

## Effects of Lead Exposure in Printing Houses on Immune and Neurobehavioral Functions of Women

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**Abstract: Effects of Lead Exposure in Printing Houses on Immune and Neurobehavioral Functions of Women: Niu QIAO, et al. Department of Occupational Health, Shanxi Medical University, China**—The object of this study was the effect of lead (Pb) exposure on immune and neurobehavioral parameters of 16 women with a mean age of 34 yr who worked for an average of 12 yr in three printing houses in Taiyuan (Shanxi Province, China). The women were not occupationally exposed to toxic agents other than Pb. Mean air Pb in the printing houses was about 25  $\mu\text{g}/\text{m}^3$ , mean blood Pb of the workers was 28.6  $\mu\text{g}/\text{dl}$  and that of the referents 12.4. The referent group of 36 women, resident in the same industrialised and traffic busy city, was of similar age, working activity, lifestyle and education of the Pb-exposed group. The Neurobehavioral Core Test Battery (NCTB) was utilised; some of the NCTB scores, including the simple reaction time, Digit Symbol, Pursuit Aiming II (PA) and correct PA, were significantly lower in the Pb exposed group, whereas all the negative Profile Mood States (POMS) scores were slightly higher in the workers (some with a significant difference) indicating a negative psychological effect of Pb. Lymphocyte subsets were determined in 16 of the Pb exposed women and in 16 referent women with similar age and lifestyle of the whole group of 36 controls. CD4<sup>+</sup>-CD45RO<sup>+</sup> “memory” T lymphocytes of the workers were significantly higher than those of control women, whereas NK and B CD3<sup>+</sup>-HLA-DR<sup>+</sup> and NK CD16<sup>+</sup>-CD56<sup>+</sup> lymphocytes of the Pb exposed women were much lower than those of the referent group. We suggest that the reduction of blood CD16<sup>+</sup>-CD56<sup>+</sup> lymphocytes in the workers in the printing houses may depend either on a direct effect of Pb exposure on the immune system or on an effect mediated by the neuroendocrine system.

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**Key words:** Lead exposure, Neuroendocrine system, Neurobehavioral tests, Immune system, Lymphocyte subpopulations

There are several experimental studies on the effects of lead (Pb) exposure on the nervous system<sup>1</sup>. Changes in morphology and reduction of sulphhydryl groups were found in astrocytes and Schwann cells “*in vitro*” incubated with Pb<sup>2</sup>. Pb, zinc (Zn) and/or copper (Cu) were significantly increased with a dose-response effect in the brain of male rats exposed to 15, 30 and 60 ppm of Pb in drinking water for 14 months<sup>3</sup>. Chronic Pb treatment induced in rats higher levels of plasma norepinephrine and epinephrine and of tissue monoamine oxidase (MAO), increased sympathetic tone in the central nervous system and increased responsiveness of peripheral catecholaminergic receptors<sup>4,5</sup>.

The effects of Pb exposure on neurobehavioral functions, and the amount of monoamine neurotransmitter in the hippocampus and of lipid peroxide in the brain were studied by Shi *et al.*<sup>6</sup>. The results suggested that changes in levels of neurotransmitters, such as monoamine and lipid peroxide, could explain the mechanism of Pb-induced damage to neurobehavioral functions.

A meta-analysis, used to deal with the data of neurobehavioral toxicological studies, revealed that Pb mainly impairs touch sense, memory, emotion and cognitive abilities<sup>7</sup>. Twenty-nine Pb-exposed printing house male workers (with blood Pb 25–40  $\mu\text{g}/\text{dl}$ , age 20–40 yr) were investigated with the Neurobehavioral Core Test Battery (NCTB), a computer-administrated neurobehavioral evaluation system, the inventory of profile of mood states (POMS) and the Autonomic Nervous System (ANS) Function Test Battery. The results indicated that the profile of mood states of Pb exposed workers was

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moving to the negative side and most of the NCTB and Computerized Neurobehavioral Evaluation System (NES-C, including some test items of the NCTB) performances (in particular those of learning, memory and reaction) were modified<sup>8</sup>). Moreover, the parasympathetic tone of Pb-exposed workers was lower than that of the controls.

The blood Pb of men (mean blood Pb 11  $\mu\text{g}/\text{dl}$ ), with exposure to an urban environment polluted by traffic, was correlated with total blood lymphocytes and several activated lymphocyte subsets ( $\text{CD4}^+\text{-CD45RO}^+$ ,  $\text{HLA-DR}^+$  and  $\text{CD25}^+$ )<sup>9</sup>). These results are in agreement with data demonstrating high levels of activated  $\text{HLA-DR}^+$  cells in male workers with moderate Pb exposure (mean blood Pb 13  $\mu\text{g}/\text{dl}$ ) in a factory producing plastic materials<sup>10</sup>). On the other hand, blood T  $\text{CD3}^+$  and  $\text{CD4}^-$  lymphocytes were reduced with a dose-response effect in two groups of firearm instructors with mean blood Pb of 15 or 30  $\mu\text{g}/\text{dl}$ <sup>11</sup>). The findings of these studies suggest that high Pb exposure may inhibit some immune functions, whereas low Pb exposure exerts an opposite effect. This may be explained by the phenomenon "hormesis" (a low dose stimulating effect and a high dose inhibitory effect<sup>12</sup>).

One "in vitro" study showed that Pb modulates immune activity with different mechanisms: Pb inhibits the Th1-like cell-mediated immune response<sup>13</sup>), but enhances a Th2-like humoral response with release of cytokines inducing B cell maturation and Ig production<sup>14</sup>). Treatment of BALB/c mice with  $\text{PbCl}_2$  was found to induce increased IgE and decreased interferon- $\gamma$  (IFN- $\gamma$ ) production<sup>15</sup>). In another experiment, BALB/c mice, subcutaneously treated with  $\text{PbCl}_2$ , had higher levels of plasma IgE and IL-4 and lower levels of plasma IFN- $\gamma$ <sup>16</sup>). T cells from the Pb-treated mice, stimulated "in vitro" by  $\text{PbCl}_2$ , also produced higher levels of IL-4 and lower levels of IFN- $\gamma$ . These experimental studies confirm the results of an investigation which showed, in atopic men, a positive linear correlation of  $\text{CD5}^+\text{-CD19}^+$  B lymphocytes either with blood Pb ( $n=17$ ;  $r=0.679$ ;  $p<0.01$ ) or serum IgE ( $n=17$ ;  $r=0.713$ ;  $p<0.01$ )<sup>9</sup>). This suggests that Pb exposure, possibly with concomitant effects of other noxious agents produced by traffic, may enhance the production of IgE by B lymphocytes contributing to increase the incidence of allergic symptoms.

Pb exposure still remains a major health problem in several countries, including China<sup>17, 18</sup>). The aim of this study was to find further evidence of the subclinical and immune effects of Pb exposure in Chinese workers.

## Materials and Methods

In the recent years Pb printing by male workers has almost disappeared in China and is now performed by women only. Three small Pb printing houses in the South District of Taiyuan City (Shanxi Province, China) were investigated. All the Pb exposed women were engaged in printing characters made from colours on plates: these colours contained Pb as a toxic agent, but there was no

exposure to solvents or other chemical compounds. The printing houses had similar equipment and production procedure with generally good hygiene conditions and the use of air ventilators. Air Pb concentrations in the premises were measured according to the method of Wang *et al.*<sup>19</sup>

Sixteen Pb-exposed women in the three printing houses were matched with 36 referent women with the same economical and social status and resident in the same area. Both groups had similar income, lifestyle, working activities and special habits (including smoking). The referent women were not occupationally exposed to toxic agents. The duration of employment in the printing houses was (mean  $\pm$  S.D.)  $12.7 \pm 5.1$  yr. The age of the Pb-exposed subjects was (mean  $\pm$  S.D.)  $34.8 \pm 4.9$  yr and that of the referents  $33.4 \pm 4.8$ . The periods of education in middle school of the referent and Pb exposed groups were (mean  $\pm$  S.D.)  $9.2 \pm 1.5$  and  $9.4 \pm 1.8$  yr, respectively. Subjects with diseases or being treated with drugs or pregnant were excluded from the study.

All the recruited women were living in the South District of Taiyuan City with exposure to traffic and environmental emissions from lead battery and chemical industries. Alkylated Pb compounds are no longer components of petrol as in the period (1993) when mean Pb levels of  $0.93 \mu\text{g}/\text{m}^3$  were determined in the environmental air of Taiyuan. There are no recent data reporting the levels of exposure to Pb and other toxic metals in the town.

Women after sleeping at night and in a fasting condition, attended the hospital in the early morning. Blood and urine samples of all the Pb-exposed women and of 16 referent women were collected at 8 a.m. The 16 referent subjects were of similar age, income, education and lifestyle of the whole group of 36 referent subjects.

The neurobehavioural tests were performed in a clinic room, with the same examiner, from 8.30 to 10.30 a.m. The test lasted about 30 minutes for each subject. The clinic room was quiet, illuminated with a daylight lamp, and the temperature in the room was  $20\text{--}22^\circ\text{C}$ .

The WHO recommended NCTB was used for interviewing all the examined subjects<sup>19, 20</sup>). The NCTB was developed in English with many versions in different languages; the differences among these versions have been discussed<sup>20</sup>). The Chinese version is widely used<sup>20, 21</sup>). The NCTB is composed of two parts; one is the POMS questionnaire including: Anger-Hostility (POMSA), Confusion-Bewilderment (POMSC), Depression-Dejection (POMSD), Fatigue-Inertia (POMSF), Tension-Anxiety (POMST), and Vigor-Activity (POMSV), which reflect psychological variations caused by neurotoxicants; the second is a test battery including six tests: Simple Reaction Time (SRT), which reflects response speed and attention; Digit Span (DSP), which reflects attention and auditory memory; Santa Anna Dexterity (SAN), which shows manual coordination and speed; Digit Symbol

(DSY), which shows cognition and motor speed; Benton Visual Retention (BVR), which reflects Visual cognition and memory, coordination and speed; and Pursuit Aiming II (PA), which shows manual coordination and speed. The SRT is divided into SRTF (fastest simple reaction time) and SRTS (slowest simple reaction time), the DSP is divided into DSPF (forward) and DSPB (backward), the SAN includes SANP (preferred hand) and SANN (non-preferred hand), the PA includes PAC (correct) and PAE (erroneous).

Blood Pb was determined according to Sabbioni *et al.*<sup>22)</sup> in two different laboratories for performing a quality control of the results; serum IgE was determined by ELISA with UniCAP (Pharmacia & Upjohn, Uppsala, Sweden). The detection limit of the method was 2 I.U./ml.

Fluorescein isothiocyanate (FITC) and phycoerythrin (PE) -conjugated antibodies (Beckton-Dickinson, San José, CA, USA) were used for lymphocyte subset determinations. The antibodies were CD4-CD45RO (to determine "memory and virgin" T helper lymphocytes<sup>13)</sup>),

CD3-CD8 (cytotoxic-suppressor cells), CD16-56 (NK cells), CD19 (B lymphocytes), CD3-HLA-DR (activated T, B and NK lymphocytes). Two-colour flow-cytometry analysis was performed by FACScan<sup>23)</sup>.

Kolmogorov-Smirnov test was used for determining the distribution of the values. The results concerning lymphocyte subsets conformed to the normal distribution less when expressed as number of cells/ $\mu$ l than as percentage of the total number of lymphocytes.

## Results

The air Pb concentration in the premises of the three printing houses was  $25 \pm 19 \mu\text{g}/\text{m}^3$  (mean  $\pm$  S.D.) (n=24). There were no significant differences among the levels of Pb in the air of the premises of the three printing houses.

The blood lead concentration of the Pb-exposed workers was  $28.6 \pm 14.6 \mu\text{g}/\text{dl}$  (mean  $\pm$  S.D.) and that of referents  $12.4 \pm 4.7 \mu\text{g}/\text{dl}$ .

The results of the neurobehavioral tests are listed in Table 1. All the negative POMS scores of the Pb-exposed subjects were higher than those of the controls, indicating

**Table 1.** Neurobehavioral tests and profile of mood states in Pb-exposed women

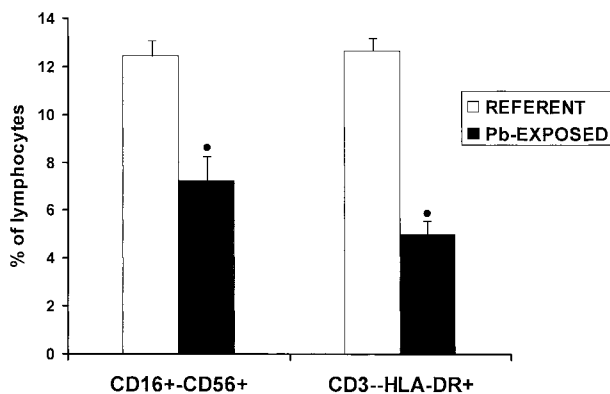
Item	Referents	Pb-exposed women	F	P
POMSA	12.50 $\pm$ 8.12	13.97 $\pm$ 7.63	1.126	0.357
POMSC	7.08 $\pm$ 3.96	8.41 $\pm$ 4.40	3.022	0.006**
POMSD	13.82 $\pm$ 3.96	15.41 $\pm$ 11.98	1.856	0.081
POMSF	6.17 $\pm$ 3.83	8.18 $\pm$ 4.74	3.615	0.001**
POMST	10.20 $\pm$ 5.29	12.38 $\pm$ 6.55	2.820	0.009**
POMSV	16.08 $\pm$ 11.51	17.45 $\pm$ 6.30	1.846	0.083
SRT	289.97 $\pm$ 86.92	325.85 $\pm$ 153.57	2.300	0.030*
SRTF	208.20 $\pm$ 69.49	227.79 $\pm$ 126.91	1.919	0.071
SRTS	513.81 $\pm$ 357.18	563.50 $\pm$ 330.52	1.124	0.358
DSPF	10.67 $\pm$ 2.56	10.43 $\pm$ 2.83	1.289	0.264
DSPB	5.82 $\pm$ 2.19	5.81 $\pm$ 2.52	0.998	0.446
DSP	16.50 $\pm$ 3.96	16.25 $\pm$ 4.66	0.791	0.613
SANP	41.79 $\pm$ 6.57	43.86 $\pm$ 5.84	1.659	0.124
SANN	40.00 $\pm$ 8.51	41.88 $\pm$ 4.21	0.912	0.512
DSY	50.38 $\pm$ 16.23	46.41 $\pm$ 11.78	4.810	0.001**
BVR	6.85 $\pm$ 2.40	7.41 $\pm$ 1.54	0.777	0.624
PAC	216.23 $\pm$ 39.76	201.22 $\pm$ 51.76	7.186	0.001**
PA	220.08 $\pm$ 41.78	209.19 $\pm$ 59.30	6.576	0.001**
PAE	3.97 $\pm$ 7.77	7.11 $\pm$ 13.10	1.198	0.313

Values are means  $\pm$  S.D. The POMS scores were processed by covariance analysis in order to exclude the effects of age, sex and education degree. \*p<0.05; \*\*p<0.01. POMSA=Anger-Hostility; POMSC=Confusion-Bewilderment; POMSD=Depression-Dejection; POMSF=Fatigue-Inertia; POMST=Tension-Anxiety; POMSV=Vigor-Activity; SRT=Simple Reaction Time; SRTF=Fastest Simple Reaction Time; SRTS=Slowest Simple Reaction Time; DSPF=Digit Span Forward; DSPB=Digit Span Backward; DSP=Digit Span; SANP=Santa Anna Dextery Preferred Hand; SANN=Santa Anna Dextery Non- Preferred Hand; DSY=Digit Symbol; BVR=Benton Visual Retention; PAC=Pursuit Aiming Correct; PA=Pursuit Aiming II; PAE=Pursuit Aiming Erroneous.

**Table 2.** Blood lymphocyte subpopulations of Pb exposed women

% of lymphocytes	referents (n=16)		Pb-exposed (n=16)	
	Mean	S.D.	Mean	S.D.
Total lymphocytes/ $\mu$ l	2112	417	2161	450
CD3 <sup>+</sup>	63.78	11.26	69.93	7.42
CD4 <sup>+</sup> -CD45RO <sup>-</sup>	14.73	4.51	16.03	5.96
CD4 <sup>+</sup> -CD45RO <sup>+</sup>	14.02	2.70	17.03	4.06*
CD3 <sup>+</sup> -CD8 <sup>+</sup>	22.75	3.95	21.35	4.00
CD16 <sup>+</sup> -CD56 <sup>+</sup>	12.45	2.94	7.25	4.30**
CD19 <sup>+</sup>	9.04	3.28	10.09	4.90
CD3 <sup>-</sup> -HLA-DR <sup>+</sup>	12.68	2.61	5.00	2.57**
CD3 <sup>+</sup> -HLA-DR <sup>+</sup>	3.00	0.62	3.12	0.65

\*p<0.05, \*\*p<0.01 by Mann-Whitney U-test.



**Fig. 1.** Blood CD16<sup>+</sup>-CD56<sup>+</sup> and CD3<sup>-</sup>-HLA-DR<sup>+</sup> lymphocytes in Pb-exposed women. Values are expressed as mean  $\pm$  S.E. \*p<0.01 by Mann-Whitney U-test.

that Pb-exposure has a negative psychological effect. The differences between the POMSC, POMSF and POMST scores for the two groups showed a statistical significance (p<0.01).

Although the differences between scores in some NCTB tests for the Pb exposed and control women were not significant, the SRT, DSY, PAC and PA of the Pb-exposed women were significantly lower than those of the controls. The results indicated that Pb exposure reduces cognition and motor speed, manual coordination and speed.

The serum mean IgE of the referents was  $48.7 \pm 17.4$  I.U./ml (mean  $\pm$  S.D.) and that of the Pb exposed subjects was  $42.5 \pm 17.4$  I.U./l. The detection limit of the method is 2 I.U./ml; values in non-atopic subjects are <100 I.U./ml.

CD3<sup>+</sup>, CD3<sup>+</sup>-CD8<sup>+</sup>, CD4<sup>+</sup>-CD45RO<sup>-</sup>, CD3<sup>+</sup>-HLADR<sup>+</sup> and CD19<sup>+</sup> lymphocyte subsets of Pb-exposed women were not statistically different from those of control

women (Table 2), but CD4<sup>+</sup>-CD45RO<sup>+</sup> "memory" T lymphocytes of Pb-exposed women were significantly higher than those of referent women, whereas CD3<sup>-</sup>-HLA-DR<sup>+</sup> and NK CD16<sup>+</sup>-CD56<sup>+</sup> lymphocytes of the Pb exposed women were significantly lower than those of the referent group (Table 2 and Fig. 1).

## Discussion

Blood Pb of the population resident in Taiyuan has to be considered high in relation to that of other Chinese and European towns<sup>9, 18, 22, 24</sup>. This may be due to emissions from industries, particularly from those producing Pb batteries. Despite the high blood Pb levels, the referent group resident in Taiyuan showed values of lymphocyte subsets similar to those recently determined in the Italian population with exposure to an urban environment<sup>8, 24</sup>. Therefore, the values for lymphocytes subsets of the Chinese referent group are comparable with those of previous studies.

In this study, blood CD4<sup>+</sup>-CD45RO<sup>+</sup> "memory" lymphocytes were higher in the Pb-exposed women than in the referents. Lower blood CD4<sup>+</sup>-CD45RO<sup>-</sup> "virgin" lymphocytes and an increased CD4<sup>+</sup>-CD45RO<sup>+</sup>/CD4<sup>+</sup>-CD45RO<sup>-</sup> "memory/virgin" lymphocyte ratio was found in atopic men in relation to those non-atopic exposed to an urban environment (with mean blood Pb 11  $\mu$ g/dl in both groups)<sup>9</sup>. In this previous study, blood CD4<sup>+</sup> and CD4<sup>+</sup>-CD45RO<sup>+</sup> "memory" lymphocytes of both atopic and non-atopic men were significantly correlated with blood Pb values. On the other hand, atopic women living in a urban environment showed only a statistically significant correlation between the values for blood Pb and CD4<sup>+</sup>-CD45RO<sup>-</sup> "virgin" or "naive" lymphocytes<sup>24</sup>. Sata *et al.* also showed in workers exposed to Pb stearate in a chemical factory a reduced number of blood CD3<sup>+</sup>-CD45RO<sup>+</sup> "memory" lymphocytes and a significant positive correlation between the percentage of CD3<sup>+</sup>-CD45RA<sup>+</sup> (naive T cells) and blood Pb<sup>25</sup>. The results of

the above reported studies suggest that the values for blood CD4<sup>+</sup>-CD45RO<sup>+</sup> and CD4<sup>+</sup>-CD45RO<sup>-</sup> lymphocytes and their ratio may depend on several factors including sex, atopy, concomitant exposure to different toxic agents (besides Pb). On the other hand, Pb exposure may induce the above reported phenomenon of “hormesis”<sup>12</sup>.

Our results showing reduced blood NK lymphocytes in Pb-exposed women are in agreement with those of Sata *et al.*<sup>26</sup> on CD16<sup>+</sup> NK lymphocytes in 29 male workers. The mean blood Pb of the workers was 18  $\mu\text{g}/\text{dl}$  (range 7–35), and the workers were divided into two groups with blood Pb higher or lower than 20  $\mu\text{g}/\text{dl}$ . The workers with higher Pb values had a smaller number and percentage of NK cytotoxic CD16<sup>+</sup> lymphocytes both in comparison to workers with lower Pb exposure and to non-exposed controls. A decreased CD16<sup>+</sup> lymphocyte count was found in workers with alexithimia, a psychological trait characterised by difficulty in verbalising feelings<sup>27</sup>. Similarly, both low blood NK activity and reduction in blood NK lymphocytes were found by Morimoto *et al.* in subjects with a poor lifestyle and/or mental instability<sup>28</sup>. Reduced blood NK CD16<sup>+</sup>-CD56<sup>+</sup> lymphocytes and “*in vitro*” production of interferon- $\gamma$  and interleukin 2 (markers of cell mediated Th1 response<sup>13</sup>) by peripheral blood mononuclear cells (PBMC) was found in women exposed to low or high frequency electromagnetic fields (ELMFs)<sup>29, 30</sup>. It was suggested that ELMFs reduce blood NK lymphocytes and their activity by effects on both immune and nervous systems.

The results of the above reported studies demonstrate that not only stress, lifestyle and mental status<sup>27, 28</sup> and ELMF exposure<sup>29, 30</sup> but also Pb exposure<sup>26</sup> influence the activity of the sympathetic system and alter the homeostasis of neurohormones which may influence the immune response<sup>13</sup>. For this reason the low blood NK lymphocyte percentage found in Pb exposed women may depend on an effect of the metal on the nervous system similar to that of stress, poor lifestyle and unstable mental status<sup>27, 28</sup>. In this regard, it was shown that the neuroendocrine and immune systems are linked by several relationships<sup>13, 31–33</sup>. Some authors suggest that they constitute a single immuno-neuroendocrine network; the immune response stimulates the nervous system, whereas stimulation of specific central nervous areas modifies the immune response.

Modification of neurobehavioral tests in the Pb exposed women in this study may reflect an impairment of neurological functions induced by the metal<sup>4, 5</sup>. Neurobehavioral tests measure integrated activities of the nervous system. For this reason they are considered markers of neurological alterations responsible for changing performance<sup>34, 35</sup>. The NCTB is the most widely used test. The sensitivity, reliability, validity and cross-cultural consistency of NCTB as an integrated battery

have been confirmed, with some disparity of different tests dependent on cultural groups, and with some influence of age and education degree<sup>36</sup>.

Many reports on neurobehavioral assessment for neurotoxicity of chemicals have been published. They are mainly concentrated on the exposure to Pb, manganese, aluminum, organic solvents and pesticides. Tang *et al.*<sup>37</sup> reported impairment of certain neurobehavioral performances, including attention/response speed, manual dexterity, perceptual-motor speed, visual perception/memory, and motor speed/steadiness in humans with mean blood Pb of 70.5  $\mu\text{g}/\text{dl}$ . Yang *et al.*<sup>38</sup> found that behavioral performances on SRT, SANP, and PA were significantly poorer in 78 Pb exposed workers (with mean blood Pb of 44.2  $\mu\text{g}/\text{dl}$ ) than in the control group. Chia *et al.*<sup>39</sup> reported that Pb exposed workers (with a geometric mean concurrent blood Pb of 37.1  $\mu\text{g}/\text{dl}$ , had significantly poorer manual dexterity, perceptual-motor speed, and motor steadiness than the referents. Hu *et al.*<sup>40</sup> showed that the scores for three negative mood states (confusion-bewilderment, fatigue-inertia and tension-anxiety) were significantly higher in workers with mean blood Pb of 52.6  $\mu\text{g}/\text{dl}$  than in the referents, whereas their activity score was significantly decreased. Maizlish *et al.*<sup>41</sup> studied the profile of the mood state of Venezuelan workers with a mean blood Pb of 42  $\mu\text{g}/\text{dl}$ ; their tension-anxiety, hostility, and depression mood scales showed a significantly poorer dose-response relationship to the blood Pb concentration in multiple linear regression models including age, education and alcohol intake as covariates. The mean blood Pb levels of the above reported investigations were all higher than those of the women investigated in this study. The results of this investigation are in agreement with those of Qiao *et al.*<sup>8</sup> who showed impairment of neurobehavioral parameters in subjects with low Pb exposure (mean blood Pb 17.2  $\mu\text{g}/\text{dl}$ ) by using a multistep regressive statistical analysis: blood Pb was selected into the formula for POMS and POMST ( $p=0.018$  and  $0.039$ , respectively) and the ALA and exposure duration were selected into the SAN, DSY and PA formulae ( $p<0.05$  in all the tests)<sup>8</sup>.

In conclusion, we have shown altered neurobehavioral tests and low NK blood lymphocytes in Pb exposed women. We suggest that the reduction in blood CD16<sup>+</sup>-CD56<sup>+</sup> lymphocytes may depend either on a direct effect of Pb exposure on the immune system or it may be mediated by an action of Pb on the neuroendocrine system, mental status and behaviour, all of which are able to influence the immune response. On the other hand, the increased blood “memory” lymphocytes in the Pb-exposed women may more likely depend on a direct effect of Pb exposure (possibly concomitant with that of other toxic agents) on the immune system.

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