Semen Quality and Reproductive Endocrine Function in Relation to Biomarkers of Lead, Cadmium, Zinc, and Copper in Men

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Blood lead (BPb), activity of δ-aminolevulinic acid dehydratase (ALAD), erythrocyte protoporphyrin (EP), blood cadmium (BCd), serum zinc (SZn), seminal fluid zinc (SfZn), serum copper (SCu), and parameters of semen quality and of reproductive endocrine function were measured in 149 healthy male industrial workers 20–43 years of age. The group contained 98 subjects with slight to moderate occupational exposure to Pb and 51 reference subjects. All of the subjects lived in Zagreb, Croatia. Significant ($p < 0.05$) correlations of BPb, ALAD, and/or EP with reproductive parameters indicated a Pb-related decrease in sperm density, in counts of total, motile, and viable sperm, in the percentage and count of progressively motile sperm, in parameters of prostate secretory function (SfZn, acid phosphatase, and citric acid in seminal fluid), and an increase in abnormal sperm head morphology, serum testosterone, and estradiol. These associations were confirmed by results of multiple regression, which also showed significant ($p < 0.05$) influence of BCd, SZn, SCu, smoking habits, alcohol consumption, or age on certain reproductive parameters. These effects were mainly of lower rank and intensity as compared to Pb-related reproductive effects, whereas BCd contributed to a decrease in sperm motility and an increase in abnormal sperm morphology and serum testosterone. No significant Pb- or Cd-related influence was found on levels of the lactate dehydrogenase isoenzyme LDH-C_4 and fructose in seminal fluid or on follicle-stimulating hormone, luteinizing hormone, and prolactin in serum. The seminal fluid concentrations of Pb (SfPb) and Cd (SfCd) were measured in 118 of the 149 subjects, and a highly significant ($p < 0.0001$) correlation was found between BPb and SfPb levels ($r = 0.571$) and between BCd and SfCd levels ($r = 0.490$). The overall study results indicate that even moderate exposures to Pb (BPb < 400 μg/L) and Cd (BCd < 10 μg/L) can significantly reduce human semen quality without conclusive evidence of impairment of male reproductive endocrine function. *Key words*: age, alcohol, δ-aminolevulinic acid dehydratase, cadmium, copper, human male reproductive capacity, lead, smoking, sperm, zinc. *Environ Health Perspect* 108:45–53 (2000). [Online 9 December 1999]
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Many recent studies have indicated an increasing prevalence of various abnormalities of the reproductive system in human males. There is growing concern about the considerable decrease in sperm density over the last 50 years in general populations worldwide, especially in the United States and in Europe (*1,2*). Possible explanations for this phenomenon include increased stress, lifestyle factors, and a variety of endocrine-altering chemicals in the environment that can be linked to decreased male reproductive capacity, as indicated mainly by the results of experimental animal studies. Of the possible causal factors considered, including the most explored hypothesis of the role of various xenoestrogens and synthetic estrogens (*3,4*), none have yet been reliably identified as the cause of a generally decreasing reproductive capacity in human males, at least in some areas.

The human male is of relatively low fertility as compared to other mammals. Thus, the human male may be at greater risk from reproductive toxicants. For example, the number of sperm per human ejaculate is typically only 2- to 4-fold higher than the number at which fertility is significantly reduced, whereas the number of sperm in rat, rabbit, or bull ejaculate is many times (up to 1,400-fold) the number that will produce maximum fertility (*5*). Human males have markedly smaller relative testis size and the lowest rate of daily sperm production per gram of testes (by a factor of more than 3) as compared to the mouse, rat, or monkey; the percentages of progressively motile sperm and morphologically normal sperm in human semen are also lower than in any of the animal models (*5*).

Pb and Cd are highly toxic metals for humans and other mammals. Both are pervasive in the human environment and accumulate in the human body over a lifetime, including prenatal life (especially Pb). Apart from numerous sources of occupational exposure to each of the metals, the most important nonoccupational sources are food (especially seafood from metal-polluted areas), water (Pb, mostly from Pb pipes in contact with soft and acidic water), air (especially Pb from gasoline in dense traffic areas), Pb-based paints of housing, smoking habits [Cd and (to a lesser extent) Pb from tobacco], and alcohol consumption (Pb-contaminated alcoholic beverages). There is also some evidence of the possible interaction of Pb and alcohol in humans, that is, an ethanol-induced increase in the biologically active or mobile fraction of Pb accumulated in the body (*6–8*).

Many experimental animal studies show that Pb and Cd can adversely affect the mammalian male reproductive system. On the other hand, relatively few data are available regarding the possible reproductive effects of Pb and/or Cd in men, and generally conflicting results have been reported in reviews on the subject (*9,10*). Nevertheless, the results of several studies suggest that relatively high occupational exposure to Pb, as indicated by blood Pb (BPb) levels, can reduce human semen quality (decreased number, motility, and altered morphology of sperm), whereas reproductive endocrine function is either not affected or is only marginally affected (*11–28*). Some data suggest that the reproductive effects of Pb in men are reversible, that is, a trend toward normalization of reproductive parameters was found in subjects treated with a Pb-chelating agent (*16,17*), or after cessation of occupational Pb exposure (*28*). Most of these studies have one or more of the following shortcomings: a small number of subjects examined, no reference group or an inadequate reference group because of relatively high BPb levels, the inclusion of subjects with current or recent urogenital tract infections, and/or a lack of control for other common factors capable of affecting reproductive parameters (such as age, stress, smoking, and alcohol). However, recent epidemiologic studies have indicated that even moderate occupational exposure to Pb can reduce the fertility of male workers (*29,30*) and increase spontaneous abortions in workers' wives (*31*).

Evidence for possible reproductive effects of Cd in men is scanty and less conclusive as

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compared to that for Pb, as indicated in reviews on the subject (*9,10*). In groups of male subjects suspected of infertility (including nonsmokers and smokers), a significant positive correlation was found between abnormal sperm morphology and blood Cd (BCd) levels (*15,32*); significant inverse correlations were found between sperm density and BCd (*27,32*) and between semen volume and either BCd (*15*) or seminal fluid Cd (SfCd) levels (*27,32*). On the other hand, significant positive correlation was found between sperm motility and linear and curvilinear velocity with respect to semen Cd (*33*), or no significant correlation was found between conventional parameters of semen quality and either semen Cd (*34*) or SfCd levels (*33,35,36*) in the same kind of subjects. No significant influence of occupational Cd exposure on testicular endocrine function (*22*) and fertility of male workers (*29*) was found.

The present study considers the interrelationship of biomarkers of Pb, Cd, Zn, and Cu to parameters of semen quality and reproductive endocrine function in men. Activity of δ-aminolevulinic acid dehydratase (ALAD) in blood and erythrocyte protoporphyrin (EP) concentration, biomarkers of Pb exposure and/or effect, were used in addition to BPb for better evaluation of long-term cumulative exposure to Pb (*37–39*). The possible influence factors of age, smoking habits, and alcohol consumption on reproductive parameters were evaluated, bearing in mind that they are commonly correlated with biomarkers of exposure to Pb and/or Cd in humans. In Croatia, as compared to other countries, the general population has relatively higher exposure to Pb because of the prevalent use of gasoline containing Pb (0.5 g Pb/L gasoline), whereas smokers have considerably higher exposure to Cd because of the relatively high Cd content in Croatian cigarettes; in heavy smokers BCd levels of up to 13 µg/L are commonly found (*40,41*). Biomarkers of Zn and Cu were included because human exposures to Pb and Cd are often accompanied by considerable exposure to Zn (and vice versa), which may act as an antagonist and thus mask the Pb- and/or Cd-related effects; both Pb and Cd can adversely affect Zn metabolism, and possibly Cu metabolism; and Cu and Zn can antagonistically influence each other's absorption rate and metabolism (*42*).

Materials and Methods

Study population. The study was cross-sectional in design; workers were invited from plants with a range of exposure to Pb (no Pb to moderate Pb) and no likely exposure to other metals and other factors that might influence reproductive parameters. The study population consisted of 149 healthy male industrial workers who had never been occupationally exposed to Cd, Zn, Cu, or other metals apart from Pb. The workers lived in Zagreb, Croatia. There were 51 subjects not occupationally exposed to Pb (all from the final product assembly department in a machine tool factory) and 98 subjects with slight to moderate occupational exposure to Pb (3 from a printing press, 5 from a Pb-products factory, 9 from a ceramics factory, 19 from a factory producing Pb-based paints, and 62 from a factory producing storage batteries). Workers from the latter organizations had been regularly controlled for Pb-exposure in our laboratory for more than 15 years (≥ 2 times per year). Their long-term average BPb values were < 400 µg/L. The selection criteria were age (20–45 years); comparable lifestyle and socioeconomic status (industrial workers); employment at the present place of work for ≥ 2 years; absence of a disease, condition, or exposure to physical and/or chemical factors other than Pb that affect or are suspected to affect spermatogenesis or semen quality; and the absence of psychologic stress (due to the death of a close relative or similar event), acute disease, or high body temperature during the preceding 4-months (period that exceeds the duration of one spermatogenesis cycle of approximately 72 ± 9 days). None of the selected subjects had ever received any chelation therapy, had hobbies involving the risk of metal exposure, or used any medication that could influence metal metabolism. The participation rate was > 90% in Pb workers from each of their organizations, as opposed to 67% in reference subjects. There appeared to be no significant difference between the participants and those who refused to participate in the study, except for the somewhat lower age of study participants. All subjects gave their informed consent before inclusion in the study. The study was performed in accordance with the ethical standards of the 1964 Helsinki declaration (*43*) and was approved by the common ethical committee of the collaborating health institutions in Zagreb (at the Vuk Vrhovac Clinic, Medical Faculty of the University of Zagreb).

A detailed questionnaire was completed by a physician for each of the 149 subjects. The structured interview was divided in two parts. Data on age, smoking habits, alcohol consumption, and professional and medical history of the subjects were collected by a specialist in occupational health, who was responsible for the health of the workers in each of the factories. Data on marital status, sexual history, and an andrologic physical examination were completed by a specialist in andrology. The andrology specialist was unaware of the exposure category of the subjects and was also responsible for the final selection of subjects. Subjects with any of the following findings were excluded: varicocele, cryptorchidism, hypogonadism, digitorectal indication of prostatitis, indication of chronic orchoepididymitis, or history of genital region trauma. However, because the incidence of varicocele was considerably greater in Pb workers as compared to reference subjects (26 and 14%, respectively), an overcontrol with regard to possible reproductive effects of Pb may have occurred.

Special care was taken to ensure identical conditions for each of the selected subjects with regard to the semen and blood sampling, storage, and analyses. The reference subjects and Pb workers were examined in random order at the same place (in the Vuk Vrhovac Clinic), at the same time (maximum four subjects/day, Tuesday and Thursday, between 0800 and 1000 hours) and in practically the same period of the year (April–June and September–November of the years 1987–1989) to minimize possible temperature influences on semen quality. The exposure assessment was performed in one of the collaborating institutions and the reproductive effect assessment in the other. The same person(s) performed the assessment and rigorously applied the same procedure for the semen sampling and analysis. There is no known reason other than exposure that may explain any significant difference in reproductive parameters between the two groups.

In all 149 subjects, the following measurements were performed for assessment of metal exposure: BPb, ALAD activity, EP, BCd, serum Zn (SZn) and serum Cu (SCu). The concomitant assessment of male reproductive capacity included:

- macroscopic and microscopic examination of semen: semen volume, color, pH, liquefaction time, sperm density, sperm count, motility, viability, and morphology of sperm
- indicators in seminal fluid: the lactate dehydrogenase isoenzymes fraction C_4 (LDH-C_4), fructose, zinc (SfZn), acid phosphatase, and citric acid
- hormones in serum: follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, and estradiol.

In 118 of the 149 subjects, relatively high semen volume enabled additional measurements of the seminal fluid concentrations of Pb (SfPb) and Cd (SfCd).

Sampling and storage of biologic specimens. The ejaculate (by masturbation) and venous blood were sampled between 0800 and 1000 hours for each subject. All subjects were required to fast in the preceding 10 hr, abstain from alcohol in the preceding 24 hr, and avoid any sexual activity in the preceding

4 days. After the ejaculate liquefaction (approximately 15 min), the seminal fluid was separated from the spermatozoa by centrifugation at 1,500*g* for 10 min at room temperature. An aliquot of the seminal fluid (approximately 300 µL) was decanted into a metal-free polypropylene microtube and stored at -20°C until required for SfPb and SfCd analyses. Venous blood (total 15 mL) was sampled into a K_2EDTA-containing tube for BPb and BCd analyses, a heparin-containing tube for ALAD and EP analyses, and an anticoagulant-free tube for analyses of serum hormones and SZn and SCu. After allowing 60 min for spontaneous blood clotting, the serum was separated from the blood cells by centrifugation at 900*g* for 20 min at room temperature. The serum was decanted and centrifuged for a second time at 900*g* for 15 min, decanted again, and stored in a metal-free polypropylene tube at -20°C until required for SZn and SCu analyses.

Special care was taken to avoid any contamination with metals during the ejaculate and blood sampling, storage, and analyses. The specimens of each subject were sampled in the Vuk Vrhovac Clinic. All laboratory ware used (glass and plastic), including containers for the specimen sampling and storage, was cleansed by soaking in 10% (mass/vol) nitric acid for 24 hr, rinsing with deionized water, then by soaking in 3% (mass/vol) K_2EDTA for 24 hr, and finally by thoroughly rinsing with deionized water.

Analytical procedures and quality assurance. The SfPb and SfCd measurements were performed by the electrothermal-atomic absorption spectrometry (AAS) method (*44*) and analytical quality control was performed daily by analyzing a sample of pooled seminal fluid (no reference samples with certified SfPb and SfCd values were available). Essentially the same method (*44*) was used for the BPb and BCd measurements, controlled daily for accuracy by analyzing three reference blood samples with certified BPb and BCd values: BCR no. 194–196 (Community Bureau of Reference, Commision of the European Communities, Brussels, Belgium). The accuracy of both BPb and BCd measurements was also controlled by regular participation in the national external quality assessment scheme (Birmingham, UK), and our mean running variance index score (MRVIS) was consistently lower than the average MRVIS for all participants. ALAD activity was measured < 5 hr after blood sampling (blood was stored at 4°C) using the European standardized method (*45*). EP was measured by spectrofluorometric method (*46*) and the accuracy controlled by regular participation in the proficiency testing program for protoporphyrin in blood (Centers for Disease Control and Prevention, Atlanta, GA). The SZn and SCu measurements were performed by the flame–AAS method (*47*) and controlled daily for accuracy by analyzing two reference serum samples with certified SZn and SCu values: Cation-Cal (American Dade, Miami, FL) and Seronorm (Nycomed Pharma, Oslo, Norway).

Macroscopic and microscopic examination of semen was performed according to World Health Organization (WHO) recommendations (*48*), where progressive motility refers to the sperm with rapid linear progression. Sperm motility was evaluated at room temperature (22–24°C) within 1 hr after the sampling of ejaculate. The LDH-C_4 (which is often called LDH-X) in seminal fluid was measured by electrophoresis (*49*), whereas fructose (*50*), SfZn and acid phosphatase (*51*), and citric acid (*52*) in seminal fluid were measured by automated methods using an ABA-100 bichromatic analyzer (Abbott, South Pasadena, CA). Serum hormones were measured by immunologic methods using commercial kits: FSH, LH, prolactin, and estradiol by fluoroimmunoassay (DELFIA, Pharmacia, Uppsala, Sweden), and testosterone by radioimmunoassay (Byk-Sangtec Diagnostica, Dietzenbach, Germany).

Statistical methods. Because of the skewed distribution of most of the measured parameters, the results within groups are presented as median and range, and the significance of the difference between groups was calculated by using the Mann-Whitney *U*-test (z, p). The results are also presented as mean ± standard deviation for better comparison with relevant data in literature. The Pearson's correlations (r, p) and regression equations were calculated for the linear, semilogarithmic, and logarithmic combinations (y/x, log y/x, y/log x, and log y/log x) between each of the measured parameters to assess the shape of relationship based on the highest r value obtained. Because the results suggested a curved rather than a straight relationship between certain parameters, the Spearman's rank correlation (r, p) between each of the measured parameters was also calculated. Stepwise multiple regression was used to calculate the interrelationship of all the parameters considered possible explanatory variables (which were simultaneously introduced in the model) with respect to each of the measured reproductive parameters.

Results

Table 1 shows relevant data in 51 male reference subjects and 98 male Pb workers. None had ever been occupationally exposed to Cd, Zn, Cu, or other metals. The data show significantly higher Pb exposure in Pb workers as compared to the reference subjects [indicated by increased BPb and EP and lowered ALAD ($p < 0.0001$)]. No significant difference between the groups was found for age, smoking habits, alcohol consumption, and levels of BCd, SZn, and SCu. The reference group included 27 smokers and 24 nonsmokers (53 and 47%, respectively); the Pb workers group had 68 smokers and 30 nonsmokers (69 and 31%, respectively). The incidence of smokers may explain the relatively higher BCd in the latter group as well as increased BCd levels in both groups. However, the difference in BCd levels was highly significant ($p < 0.0001$) between the 95 smokers and 54 nonsmokers: 4.33 (0.49–13.33) µg/L and 0.46 (0.16–2.85) µg/L, respectively.

Table 2 shows data for the parameters of semen quality in the groups of reference subjects and Pb workers. As compared to the

Table 1. Median and range values and mean ± standard deviation for relevant parameters in 51 reference subjects and 98 lead workers, and the significance of the difference between the groups (z, p).

Parameter	Reference subjects	Lead workers	z	p
Occupational Pb exposure duration (years)	0 0	5 (2–21) 7 ± 5	–	–
Age (years)	31 (20–43) 31 ± 5	30 (20–43) 30 ± 5	-0.601	> 0.50
Smoking (cigarettes/day)	7 (0–40) 12 ± 14	20 (0–40) 16 ± 13	1.767	> 0.05
Alcohol (drinks[a]/week)	2 (0–28) 4 ± 5	2 (0–38) 4 ± 6	0.140	> 0.80
BPb (µg/L)	103 (67–208) 109 ± 30	367 (119–659) 387 ± 125	9.850	$< 10^{-16}$
ALAD (European units)	52.4 (24.9–79.4) 52.6 ± 12.3	21.8 (5.2–58.2) 23.6 ± 11.3	-9.098	$< 10^{-16}$
EP (mg/L red blood cells)	0.56 (0.42–1.38) 0.59 ± 0.15	1.62 (0.41–7.84) 2.18 ± 1.66	8.497	$< 10^{-14}$
BCd (µg/L)	1.83 (0.20–10.80) 2.82 ± 2.72	3.40 (0.16–13.33) 3.86 ± 3.30	1.560	> 0.10
SZn (µg/L)	960 (733–1,245) 971 ± 125	950 (601–1,235) 946 ± 119	-0.948	> 0.30
SCu (µg/L)	1,175 (918–1,583) 1,176 ± 144	1,132 (831–1,772) 1,136 ± 178	-1.642	> 0.10

[a]One drink = 3 dL beer, 1 dL wine, or 0.3 dL brandy.

reference subjects, Pb workers had significantly lower sperm density ($p < 0.02$); lower counts of total ($p < 0.02$), motile ($p < 0.05$) and viable ($p < 0.05$) sperm; lower percentage ($p < 0.0005$) and count ($p = 0.001$) of progressively motile sperm; a higher prevalence of morphologically abnormal sperm head ($p < 0.05$); and a lower level of indicators of prostate secretory function: SfZn ($p = 0.001$), acid phosphatase ($p < 0.01$), and citric acid ($p < 0.005$) in seminal fluid. No significant difference between the groups was found for semen volume; percentages of motile, viable, and pathologic sperm; and levels of LDH-C_4 and fructose in seminal fluid.

Apart from the results presented in Table 2, normal semen color, liquefaction time, and the absence of bacteria and erythrocytes in semen were found in all 149 subjects. In the Pb-worker group, one subject had slightly increased semen pH, three had slightly increased semen leukocytes, and four had an increased percentage of immature sperm cells.

Table 3 shows data for the parameters of reproductive endocrine function in the groups of reference subjects and Pb workers. Significantly higher serum estradiol ($p < 0.01$) was found in Pb workers as compared to the reference subjects, whereas no significant difference between the groups was found for serum FSH, LH, prolactin, and testosterone levels.

Results of the Spearman's rank correlation in all 149 subjects were highly significant ($p < 0.0001$) between each of the biomarkers of Pb exposure: ALAD and BPb ($r = -0.833$), EP and BPb ($r = 0.758$), and EP and ALAD ($r = -0.760$), as well as between BCd and smoking habits ($r = 0.777$). Table 4 shows the correlations between each of the measured reproductive parameters with respect to BPb, ALAD, EP, BCd, SZn, SCu, smoking, alcohol, age, and occupational Pb exposure duration (in the latter case by assuming the value of zero years in the 51 reference subjects). A significant ($p < 0.05$) correlation was found between one or more biomarkers of increased Pb exposure (a decrease in ALAD and/or an increase in BPb and EP) with respect to a decrease in sperm density; counts of total, motile, and viable sperm; both the percentage and count of progressively motile sperm; the parameters of prostate secretory function (SfZn, acid phosphatase, and citric acid in seminal fluid); and an increase in abnormal sperm head morphology, serum testosterone, and estradiol. In addition, a significant ($p < 0.05$) correlation was observed between BCd and an increase in percentage of pathologic sperm, serum LH and testosterone, and a decrease in serum prolactin; between SZn and an increase in counts of total and viable sperm, both the percentage and count of motile and progressively motile sperm, parameters of prostate secretory function (SfZn, acid phosphatase, and citric acid in seminal fluid), and a decrease in percentage of pathologic sperm; between SCu and a decrease in counts of motile and viable sperm; between smoking and a decrease in LDH-C_4 in seminal fluid and serum prolactin, and an increase in serum LH, testosterone, and estradiol; between alcohol and a decrease in semen volume and serum prolactin; and between age and an increase in serum FSH. Occupational Pb exposure duration appears to have contributed mostly to a decrease in sperm density, in percentage and count of progressively motile sperm, and in levels of SfZn, acid phosphatase, and citric acid in seminal fluid.

Figures 1 and 2 show the relationship between sperm density and BPb (log-transformed) and ALAD, respectively, in the 149 subjects. The data suggest a curved relationship of BPb with sperm density, because log-transformed BPb values approximated to a straight relationship with the reproductive parameter, and thus a steeper slope of decreasing sperm density at very low as compared to higher BPb values. On the other hand, ALAD showed a straight relationship with sperm density in accordance with its

Table 2. Median and range values and mean ± standard deviation for parameters of semen quality in 51 reference subjects and 98 lead workers, and the significance of the difference between the groups (*z*, *p*).

Parameter	Reference subjects	Lead workers	*z*	*p*
Semen				
Semen volume (mL)	2.6 (1.1–5.2) 2.8 ± 1.0	2.5 (0.7–6.3) 2.6 ± 1.0	-0.581	> 0.50
Sperm density (million/mL)	72.0 (15.0–168.0) 79.1 ± 36.7	60.5 (3.8–163.0) 63.4 ± 28.4	-2.457	< 0.02
Sperm count (million)	176.4 (27.5–847.6) 217.6 ± 141.5	141.7 (7.6–582.4) 167.6 ± 101.7	-2.474	< 0.02
Motile sperm, % (per 100 sperm)	41 (15–60) 42 ± 9	41 (9–59) 40 ± 10	-0.795	> 0.40
Motile sperm count (million)	85.3 (13.7–339.0) 91.6 ± 62.9	54.4 (0.7–332.0) 71.6 ± 53.2	-2.286	< 0.05
Progressively motile sperm, % (per 100 sperm)	31 (8–49) 31 ± 9	27 (1–42) 26 ± 8	-3.601	< 0.0005
Progressively motile sperm count (million)	58.3 (6.9–259.4) 69.5 ± 51.2	37.0 (0.1–207.2) 48.0 ± 39.2	-3.227	= 0.001
Viable sperm, % (per 100 sperm)	48 (22–64) 47 ± 9	46 (15–67) 47 ± 11	-0.234	> 0.80
Viable sperm count (million)	94.8 (14.3–457.7) 105.5 ± 77.1	63.9 (1.1–366.9) 82.8 ± 60.8	-2.150	< 0.05
Pathologic sperm, % (per 100 sperm)	33 (16–55) 33 ± 7	34 (16–68) 34 ± 10	0.701	> 0.40
Head % pathologic sperm (per 100 pathologic sperm)	55.3 (29.6–72.7) 54.3 ± 9.2	58.0 (38.5–100) 60.1 ± 13.2	2.087	< 0.05
Seminal fluid				
LDH-C_4 (relative %)	20.3 (2.8–34.2) 19.9 ± 7.2	19.2 (0–41.1) 18.8 ± 8.7	-1.308	> 0.10
Fructose (mmol/L)	13.3 (2.5–25.1) 13.5 ± 5.0	13.9 (2.1–27.9) 14.0 ± 5.5	0.758	> 0.40
SfZn (mg/L)	146.5 (9.1–320.4) 154.6 ± 78.1	99.4 (6.5–332.8) 111.8 ± 72.3	-3.219	= 0.001
Acid phosphatase (× 1000 U/L)	480 (144–1,510) 600 ± 357	386 (32–1,296) 433 ± 273	-2.763	< 0.01
Citric acid (mmol/L)	27.9 (9.8–66.5) 28.5 ± 12.2	20.2 (2.9–76.4) 23.0 ± 12.3	-2.959	< 0.005

Table 3. Median and range values and mean ± standard deviation for parameters of reproductive endocrine function in 51 reference subjects and 98 lead workers, and the significance of the difference between the groups (*z*, *p*).

Parameter (serum)	Reference subjects	Lead workers	*z*	*p*
FSH (U/L)	3.20 (1.50–10.00) 3.65 ± 1.82	3.35 (0.63–15.50) 4.02 ± 2.73	0.170	> 0.80
LH (U/L)	4.0 (2.1–13.2) 4.8 ± 2.5	4.3 (1.9–11.3) 4.9 ± 2.2	0.796	> 0.40
Prolactin (µg/L)	5.3 (2.3–32.7) 6.3 ± 4.5	5.5 (1.4–34.6) 6.6 ± 4.2	0.736	> 0.40
Testosterone (nmol/L)	30.6 (13.1–48.0) 30.1 ± 9.2	32.6 (8.4–56.0) 33.4 ± 10.0	1.880	> 0.05
Estradiol (nmol/L)	0.14 (0.02–0.37) 0.14 ± 0.07	0.17 (0.02–0.33) 0.17 ± 0.08	2.701	< 0.01

curved relationship with BPb (ALAD/log BPb) (Figure 3) and a relatively more pronounced decrease in ALAD, as well as sperm density, at relatively low BPb values. The same shape was characteristic for the relationships of BPb and ALAD with other reproductive parameters, and changes were relatively more pronounced in the range of BPb values < 350 µg/L. The only exception was a curved relationship between semen volume and ALAD (semen volume/log ALAD: r = 0.188, $p < 0.05$), suggesting that a decrease in semen volume may occur at ALAD values < 20 European units, which is equivalent to BPb values > 400 µg/L (Table 5).

Figures 4 and 5 show the relationship between sperm count values with respect to the mean values of BPb and ALAD in six subgroups (I–VI) of the 149 subjects. These subjects were divided according to increasing BPb or decreasing ALAD values. There were 24 subjects in a subgroup with the lowest BPb (< 100 µg/L) or the highest ALAD (> 54 European units) values (I) and 25 subjects in each of the remaining subgroups (II–VI). The subgroups of the same rank (e.g., VI for BPb and VI for ALAD, indicating the highest intensity of Pb exposure) do not necessarily consist of the same subjects. A significant difference in sperm count values was found between the subgroups: III and I (p = 0.030), IV and I (p = 0.011), and VI and I (p = 0.004) with regard to BPb values; and V and I (p = 0.016) and VI and I (p = 0.033) with regard to ALAD values. A quantitative relationship between the sperm count and BPb and ALAD values, respectively, in all 149 subjects is also presented (Figures 4 and 5). These results indicate an average decrease of approximately 65 million sperm in the BPb range of 50–350 µg/L and in the corresponding ALAD range of 65–25 European units. This is well below the WHO-recommended health-based limit for occupational Pb exposure in male subjects [BPb of 400 µg/L (*53*)]. Moreover, the results suggest that a decrease in human sperm count may occur even within the Pb exposure range that is common for general populations worldwide.

Table 5 shows the results of stepwise multiple regression in 149 subjects when each of the measured reproductive parameters was considered with respect to all of the following explanatory variables: BPb (log-transformed), ALAD, EP, BCd, SZn, SCu, smoking, alcohol, and age. These explanatory variables were simultaneously introduced in the model, and none of them was removed from the model throughout the stepwise procedure. With all of its inherent limitations, the model identified the best predictor(s) of the reproductive parameters in the statistical sense, with no implication of a causal association. Although actual BPb, ALAD, or EP cannot be used to represent

Table 4. The Spearman's rank correlation coefficient and the level of significance (r, p) for relationships between reproductive parameters with respect to biomarkers of lead (BPb, ALAD, and EP), cadmium (BCd), zinc (SZn) and copper (SCu), smoking habits, alcohol consumption, age, and occupational lead exposure duration (ED) in 149 subjects.

Parameter	BPb	ALAD	EP	BCd	SZn	SCu	Smoking	Alcohol	Age	ED
Semen volume	-0.052	0.142	-0.095	0.052	0.140	-0.145	0.018	-0.172*	0.091	-0.028
Sperm density	-0.137	0.190*	-0.197*	-0.087	0.129	-0.045	-0.058	0.128	-0.032	-0.212**
Sperm count	-0.177*	0.273##	-0.217**	-0.073	0.236#	-0.152	-0.047	-0.011	0.037	-0.193*
Motile sperm (%)	0.028	0.046	-0.034	-0.114	0.161*	-0.123	0.028	0.133	-0.009	-0.056
Motile sperm count	-0.134	0.243#	-0.187*	-0.112	0.225**	-0.168*	-0.038	0.030	0.033	-0.185*
Progressively motile sperm (%)	-0.128	0.172*	-0.197*	-0.100	0.167*	-0.038	0.003	0.053	0.101	-0.224**
Progressively motile sperm count	-0.179*	0.267##	-0.240##	-0.115	0.235#	-0.140	-0.036	0.032	0.071	-0.237#
Viable sperm (%)	0.080	0.000	0.004	-0.074	0.144	-0.147	0.053	0.113	-0.002	-0.015
Viable sperm count	-0.126	0.235#	-0.188*	-0.100	0.229#	-0.170*	-0.037	0.030	0.031	-0.178*
Pathologic sperm (%)	0.112	-0.120	0.126	0.158*	-0.191*	0.075	0.033	-0.063	-0.015	0.056
Head/pathologic sperm (relative %)	0.209**	-0.155	0.225**	0.086	-0.109	0.012	-0.041	-0.083	0.046	0.127
LDH-C_4	-0.022	0.066	-0.078	-0.116	-0.052	-0.062	-0.207**	0.137	-0.050	-0.100
Fructose	-0.056	0.051	-0.085	-0.092	-0.035	-0.133	-0.110	-0.118	-0.022	0.013
SfZn	-0.222**	0.290##	-0.192*	0.083	0.251#	0.054	0.126	-0.011	0.040	-0.221**
Acid phosphatase	-0.202**	0.229#	-0.158*	0.006	0.216**	0.153	0.065	0.038	-0.004	-0.211**
Citric acid	-0.217**	0.229#	-0.177*	0.038	0.215**	0.094	0.110	0.019	0.054	-0.201**
FSH	0.014	-0.067	-0.059	0.142	-0.057	0.109	0.143	-0.031	0.270##	0.057
LH	0.065	-0.012	-0.018	0.185*	-0.047	0.135	0.193*	-0.014	0.009	0.090
Prolactin	0.070	-0.030	0.030	-0.168*	-0.070	0.115	-0.305##	-0.163*	-0.060	-0.001
Testosterone	0.188*	-0.267##	0.108	0.295##	-0.111	0.110	0.193*	0.139	0.037	0.110
Estradiol	0.201**	-0.279##	0.200**	0.146	-0.037	-0.022	0.180*	0.145	-0.029	0.167*

*$p \le 0.05$. **$p \le 0.01$. #$p \le 0.005$. ##$p \le 0.001$.

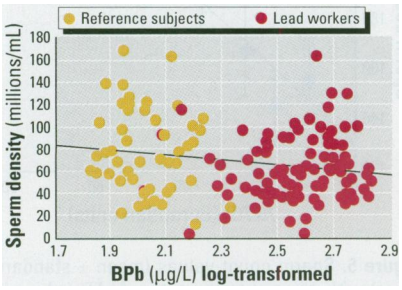

Figure 1. Relationship between sperm density and BPb (log-transformed) in 51 reference subjects and 98 lead workers. Sperm density = -21.24 log BPb + 119.29; r = -0.194, p = 0.018.

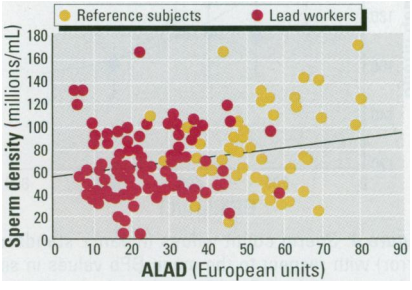

Figure 2. Relationship between sperm density and ALAD in 51 reference subjects and 98 lead workers. Sperm density = 0.44 ALAD + 54.10; r = 0.245, p = 0.003.

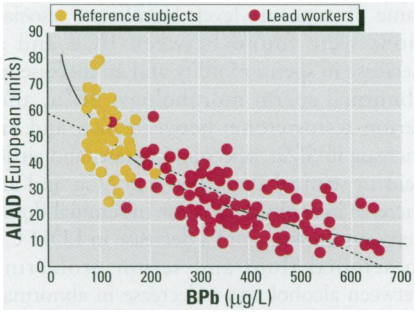

Figure 3. Relationship between ALAD and BPb in 51 reference subjects and 98 lead workers. The solid line is ALAD = -51.59 log BPb + 156.13; r = -0.849, $p < 0.0001$. The dotted line is ALAD = -0.087 BPb + 58.90; r = -0.814, $p < 0.0001$.

the true internal Pb dose that caused the reproductive effect, when combined they may serve as a reliable indirect assessment of the Pb amount at the site(s) of its effect in the body. The results confirmed the aforementioned Pb-related reproductive effects (Table 4) and showed their more significant association with ALAD as compared to BPb, and particularly EP. In this regard it must be stressed that BPb consistently showed a curved relationship with each of the reproductive parameters; the highest Pearson's *r*-value was obtained when BPb was log-transformed. On the other hand, ALAD consistently showed a straight relationship with reproductive parameters (the only exception was the curved relationship with semen volume), and the same was generally the case for BCd, SZn, SCu, smoking, alcohol, and age. Only EP showed an inconsistent trend and mainly a kind of curved relationship with reproductive parameters.

The individual models evaluating the association of BPb (log-transformed), ALAD, and EP with the reproductive parameters were calculated by adjusting for potentially confounding variables. Log BPb was a significant predictor of sperm density, counts of total and progressively motile sperm, abnormal sperm head morphology, SfZn, and acid phosphatase and citric acid in seminal fluid. ALAD was a significant predictor of semen volume; sperm density; counts of total, motile, and viable sperm; both the percentage and count of progressively motile sperm; abnormal sperm head morphology; SfZn; acid phosphatase and citric acid in seminal fluid; and serum testosterone and estradiol. EP was a significant predictor of counts of total and progressively motile sperm, SfZn, and acid phosphatase in seminal fluid.

The results (Table 5) also show significant ($p < 0.05$) influence of BCd, SZn, SCu, smoking, alcohol, or age on certain reproductive parameters, which could have contributed to interindividual differences in the values of reproductive parameters at the same Pb-exposure level. Significant associations were found between BCd and a decrease in sperm motility and an increase in abnormal sperm morphology, SfZn, and serum testosterone; between SZn and an increase in SfZn, acid phosphatase, and citric acid in seminal fluid; between SCu and an increase in acid phosphatase in seminal fluid; between smoking and a decrease in LDH-C_4 in seminal fluid and serum prolactin; between alcohol and a decrease in abnormal sperm head morphology and an increase in sperm motility and viability, SfZn, acid phosphatase, and citric acid in seminal fluid, and serum estradiol; and between age and an increase in serum FSH. These effects were of lower rank and intensity as compared to Pb-related effects on reproductive parameters, with the exception of the contribution of BCd to an increase in the percentage of pathologic sperm (regardless of the site of abnormal sperm morphology, as opposed to Pb effect) and serum testosterone.

Results related to seminal fluid concentrations of Pb and Cd. Additional SfPb and SfCd measurements were carried out in 118 of the 149 subjects. As compared to the remaining 31 subjects without SfPb and SfCd measurements, these 118 subjects had a significantly higher semen volume ($p < 0.02$) and fructose in seminal fluid ($p < 0.005$), and a tendency ($p < 0.10$) toward higher ALAD, SfZn, acid phosphatase, and citric acid in seminal fluid. No other variables (from age to estradiol) differed significantly.

Table 6 presents the results of SfPb and SfCd in 118 subjects, as well as the concomitant BPb and BCd levels in the same

Table 5. The stepwise multiple regression results for significant ($p < 0.05$) relationships between each of the reproductive parameters considered with respect to all of the variables [BPb (log-transformed), ALAD, EP, BCd, SZn, SCu, smoking habits, alcohol consumption, and age] in 149 subjects.

Parameter	Equation (respects the sequence of significant variables entered in the equation)	*r*	*p*
Semen volume	Nonsignificant[a]	–	–
Sperm density	= 0.439 ALAD + 54.103	0.245	0.0027
Sperm count	= 1.763 ALAD + 126.129	0.269	0.0010
Motile sperm (%)	= 0.372 alcohol - 0.573 BCd + 41.292	0.282	0.0024
Motile sperm count	= 0.704 ALAD + 55.087	0.223	0.0068
Progressively motile sperm (%)	= 0.099 ALAD + 25.015	0.194	0.0178
Progressively motile sperm count	= 0.611 ALAD + 35.079	0.248	0.0025
Viable sperm (%)	= 0.377 alcohol + 45.573	0.201	0.0145
Viable sperm count	= 0.780 ALAD + 64.698	0.218	0.0109
Pathologic sperm (%)	= 0.630 BCd + 31.290	0.224	0.0061
Head/pathologic sperm (relative %)	= 11.364 log BPb - 0.457 alcohol + 32.944	0.321	0.0004
LDH-C_4	= -0.122 smoking + 21.015	0.196	0.0167
Fructose	Nonsignificant	–	–
SfZn	= 1.373 ALAD + 3.857 alcohol + 0.131 SZn + 4.380 BCd - 83.686	0.458	0.0000
Acid phosphatase	= -258.136 log BPb + 0.647 SZn + 12.318 alcohol + 0.297 SCu + 97.358	0.437	0.0000
Citric acid	= 0.022 SZn - 9.064 log BPb + 0.482 alcohol + 23.074	0.362	0.0001
FSH	= 0.090 Age + 1.154	0.194	0.0178
LH	Nonsignificant	–	–
Prolactin	= -0.090 smoking + 7.809	0.276	0.0007
Testosterone	= 0.756 BCd - 0.100 ALAD + 33.100	0.343	0.0001
Estradiol	= -0.001 ALAD + 0.002 alcohol + 0.186	0.325	0.0003

[a]Significant relationship for semen volume was found only with log-transformed ALAD (semen volume = 0.699 log ALAD + 1.683; $r = 0.188$, $p = 0.022$), indicating that a decrease in semen volume may occur at ALAD < 20 European units, which is equivalent to BPb > 400 µg/L in the population studied.

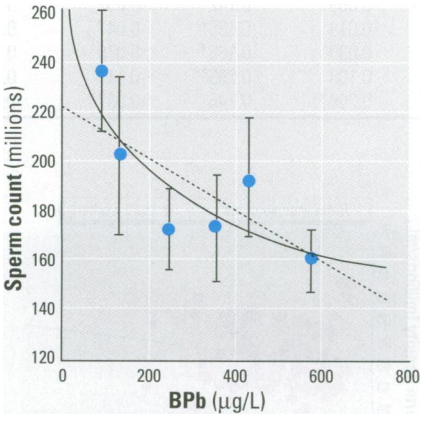

Figure 4. Sperm count values (mean ± standard error) with respect to the mean BPb values in six subgroups of the 149 subjects divided according to increasing BPb. The relationship between sperm count and BPb in all 149 subjects is shown by the solid line (sperm count = -74.08 log BPb + 360.88; $r = -0.184$, $p = 0.025$) and the dotted line (sperm count = -0.12 BPb + 219.01; $r = -0.166$, $p = 0.043$).

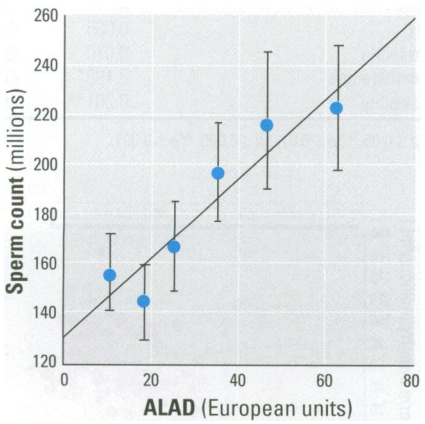

Figure 5. Sperm count values (mean ± standard error) with respect to the mean ALAD values in six subgroups of the 149 subjects divided according to decreasing ALAD. The relationship between sperm count and ALAD in all 149 subjects: sperm count = 1.76 ALAD + 126.13; $r = 0.269$, $p = 0.001$.

subjects, divided into subgroups with regard to occupational Pb exposure and smoking habit. A highly significant ($p < 0.0001$) difference was found for the SfPb and BPb levels between Pb workers and reference subjects and for the SfCd and BCd levels between smokers and nonsmokers. In addition, considerably higher BCd was found in Pb workers as compared to reference subjects and BPb in smokers as compared to nonsmokers, both of which can mainly be ascribed to the higher incidence of smokers among the Pb workers than among the reference subjects (70 and 51%, respectively). The same trend was relatively less pronounced for the corresponding SfCd and SfPb levels, which may be explained by the fact that blood indicators (BPb and BCd) mainly reflect current or recent exposure to the metal, whereas seminal fluid indicators (SfPb and SfCd) appear to better reflect long-term cumulative exposure to the metal (*41,54*).

Results of the Spearman's rank correlation in 118 subjects were highly significant ($p < 0.0001$) between SfPb and BPb levels ($r = 0.571$) and between SfCd and BCd levels ($r = 0.490$). In addition, a highly significant ($p < 0.0001$) correlation was found between SfPb and ALAD ($r = -0.501$), SfPb and EP ($r = 0.461$), SfPb and SfCd ($r = 0.430$), SfCd and smoking habits ($r = 0.499$), and BCd and smoking habits ($r = 0.787$). Table 7 shows the correlations between each of the measured parameters of semen quality with respect to BPb, SfPb, ALAD, EP, BCd, SfCd, SZn, SCu, smoking, alcohol, age, and occupational Pb exposure duration (in the latter case by assuming the value of zero years in the 35 reference subjects). A significant ($p < 0.05$) correlation was found between several parameters of decreased semen quality with respect to BPb, ALAD, and/or EP. However, SfPb significantly correlated only with a decrease in the percentage and count of progressively motile sperm. An even more striking difference was found between BCd and SfCd concerning their correlations with parameters of semen quality. Although BCd significantly correlated with an increase in percentage of pathologic sperm ($p < 0.005$), this was not the case with respect to SfCd. Moreover, a significant correlation was found between an increase in SfZn, acid phosphatase, and citric acid in seminal fluid with respect to SfCd ($p < 0.001$, $p < 0.01$, and $p < 0.0001$, respectively), which might suggest even a beneficial effect of Cd on prostate secretory function, as opposed to the nonsignificant correlation of these parameters with BCd. Data on significant ($p < 0.05$) correlations between several other parameters in 118 subjects (Table 7) are similar to those found in all 149 subjects (Table 4). Nevertheless, in the present group of subjects with relatively greater semen volume than the rest of study population, SZn showed a relatively more pronounced beneficial influence on several parameters of semen quality, that is, an inverse effect of Zn as compared to that of Pb (Table 7).

Table 6. Median and range values of SfPb, BPb, SfCd, and BCd within subgroups with regard to occupational lead exposure and smoking habit in 118 subjects.

Subgroups	SfPb (µg/L)	BPb (µg/L)	SfCd (µg/L)	BCd (µg/L)
Reference subjects ($n = 35$)	8.6 (4.2–16.6)	103 (67–208)	0.67 (0.17–3.56)	1.05 (0.20–10.80)
Lead workers ($n = 83$)	15.3 (6.5–48.3)*	363 (119–659)*	0.75 (0.20–2.47)	3.60 (0.19–13.33)
Nonsmokers ($n = 42$)	12.5 (4.8–32.3)	253 (70–624)	0.54 (0.17–1.67)	0.46 (0.19–1.49)
Smokers ($n = 76$)	13.9 (4.2–48.3)	325 (67–659)	0.85 (0.29–3.56)**	4.35 (0.49–13.33)**

*$p < 0.0001$ as compared to reference subjects. **$p < 0.0001$ as compared to nonsmokers.

Discussion

The overall results of this study indicate that even moderate exposure to Pb (BPb < 400 µg/L, exposure duration ≥ 2 years) can significantly reduce human semen quality and can influence reproduction hormones, although there was no conclusive evidence of impairment of male reproductive endocrine function. Better correlations of reproductive parameters were generally found with ALAD activity than with BPb, EP, or SfPb. This can be ascribed to the fact that ALAD activity is considerably more sensitive than EP in discriminating between different relatively low Pb exposure levels, especially at BPb < 250 µg/L (*38,39*). ALAD activity better reflects long-term cumulative Pb exposure in humans, as compared to BPb, which mainly reflects current or recent Pb exposure level (*37,38*). This characteristic feature of BPb may help explain why the log-transformed BPb values correlated better than original (nontransformed) BPb with each of the reproductive parameters (which is related to the Pearson's correlations); in the study population, actual levels of BPb > 400 µg/L represent merely an excessive Pb exposure episode rather than long-term average Pb exposure level. Under circumstances of fluctuating Pb exposure and BPb levels (typical for occupationally exposed subjects), ALAD activity may provide better assessment than BPb of the amount of accumulated Pb at the site(s) of its effect(s) in the body because it better predicts the amount of chelatable Pb excreted in urine, which originates from peripheral blood and soft tissues (*37*). At a same level of BPb, the simultaneous ALAD

Table 7. The Spearman's rank correlation coefficient and the level of significance (r, p) for relationships between the parameters of semen quality with respect to biomarkers of lead (BPb, SfPb, ALAD, EP), cadmium (BCd, SfCd), zinc (SZn), and copper (SCu), smoking habits, alcohol consumption, age, and occupational lead exposure duration (ED) in 118 subjects.

Parameter	BPb	SfPb	ALAD	EP	BCd	SfCd	SZn	SCu	Smoking	Alcohol	Age	ED
Semen volume	0.037	-0.133	0.077	-0.036	0.058	-0.099	0.150	-0.137	-0.003	-0.179*	0.037	-0.052
Sperm density	-0.124	-0.028	0.212*	-0.201*	-0.104	0.005	0.265#	-0.088	-0.082	0.112	-0.090	-0.214*
Sperm count	-0.103	-0.148	0.230**	-0.184*	-0.064	-0.081	0.318##	-0.162	-0.071	-0.008	-0.045	-0.204*
Motile sperm (%)	0.024	0.000	0.065	-0.039	-0.158	-0.088	0.148	-0.187*	-0.028	0.090	0.077	-0.001
Motile sperm count	-0.069	-0.124	0.210*	-0.150	-0.123	-0.106	0.296##	-0.192*	-0.078	0.035	-0.016	-0.171
Progressively motile sperm (%)	-0.134	-0.212*	0.197*	-0.190*	-0.139	-0.172	0.132	-0.050	-0.070	0.042	0.144	-0.193*
Progressively motile sperm count	-0.131	-0.197*	0.252**	-0.206*	-0.129	-0.156	0.290#	-0.138	-0.094	0.047	0.020	-0.237**
Viable sperm (%)	0.066	-0.004	0.030	-0.011	-0.116	-0.093	0.140	-0.220*	-0.009	0.066	0.066	0.024
Viable sperm count	-0.060	-0.122	0.201*	-0.156	-0.107	-0.107	0.298##	-0.193*	-0.075	0.040	-0.022	-0.168
Pathologic sperm (%)	0.143	-0.081	-0.151	0.119	0.260#	-0.082	-0.140	0.080	0.141	-0.092	-0.075	0.018
Head/pathologic sperm (relative %)	0.204*	-0.006	-0.146	0.208*	0.171	0.048	-0.127	0.085	0.017	-0.010	0.025	0.090
LDH-C_4	-0.013	-0.089	0.085	-0.029	-0.124	-0.158	0.025	-0.113	-0.244**	0.118	-0.115	-0.122
Fructose	-0.079	-0.117	0.095	-0.122	-0.108	-0.160	-0.080	-0.059	-0.160	-0.107	0.008	-0.069
SfZn	-0.170	-0.007	0.231**	-0.163	0.140	0.310##	0.248**	0.025	0.173	-0.071	0.071	-0.212*
Acid phosphatase	-0.208*	-0.007	0.229**	-0.178*	0.035	0.253**	0.245**	0.118	0.098	-0.016	0.039	-0.249**
Citric acid	-0.222*	0.015	0.217*	-0.203*	0.079	0.376##	0.210*	0.062	0.156	-0.033	0.088	-0.230**

*$p \leq 0.05$. **$p \leq 0.01$. #$p \leq 0.005$. ##$p \leq 0.001$.

activity is relatively less depressed in subjects with short-term Pb exposure or recently increased exposure level, for example, in most of the subjects with actual BPb > 400 µg/L in the present study (Figure 3), as compared to those who had long-term exposure to a similar Pb exposure level or who had been excessively exposed in the past (*37,38*). This can explain why, despite the highly significant correlation between BPb and ALAD ($p < 0.0001$), the ranking of the subjects in a sequence of increasing Pb exposure intensity may not be identical when it is based on actual BPb and ALAD levels of an individual (Figures 4 and 5). Furthermore, exposure to Pb can decrease the absorption rate and biologic availability of Zn in the body, mainly because of their competition for binding to the sulfhydryl (-SH) group site(s) in various enzymes, other proteins [especially metallothionein (MT)], and tissues (*42*). Because ALAD is a Zn-containing enzyme that is extremely sensitive to Pb, and because the inhibition of ALAD activity results from direct substitution of Zn by Pb, ALAD activity may better correlate than BPb with Pb-related effects which are susceptible to alterations of Zn status in the body. For example, the quantitative relationships between sperm density and sperm count with respect to ALAD (Figures 2 and 5) were persistent, and the correlations remained significant ($p < 0.05$) even within the relatively small group of 98 Pb workers, which was not the case for BPb. The polymorphism of human ALAD, which was occasionally suspected of modifying susceptibility to Pb, has no influence on ALAD activity at comparable BPb levels (*55*) and no significant relationship with sperm density and sperm count in men (*12*).

The observed Pb-related effects on semen quality have also been indicated, to a certain extent, in the studies of other authors. These effects include a decrease in semen volume (*17,21,26*), a decrease in sperm density and sperm count (*11,13,14,16,17,20,21*), a decrease in sperm motility (*15,16,20,21,28*) and the quality of motility (*17,28*), an increase in abnormal sperm morphology (*16,17,20,21,26*) [particularly at the head of the sperm (*17,21,26*)], and impairment of the prostate secretory function as indicated by decreased SfZn (*26*). In most of these studies, significant alterations of reproductive parameters were observed at relatively higher BPb levels (> 400 µg/L), and only on a group basis (or in a few individuals); therefore, insufficient evidence was presented for establishing relevant dose–response relationships. The present study shows no significant Pb-related influence on serum levels of FSH, LH, and prolactin, and the observed slight increases in serum testosterone and estradiol can hardly be regarded as impairment of male reproductive endocrine function. The Pb-related increases in serum testosterone and estradiol are difficult to explain, although it is possible that they were due to the increase in sex-hormone binding globulin (which unfortunately was not measured in the present study) (*22,24*) and would have no adverse effect on spermatogenesis. The published data concerning Pb-related effects on reproductive endocrine function in men are generally controversial (*10,11,13,14,16,18–20,22–24*). However, our results corroborate the results of other studies that showed a Pb-related decrease in human semen quality without reproductive endocrine dysfunction (*11,13,16,20*).

The study results indicate that even moderate exposure to Cd (BCd < 10 µg/L) can increase abnormal sperm morphology, as shown by a significant correlation of increasing percentage of pathologic sperm with BCd (Tables 5 and 7), although not with SfCd (Table 7), which is in accordance with the results of other authors (*15,32*). The results also show a significant correlation of BCd with a decrease in sperm motility and an increase in serum testosterone (Table 5). In the study population, increased exposure to Cd was mostly because of the smoking habit, which itself may adversely affect reproductive parameters by mechanisms of oxidative stress involving other compounds present in cigarette smoke (*42*). However, the increase in abnormal sperm morphology and in serum testosterone and the decrease in sperm motility correlated considerably better with BCd levels than with smoking habits (Tables 4 and 7) and thus appear to be independent of possible influence of other cigarette smoke constituents. This was not the case for LDH-C_4 in seminal fluid and for serum prolactin, which correlated better with smoking habits than with BCd levels (Tables 4 and 7).

The observed differences between SfPb and BPb and between SfCd and BCd, with regard to their correlation with parameters of semen quality (particularly those concerning prostate secretory function) (Table 7), appear to suggest possible toxicokinetic interaction through the influence of MT, especially as it may affect the levels of SfCd and SfZn, and to a lesser extent SfPb. Both Cd and Zn are powerful inducing agents for MT biosynthesis, and MT binds various metals including Pb, Cd, Zn, and Cu (*42*). Thus, any alteration of the MT amount in a certain body compartment may also influence the concentration of elements other than the inducing agent (*34*). In this way, actual SfPb, SfCd, and SfZn levels may be insufficiently representative of the metal bioavailability because binding of Pb and Cd to MT decreases the toxicity of Pb and Cd and also decreases the capacity of MT for its main purpose of storage and supply of intracellular Zn (*42*). It is also possible that SfPb and BPb and SfCd and BCd levels of an individual may not follow the same dynamic pattern with regard to recent changes in intensity of metal exposure (*41,54*) and may not be equally representative of the metal amount at the site(s) of its effect in the body and/or the time when the effect could have developed. In general, such findings are difficult to explain because of the limited knowledge about toxicokinetics of metals in human male reproductive organs under chronic exposure conditions.

The observed reproductive effects of both Pb and Cd appear to involve their interaction with Zn, which is essential for male reproductive function. Although Pb appears to produce relative Zn deficiency, Cd appears to mainly affect the distribution of Zn in the body. Because Zn is required for optimum activity of > 200 enzymes including those involved in the synthesis and repair of DNA and RNA, and thus related protein synthesis and tissue repair response, this may have multiple adverse consequences (*42*). However, alterations in the amount and/or biologic availability of Zn in certain body compartments (e.g., through the Pb- and Cd-related decrease in the capacity of MT to provide optimum supply of Zn to the cell) may influence sperm proliferation, maturation, and viability.

Conclusions

With regard to the WHO-proposed no-adverse-effect levels of BPb and BCd in adult male subjects (*53*), the results of this study indicate that even moderate exposure to Pb (BPb < 400 µg/L) and Cd (BCd < 10 µg/L) can significantly reduce reproductive capacity in men. This appears to be at least partly mediated through their interference with Zn metabolism, where the Pb- and Cd-exposure duration can have a highly important role. No threshold could be observed for the Pb- and Cd-related decrease in semen quality. Smoking habits, alcohol consumption, and age may have an independent influence on certain reproductive parameters apart from their contribution to the increase in cumulative exposure to Pb and Cd in the individual. These influences may result in interindividual differences in several reproductive parameters at the same BPb and BCd levels. Blood indicators of individual exposure to Pb and Cd (BPb and BCd) may be superior to seminal fluid indicators (SfPb and SfCd) with regard to their correlation with parameters of decreased semen quality in men.

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