations, however, these findings were consistent with those of previous studies; anyway, the present report suggests the need to define an occupational exposure limit for PbB lower than 30 μg/dL [39].

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All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript. The Authors have no conflict of interest to declare.

Authors' contributions

C.F. developed and planned the whole study by coordinating the various stages of research.
S.G. performed the medical examination of subjects.
A.A. made data processing and statistical analysis.
C.C. has performed the sampling and laboratory analysis of the exposed and control groups.
E.M. has chosen, administered and rated the psychodiagnostic protocol.

References

[1] Mason LH, Harp JP, Han DY. *Pb neurotoxicity: neuropsychological effects of lead toxicity*. Biomed Res Int 2014;2014:840547. doi: 10.1155/2014/840547.

[2] Schwartz J. *Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold*. Environ Res 1994;65(1):42-55. doi: 10.1006/enrs.1994.1020.

[3] Koller K, Brown T, Spurgeon A, Levy L. *Recent developments in low-level lead exposure and intellectual impairment in children*. Environ Health Perspect 2004;112(9):987-94. doi: 10.1289/ehp.6941.

[4] Onalaja AO, Claudio L. *Genetic susceptibility to lead poisoning.* Environ Health Perspect 2000 Mar;108(Suppl 1):23-8.

[5] Balbus-Kornfeld JM, Stewart W, Bolla KI, Schwartz BS. *Cumulative exposure to inorganic lead and neurobehavioural test performance in adults: an epidemiological review.* Occup Environ Med 1995;52(1):2-12.

[6] Goodman M, LaVerda N, Clarke C, Foster ED, Iannuzzi J, Mandel J. *Neurobehavioural testing in workers occupationally exposed to lead: systematic review and meta-analysis of publications.* Occup Environ Med 2002;59(4):217-23. doi:10.1136/oem.59.4.217.

[7] Meyer-Baron M, Seeber A. *A meta-analysis for neurobehavioural results due to occupational lead exposure with blood lead concentrations < 70 microg/100 ml.* Arch Toxicol 2000;73(10-11):510-8.

[8] Chertkow H, Massoud F, Nasreddine Z, Belleville S, Joanette Y, Bocti C, Drolet V, Kirk J, Freedman M, Bergman H. *Diagnosis and treatment of dementia: 3. Mild cognitive impairment and cognitive impairment without dementia.* CMAJ 2008;178(10):1273-85. doi: 10.1503/cmaj.070797.

[9] Weisskopf MG, Proctor SP, Wright RO, Schwartz J, Spiro A 3rd, Sparrow D, Nie H, Hu H. *Cumulative lead exposure and cognitive performance among elderly men.* Epidemiology 2007;18(1):59-66. doi: 10.1097/01.ede.0000248237.35363.29.

[10] Basha MR, Murali M, Siddiqi HK, Ghosal K, Siddiqi OK, Lashuel HA, Ge YW, Lahiri DK, Zawia NH. *Lead (Pb) exposure and its effect on APP proteolysis and Abeta aggregation.* FASEB J 2005;19(14):2083-4. doi: 10.1096/fj.05-4375fje.

[11] Sun L, Zhou XL, Yi HP, Jiang SJ, Yuan H. *Lead-induced morphological changes and amyloid precursor protein accumulation in adult rat hippocampus.* Biotech Histochem 2014;89(7):513-7. doi: 10.3109/10520295.2014.904926.

[12] Wu J, Basha MR, Brock B, Cox DP, Cardozo-Pelaez F, McPherson CA, Harry J, Rice DC, Maloney B, Chen D, Lahiri DK, Zawia NH. *Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD.* J Neurosci 2008;28(1):3-9. doi: 10.1523/JNEUROSCI.4405-07.2008.

[13] Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. *Lead exposure and cardiovascular disease-a systematic review*. Environ Health Perspect 2007;115(3):472-82. doi: 10.1289/ehp.9785.

[14] Vaziri ND. *Mechanisms of lead-induced hypertension and cardiovascular disease.* Am J Physiol Heart Circ Physiol 2008;295(2):H454-65. doi: 10.1152/ajpheart.00158.2008.

[15] Fenga C, Cacciola A, Martino LB, Calderaro SR, Di Nola C, Verzera A, Trimarchi G, Germanò D. *Relationship of blood lead levels to blood pressure in exhaust battery storage workers.* Ind Health 2006;44(2):304-9. http://doi.org/10.2486/indhealth.44.304.

[16] Rapisarda V, Ledda C, Ferrante M, Fiore M, Cocuzza S, Bracci M, Fenga C. *Blood pressure and occupational exposure to noise and lead (Pb): a cross-sectional study*. Toxicol Ind Health 2016;32(10):1729-36. doi: 10.1177/0748233715576616.

[17] Patterson C, Feightner JW, Garcia A, Hsiung GY, MacKnight C, Sadovnick AD. *Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of Alzheimer disease.* CMAJ 2008;178(5):548-56. doi: 10.1503/cmaj.070796.

[18] Reddy GR, Devi BC, Chetty CS. *Developmental lead neurotoxicity: alterations in brain cholinergic system.* Neurotoxicology 2007;28(2):402-7. doi.org/10.1016/j.neuro.2006.03.018.

[19] Wang L, Luo L, Luo YY, Gu Y, Ruan DY. *Effects of Pb2+ on muscarinic modulation of glutamatergic synaptic transmission in rat hippocampal CA1 area.* Neurotoxicology 2007;28(3):499-507. doi: 10.1016/j.neuro.2006.11.003.

[20] ACGIH. 2015 TLVs® and BEIs. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; 2015.

[21] Lebel J, Mergler D, Branches F, Lucotte M, Amorim M, Larribe F, Dolbec J. *Neurotoxic effects of low-level methylmercury contamination in the Amazonian Basin.* Environ Res 1998;79(1):20-32. doi: 10.1006/enrs.1998.3846.

[22] Rohlman DS, Lucchini R, Anger WK, Bellinger DC, van Thriel C. *Neurobehavioral testing in human risk assessment.* Neurotoxicology 2008;29(3):556-67. doi: 10.1016/j.neuro.2008.04.003.

[23] Wechsler D. *WAIS - Scala d'intelligenza Wechsler per adulti.* Firenze: Organizzazioni Speciali 1999.

[24] McNair DM, Lorr M, Droppleman LF. *POMS - Profile of Mood States.* Firenze: Organizzazioni Speciali 1991.

[25] Pesarin F. *Multivariate permutation test: with applications in biostatistics*. New York: Wiley 2001.

[26] Griffin D, Gonzalez R. *Correlational analysis of dyad-level data in the exchangeable case.* Psychol Bull 1995;118:430-9.

[27] Kleinbaum DG. *Logistic regression: a self-learning test.* New York: Springer 1994.

[28] Mc Cullagh P, Nelder JA. *Generalized Linear Models*. 2nd ed. USA: Chapman and Hall CRC 1989.

[29] Wilson RS, Barnes LL, Mendes de Leon CF, Aggarwal NT, Schneider JS, Bach J, Pilat J, Beckett LA, Arnold SE, Evans DA, Bennett DA. *Depressive symptoms, cognitive decline, and risk of AD in older persons*. Neurology 2002;59:364-70.

[30] Lindgren KN, Masten VL, Ford DP, Bleecker ML. *Relation of cumulative exposure to inorganic lead and neuropsychological test performance*. Occup Environ Med 1996;53:472-7.

[31] Shih RA, Hu H, Weisskopf MG, Schwartz BS. *Cumulative lead dose and cognitive function in adults: a review of studies that measured both blood lead and bone lead. Environ Health Perspect.* 2007;115(3):483-92. doi: 10.1289/ehp.9786.

[32] Weuve J, Korrick SA, Weisskopf MG, Ryan LM, Schwartz J, Nie H, Grodstein F, Hu H. *Cumulative exposure to lead in relation to cognitive function in older women.* Environ Health Perspect 2009;117(4):574-80. doi: 10.1289/ehp.11846.

[33] White LD, Cory-Slechta DA, Gilbert ME, Tiffany-Castiglioni E, Zawia NH, Virgolini M, Rossi-George A, Lasley SM, Qian YC, Basha MR. *New and evolving concepts in the neurotoxicology of lead.* Toxicol Appl Pharmacol 2007;225(1):1-27. doi: 10.1016/j.taap.2007.08.001.

[34] Praticò D. *Oxidative stress hypothesis in Alzheimer's disease: a reappraisal.* Trends Pharmacol Sci 2008;29(12):609-15. doi: 10.1016/j.tips.2008.09.001.

[35] Ahamed M, Siddiqui MK. *Low level lead exposure and oxidative stress: current opinions.* Clin Chim Acta 2007;383(1-2):57-64. doi: 10.1016/j.cca.2007.04.024.

[36] Murata K, Iwata T, Dakeishi M, Karita K. *Lead toxicity: does the critical level of lead resulting in adverse effects differ between adults and children?* J Occup Health 2009;51(1):1-12. http://doi.org/10.1539/joh.K8003.

[37] Counter SA, Buchanan LH, Ortega F. *Neurocognitive screening of lead-exposed andean adolescents and young adults.* J Toxicol Environ Health A 2009;72(10):625-32. doi: 10.1080/15287390902769410.

[38] Lucchini RG, Zoni S, Guazzetti S, Bontempi E, Micheletti S, Broberg K, Parrinello G, Smith DR. *Inverse association of intellectual function with very low blood lead but not with manganese exposure in Italian adolescents.* Environ Res 2012;118:65-71. doi: 10.1016/j.envres.2012.08.003.

[39] Landrigan P, Nordberg M, Lucchini R, Nordberg G, Grandjean P, Iregren A, Alessio L; *International Workshop on neurotoxic metals: lead, mercury, and manganese - from research to prevention (NTOXMET). The Declaration of Brescia on prevention of the neurotoxicity of metals June 18, 2006.* Am J Ind Med.2007;50(10):709-11. doi: 10.1002/ajim.20404.

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