# CENTRAL NERVOUS SYSTEM EFFECTS OF COMBINED NANOPARTICULATE LEAD EXPOSURE

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## ABSTRACT

Occupational lead exposure occurs mostly by inhalation while the general population can is exposed by ingestion. In the experiment presented, the two exposure routes were modelled in rats using different physicochemical forms of lead. To mimic airborne exposure, PbO nanoparticles (mean diameter ca. 20 nm) were instilled into the trachea of the rats (2 or 4 mg/kg b. w.) five times a week for 6 weeks. In per os treatment, 80 or 320 mg/kg b. w. Pb-acetate was given to the rats by gavage (also five times a week for 6 weeks). To combine the two routes of exposure, rats were treated per os with Pb acetate for 3 weeks, followed by intra-tracheal instillation of Pb-oxide nanoparticles for another 3 weeks, with the same timing and doses as above. After the 3 or 6 weeks of exposure, the effects of lead on the central nervous system of the rats were investigated by behavioural and electrophysiological methods. Both chemical forms and routes of exposure affected differently the outcome of central nervous system investigations.

**Key words**: lead, nanoparticle, behavioural toxicity, electrophysiology, rat

### INTRODUCTION

Lead is one of the oldest known metals. The Romans widely used this metal in everyday life by face powder, lipstick, mask, wine, dishes, coins or water pipes. Today, the popularity of lead has changed; it is mainly used in metallurgy, glass industry, corrosion protection, for manufacturing battery, as fuel additives, or applied in older paints and ceramics. Lead is one of the most common environmental xenobiotics, is present in the soil, groundwater, air, and foodstuffs. The general population is exposed mainly by contaminated drinking water or food: roots, leafy vegetables, meats, dairy products or fishes. Tobacco smoke is another notable source of Pb exposure (1).

By per os exposure, 20-70% of lead is absorbed; by inhalation route, almost all can be absorbed. Absorbed lead is transported to various organs (liver, kidneys, lungs and brain) and finally mineralized in certain tissues like bones, teeth (2). Lead is useless to the human body; it is one of the heavy metals notoriously harmful for human health. The main target of lead is the nervous system, with consequences like occupational neuropathy and delayed mental development of children. In children, acute exposure to 70-80 μg/dL levels of lead may cause encephalopathy with different degree symptoms such as hyperirritability, ataxia, convulsion, stupor, and coma. Chronic low level exposure of lead may produce decrement in intelligence quotient; hearing, balance, or peripheral functional impairment in childhood. In adulthood, acute exposure to lead (460 μg/dL) results in encephalopathy with initiative sings as dullness, irritability, poor attention, muscular tremor, loss of memory, hallucination. In workers with chronic lead exposure less severe neurologic effects were documented with headache, lethargy, dizziness, diminished reaction time, cognitive and visual motor performance and slowed nerve conduction (3).

Industrial high-temperature processes (smelting, casting, welding, cutting, grinding etc.) generate airborne metal-containing particles, including those in the submicron range (so-called nanoparticles, NPs). Compared to microscopic particles, NPs have higher mobility within the organism, including direct access to the CNS by penetrating tissue boundaries like the alveolar and capillary wall, and the blood-brain barrier (4).

In this work, the above mentioned general (food-borne) and occupational (inhalation) exposure was modelled by giving lead to rats in two different chemical forms, and combining exposure via the airways and the gastrointestinal tract. Our aim was to study the adverse effects caused by lead in different physico-chemical forms by behavioural and electrophysiological methods.

### MATERIALS AND METHODS

Young adult male Wistar rats (200±20 g, 9 groups of 8 rats each) were obtained from the university's breeding centre and were housed in a GLP-rated animal house (22±1 ºC, 30-60 % relative humidity, 12-h light/dark cycle with light on at 06:00) with free access to tap water and standard pellet. Body weight of the animals was measured before every treatment. Treatments were performed once daily, 5 times a week, and lasted 6 weeks (see Table I). For modelling airborne exposure, PbO nanoparticles (2 or 4 mg/kg b. w.) were suspended in 1% hydroxy ethyl cellulose (HEC, pH 7.4) vehicle and instilled into the trachea (1 ml/kg

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b. w.) of the rats (5). Intratracheal instillation was carried out in brief diethyl-ether anaesthesia. In per os treatment, 80 or 320 mg/kg b. w. Pb-acetate (dissolved in distilled water) was given to the rats by gavage (6). To combine the two routes of exposure, rats were treated per os with Pb acetate for 3 weeks, followed by intratracheal instillation of Pb-oxide nanoparticles for another 3 weeks, with the same timing and doses as above. For intratracheal application, NPs of $PbO_2$ with 20±12.4 nm diameter were synthesized at the Department of Applied Chemistry, University of Szeged.

The rats' spontaneous locomotor behaviour pattern was assessed in an open field (OF) instrument at the end of the treatment period, 2 days after the last Pb administration. The OF box used was of 48x48x40 cm size and was equipped with two arrays of infrared beam gates at floor level and at 12 cm height (Conducta 1.0 System; Experimetria Ltd., Budapest, Hungary). After 20–30 min accommodation in the test room, the animals were put into the centre of the box one by one for a 10 min session. From the beam interruptions, counts and time of the horizontal (running), vertical (rearing) and local (grooming etc.) activity as well as horizontal run length were calculated.

Electrophysiological recording was done on the day following the OF test. Preparation for recording, and the recording itself, was performed in urethane anaesthesia (1000 mg/kg ip). The left hemisphere was exposed, and spontaneous electrical activity (electrocorticogram, ECoG) was recorded from the primary somatosensory, visual and auditory areas for 6 minutes. From this, band spectrum according to the standard human EEG bands (delta to gamma: Kandel and Schwartz, 1985) was calculated. Then, evoked potentials (somatosensory, visual and auditory) were recorded from the same sites by applying the stimuli in trains of 50. Somatosensory stimulation (SS) was done by square pulses (3-4 V; 0.05 ms; 1, 2 and 10 Hz frequency) delivered through a pair of needles inserted into the contralateral whiskery skin. Visual stimulation (VIS) was performed by flashes (1 Hz) of a high-luminance white LED placed to the contralateral eye of the rat. For acoustic stimulation (AUD), clicks (1 Hz) were applied to the contralateral ear through the hollow ear bar of the stereotaxic frame. Latency and duration of the EPs was measured after averaging. The complete electrophysiological work was performed by means of the software Neurosys 1.11 (Experimetria Ltd., Budapest, Hungary). Finally, the rats were sacrificed by an overdose of urethane, dissected, and organ weights were measured. Relative organ weights, on the basis of 1/100 body weight, were calculated. During the whole procedure, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

The distribution of data was checked for normality by means of the Kolmogorov-Smirnov test. Data analysis was done by one-way ANOVA. Post hoc analysis of group differences was performed by LSD test, setting the probability level at $p>0.05$.

**Table I.** Treatment scheme with group codes, doses and treatment time

| Group code | Treatment |
|---|---|
| **VC6 po.** | 6 weeks, distilled water, per os |
| **PL6 po.** | 6 weeks 80 mg/b.w. kg lead(II)acetate, per os |
| **PH6 po.** | 6 weeks 320 mg/b.w. kg lead(II)acetate, per os |
| **VC33** | 3 weeks, distilled water, per os + 3 weeks HEC intra-tracheal |
| **PL33** | 3 weeks 80 mg/b.w. kg lead(II)acetate, per os + 3 weeks lead(II) oxide NPs 2 mg/b.w.kg intra-tracheal |
| **PH33** | 3 weeks 320 mg/b.w. kg lead(II)acetate, per os + 3 weeks lead(II) oxide NPs 2 mg/b.w.kg intra-tracheal |
| **VC6 it.** | 6 weeks HEC intra-tracheal |
| **PL6 it.** | 6 weeks lead(II) oxide NPs 2 mg/b.w.kg intra-tracheal |
| **PH6 it.** | 6 weeks lead(II) oxide NPs 4 mg/b.w.kg intra-tracheal |

VC: vehicle control, PL: low dose lead PH: high dose lead
HEC: 1% hydroxyethyl cellulose (HEC) dissolved in PBS

## RESULTS

Fig. 1 shows the body weight change seen on the 6th week. The weight decrease was mainly observed in the high dose treated groups, and most significantly in the single orally and intra-tracheal treated groups.

Among the relative organ weights, that of the lung, brain thymus, heart, spleen kidney and liver showed significant changes (Table II). The weight increase of the lungs was significant vs. the corresponding control both in the combined and intra-tracheal treated group. The relative liver, thymus weight was strongly raised in only orally treated group vs. own control. The relative kidney weight was significantly raised in oral and intra-tracheal groups

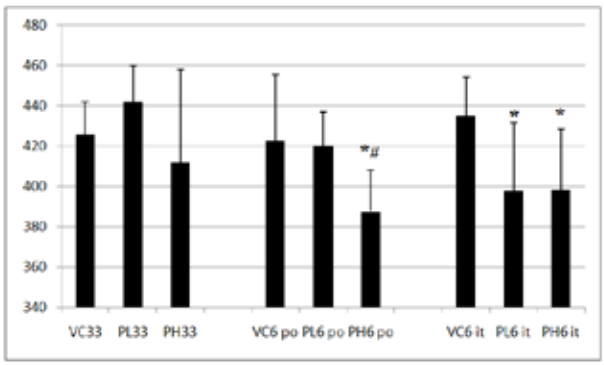


**Fig.1.** Average body weight on the 6th weeks Mean+SD, n=8, *: $p<0.05$ vs. own control; #: $p<0.05$, PH6 po vs PL6 po.

**Table II.** Relative organ weights (related to 1/100 body weight). Mean± SD, n=8. *;**, ***: p<0.05, 0.01; 0.001 vs. own control; #p<0.05, PH 6 po vs.PL6 po; PH6 it vs PL6 it; PH33 vs. PL33

| | Brain | Thymus | Heart | Lung | Liver | Spleen | Kidney |
|---|---|---|---|---|---|---|---|
| VC33 | 0,48 ± 0,02 | 0,09 ± 0,02 | 0,25 ± 0,02 | 0,58 ± 0,13 | 3,09 ± 0,39 | 0,17 ± 0,02 | 0,69 ± 0,06 |
| PL33 | 0,49 ± 0,02 | 0,14 * ± 0,04 | 0,30 *** ± 0,02 | 1,22 *** ± 0,11 | 3,48 ± 0,31 | 0,19 ± 0,03 | 0,73 ± 0,05 |
| PH33 | 0,50 ± 0,04 | 0,10 ± 0,02 | 0,28 **# ± 0,01 | 1,19 *** ± 0,19 | 3,05 # ± 0,19 | 0,17 ± 0,02 | 0,77 ± 0,11 |
| VC6 po | 0,48 ± 0,05 | 0,10 ± 0,02 | 0,25 ± 0,02 | 0,32 ± 0,02 | 2,96 ± 0,18 | 0,15 ± 0,02 | 0,62 ± 0,02 |
| PL6 po | 0,48 ± 0,02 | 0,10 ± 0,01 | 0,27 ± 0,01 | 0,41 ± 0,14 | 3,21 ± 0,20 | 0,19 * ± 0,03 | 0,72 ** ± 0,06 |
| PH6 po | 0,51 ± 0,03 | 0,12 *# ± 0,02 | 0,29 ** ± 0,03 | 0,41 ** ± 0,05 | 3,45 *** ± 0,18 | 0,20 ± 0,03 | 0,89 * ± 0,21 |
| VC6 it | 0,48 ± 0,02 | 0,09 ± 0,02 | 0,26 ± 0,02 | 0,34 ± 0,03 | 3,20 ± 0,33 | 0,18 ± 0,03 | 0,62 ± 0,04 |
| PL6 it | 0,53 * ± 0,06 | 0,10 ± 0,02 | 0,28 ± 0,03 | 0,52 *** ± 0,08 | 3,34 ± 0,37 | 0,20 ± 0,04 | 0,72 ** ± 0,10 |
| PH6 it | 0,52 ± 0,05 | 0,10 ± 0,03 | 0,27 ± 0,02 | 0,58 ***# ± 0,06 | 3,32 ± 0,16 | 0,19 ± 0,02 | 0,69 *** ± 0,04 |

Total spontaneous locomotors activity decreased dose-dependently in the three experimental schemes, however without significance. The decline of locomotors activity resulted mainly from the reduced ambulation, and rearing time, and the increased local activity and immobility.

In the spectral distribution of the spontaneous cortical activity, slow waves increased and fast waves decreased (most significantly in visual and auditory spontaneous cortical activity) after per os treatment, and to a lesser extent also after the combined treatment. Intra-tracheal treatment also showed significance, but only in case of high dose nano-lead treatment (Fig. 2).

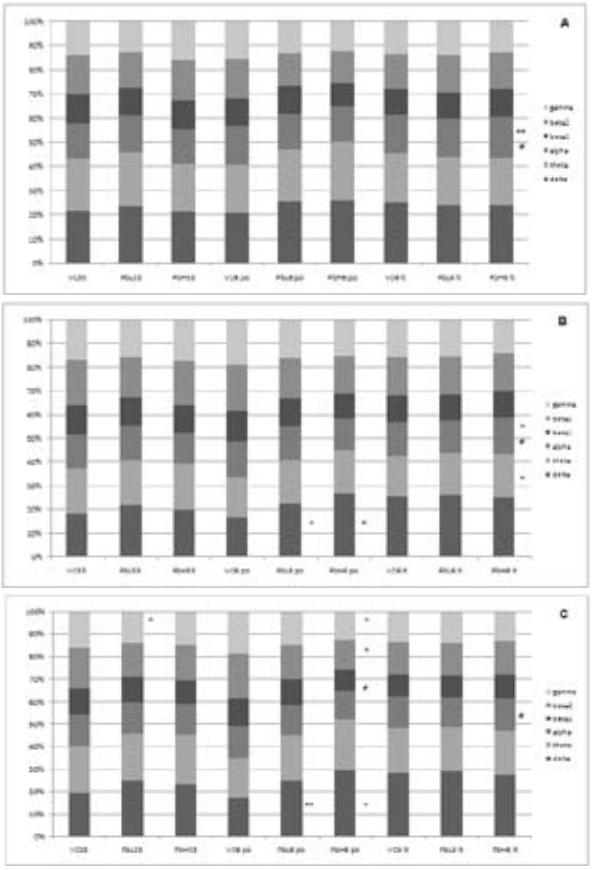


**Fig. 2.** Frequency spectrum of the spontaneous cortical activity after the treatment. A: somatosensory area B: visual area C: auditory area Mean+SD, n=8. *;**,: p<0.05, 0.01 vs. own control;#,##: p<0.05, PH6 po vs PL6 po. ; PH6 it vs PL6 it

The cortical sensory evoked potentials showed mostly dose dependently and significantly increased latency, which was seen in all experimental schemes (Fig. 3).

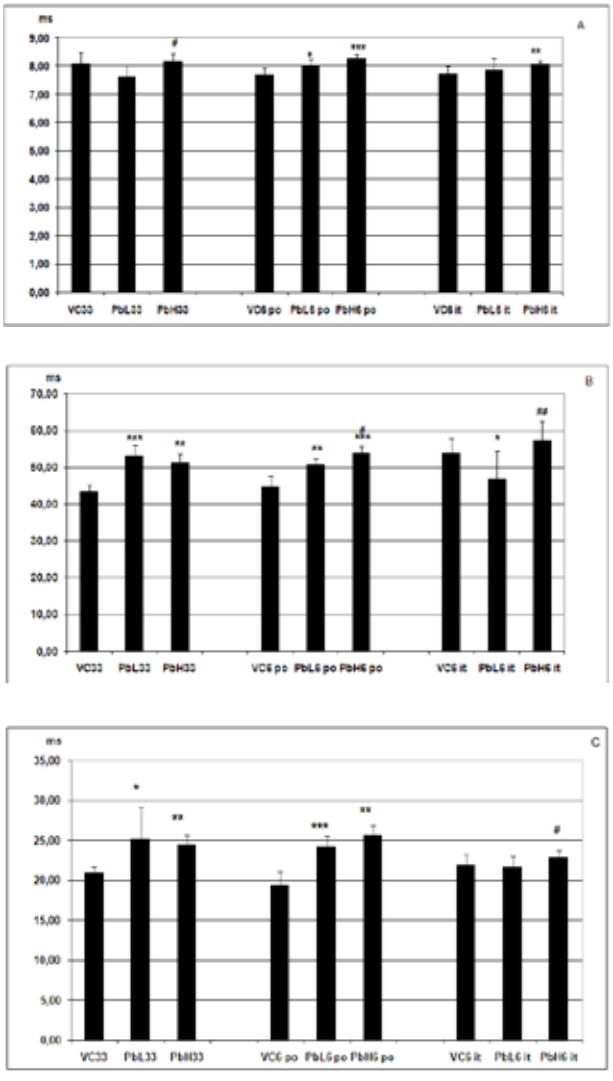


**Fig. 3.** Latency of the cortical evoked potentials. A, somatosensory response evoked with 10 Hz stimulation; B, visual and C auditory response. Mean+SD, n=8. *;**, ***: p<0.05, 0.01; 0.001 vs. own control; #,##: p<0.05, PH6 po vs PL6 po. ; PH6 it vs PL6 it; PH33 vs.PL33

**CONCLUSION**

Electrophysiological results seemed more sensitive to the effects of lead exposure than the behavioural investigations. Comparing the different exposure routes it can be concluded, that per os administration resulted in more marked alterations, which is reflected in the cortical evoked potentials. Comparing results of the combined vs. individual per os or intratracheal administration, it can be seen, that changes in cortical evoked potentials reveal some influence due to combined exposure, but not as notable as in case of the other two treatment schemes, moreover in body weight and spontaneous cortical activity almost any alteration can be observed by combined treatment. The differences indicated that chemical form and route of exposure may have their own influence on the functional alterations seen in the CNS.

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## EFECTELE INDUSE LA NIVELUL SISTEMULUI NERVOS CENTRAL DE EXPUNEREA COMBINATA LA NANOPARTICULE DE PLUMB

## REZUMAT

Expunerea ocupationala la plumb apare cel mai adesea prin inhalare, in timp ce populatia generala poate fi expusa prin ingestie. In experimentul prezentat, au fost evidentiate cele doua cai de expunere la animalul de laborator (sobolan), folosind forme fizico-chimice ale plumbului. Pentru a mima expunerea la particulele din aer, nanoparticulele de PbO (iametrul mediu de 20 nm) au fost instilate la nivelul traheei de sobolan (2 sau 4 mg/kg corp), de cinci ori pe saptamana, timp de 6 saptamani. In tratamentul per os, au fost administrate prin gavaj 80 or 320 mg/kg corp acetate de Pb (de asemenea, de 5 ori pe saptamana, timp de 6 saptamani). Pentru a combina cele doua cai de expunere, sobolanii au fost tratati per os cu acetat de Pb timp de 3 saptamani, urmat de instilarea intra-traheala a nanoparticulelor de oxid de Pb, pentru inca 3 saptamani, cu aceeasi frecventa si pentru aceasi perioada de timp. Dupa expunere timp de 3 sau 6 saptamani, efectele plumbului asupra sistemului nervos central au fost investigate prin metode comportamentale si electrofiziologice. Ambele forme chimice si cai de administrare au influentat diferit rezultatele investigatiilor la nivelul sistemului nervos central.

**Cuvinte cheie:** plumb, nanoparticule, toxicitate comportamentala, electrofiziologie, sobolan

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