REVIEW

Male reproductive toxicity of lead in animals and humans

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Abstract

***Objective*—To critically review the literature on male reproductive toxicity of lead in animals and humans.**

***Methods*—A systematic literature search identified a total of 32 experimental studies in animals and 22 epidemiological studies, one case report on humans and five review articles or documents. The studies were evaluated by paying attention mainly to sample size, study design, exposure, and dose characterisation, analytical method standardisation, and quality assurance.**

***Results*—Several studies on rats and other rodents indicated that blood lead concentrations >30–40 μg/dl were associated with impairment of spermatogenesis and reduced concentrations of androgens. However, other animal studies, mainly about histopathological, spermatozoal, and hormonal end points, indicated that certain species and strains were quite resistant to the reproductive toxicity of lead and that different testicular lead concentrations could account for these differences. The human studies focused mainly on semen quality, endocrine function, and birth rates in occupationally exposed subjects, and showed that exposure to concentrations of inorganic lead >40 μg/dl in blood impaired male reproductive function by reducing sperm count, volume, and density, or changing sperm motility and morphology. No relevant effects were detected on endocrine profile.**

***Conclusion*—Several factors make it difficult to extrapolate the animal data to the human situation. The difficulties are mainly due to differences between species in reproductive end points and to the level of exposure. Concentrations of blood lead >40 μg/dl seemed to be associated with a decrease in sperm count, volume, motility, and morphological alterations and a possible modest effect on endocrine profile. Dose-response relation, in particular at a threshold level, is poorly understood, and site, mode, or mechanism of action are unknown. Also, the effects were not always the same or associated in the same way, although the prevalent effects were on sperm count and concentration. Some methodological issues and indications for future studies are discussed.**

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The toxicity of lead (Pb) has been known for millennia and has become the most complete and useful model for industrial toxicology studies.[1]

In modern industrial activities, preventive measures and technological changes have reduced occupational exposure to the metal, especially in traditional settings—such as manufacture of batteries and ceramics or work in non-ferrous alloy foundries. Moreover, the use of Pb in gasoline should be mentioned as an important environmental source of exposure, even if it has decreased in the past decades.

On the other hand, scientific research has acquired knowledge that Pb concentration in the blood (PbB) in the range 20–50 μg/dl can cause adverse effects. The organs or systems which may be affected at such exposures can be the haemopoietic, nervous, cardiovascular, reproductive, and immune systems.

It is known that Pb influences biological enzyme systems and it can be assumed that numerous mechanisms of interaction are yet to be elucidated. Furthermore, the regulatory mechanisms of the male reproductive system are very complex and also not completely understood. It is likely that Pb interacts with one or more of those mechanisms: it could be on reproductive organs at different levels, or on endocrine control of reproduction, or both.

The question of the existence of a threshold dose for these effects is still a matter of scientific and regulatory debate, with social and research implications.[2–5]

The present paper critically reviews experimental and epidemiological studies of reproductive toxicity of Pb in males to summarise current knowledge and to suggest possible further research in this field.

Methods

We identified 32 experimental studies, 22 epidemiological studies, and one case report on male reproductive toxicity of lead and five

review articles or documents (generally not concerned with lead effects in particular).[6–10]

The literature search was carried out on the most relevant medical and toxicological data bases (OSHROM, Medline), with combinations of the following key words: lead; toxicity; occupational exposure; male; reproduction; epidemiological studies; animal studies. Besides the data base search, the reference lists of the selected papers were screened for other articles that could be useful. The search showed that the most scientifically important papers started from the early 1970s. Occasional articles appeared before 1960, but their validity is severely limited by the methods of evaluation of Pb dose and effects, which are not comparable with the ones adopted in the past two decades.

For the animal data, only in vivo experiments were considered which focused on hormonal and spermatozoal end points, and lead exposure had to have occurred after birth; papers with intrauterine exposure alone were not selected.

For the human data, practically all the articles concerning spermatozoal, histopathological, and hormonal aspects, and birth rates in workers exposed to lead which appeared after the early 1970s were selected; these were also verified by cross checking the reference lists of the various articles.

The papers were reviewed in accordance with quality criteria defined for experimental and epidemiological studies.

In particular, animal studies were evaluated by paying attention to the strain of the investigated animals, the dosage schedule, and internal dose monitoring, the age at start of the experiment, signs of systemic intoxication, and the number of animals in each exposure group.

The epidemiological and clinical papers were characterised for the presence or absence of quality control in the analytical procedures, the use of standardised criteria for semen evaluation, the size of the study population, the presence of a control group, and its accuracy for the outcome measured, the definition and assessment of dose and exposure variables, the range of exposures of interest with particular attention to dose-response evaluation, the adequacy of the statistical analysis, and control for relevant confounding variables.

Results and discussion

ANIMAL STUDIES

Table 1 summarises the results of studies in rats, mice, rabbits, and monkeys. The male reproductive organs of Sprague-Dawley rats and NMRI mice are apparently rather resistant to the toxicity of inorganic Pb. However, several studies of other rat strains and other rodents indicate fairly consistently that blood Pb concentrations >30–40 μg/dl during at least 30 days are associated with impairment of spermatogenesis and reduced concentrations of circulating androgens. There is no indication that exposure during prenatal life and before puberty causes more severe reproductive toxicity than exposure after sexual maturity has been reached. On the contrary, few studies failed to show reproductive effects after exposure during gestation and the prepubertal period. Most results are compatible with direct toxic effects of Pb on seminiferous tubules or Leydig cells, but one study reported simultaneous impairment of spermatogenesis and reduced pituitary content of follicle stimulating hormone (FSH), which points to a primary action at the pituitary level. Neither the mode nor the mechanism of male reproductive toxicity of Pb is unravelled by existing publications.

In the animal experiments, several factors can interfere with the results: type of Pb compound, presence of systemic intoxication, age at start of the experiment, duration of exposure, variability between and within species, and biological variation in hormone concentrations.

Most of the studies used Pb acetate as the Pb derivative, and as most also measured internal dose by monitoring PbB both Pb derivative and administration route are not considered as major interfering factors for absorption. However, different solubility profiles of Pb compounds could cause different Pb concentrations in the target organs. Except for the few studies that assessed testicular Pb concentrations, this factor cannot be controlled for.

Systemic intoxication is important to consider when interpretating the results: three of the 32 studies did not assess systemic toxicity and eight reported signs of systemic intoxication, of which seven studies found negative effects on male reproduction.

Age and maturity of the animal may have bearings for the results in several ways. It has been shown that prepubertal rats are less sensitive to the toxic effects of Pb on testosterone and sperm production than animals with exposure to Pb beginning after puberty has been initiated.[26] The distribution patterns of Pb in tissues may differ significantly when lead exposure occurs during the later stages of life.[43] Similarly, Momcilovic and Kostial found marked differences in Pb distribution in suckling rats compared with adult rats.[44] Thus, the same exposures can cause different tissue concentrations at different ages. Therefore we can assume that in rats different organs can be at risk at various ages. Moreover, spontaneous diseases of age should also be considered; up to 7% of rats maintained for 52 weeks show spermatogenesis not proceeding beyond the spermatocyte stage. At 104 weeks 20% of rats develop atrophy of the seminiferous epithelium.[45] In rat studies it is therefore important to specify the age at the start of the experiment. The experiments performed on animals before maturity is reached have exposures more similar to environmental than occupational, as higher concentrations of PbB are reached. The experiments on mature animals seem to be more relevant for occupational exposure. Of the 21 rat studies reviewed in this paper, 15 have stated adequately (numerically) the age at start of the experiment. However, only in two studies was sexual maturity (90 days) reached. In another four studies, age at start was only described as "mature". The remaining three experiments

Table 1 Studies on histopathological and spermatozoal end points in animals

Reference	*Strain*	*(a) Dosage schedule† (b) Corresponding mean blood lead concentration† (c) Corresponding testicular lead concentration†*	*Age at start*	*Duration of exposure*	*Signs of systemic intoxication*	*Observed effects (n)*
Rats:						
Hilderbrand *et al* 1973[11]	Sesco	(a) 0-5-100 µg la in drinking water daily (b) 14-19-30 µg/dl (c) NA	Mature	30 days	None	At 30 µg/dl: decreased sperm motility; prostatic hyperplasia (20) At 50 µg/dl: atrophy of the seminiferous tubules with inhibition of spermatogenesis (4) No changes in weight of testes and seminal vesicles (20) Impotence
Der *et al* 1976[12]	Sprague-Dawley	(a) 0-50-250 µg la ip daily (b) 23-23-73 µg/dl (c) NA	NS	70 days	None	No weight change, nor histopathological changes in testis, epididymis, seminal vesicles, and prostate (10)
Krasovskii *et al* 1979[13]	NS	(a) 0-0.0015-0.005-0.05 mg la/kg/d in drinking water (b) NA (c) NA	NS	20–30 days	NS	Decreased motility, lower osmotic stability and reduced time of motility of spermatozoa for the 0.05 mg/kg group (NS)
		(a) 0-0.0015-0.005-0.05 mg la/kg/d in drinking water (b) NA (c) NA	NS	180–360 days	NS	Reduced spermatogenesis in the 0.05 mg/kg group (NS)
Petrusz *et al* 1979[14]	Long-Evans hooded	(a) 0-25-100-200 mg la/kg/d by gastric gavage (b) 7-203-1013-1055 µg/dl (at 15 days) (c) NA	Neonatal	20 days	None	Unchanged pituitary LH content and serum FSH concentrations (4–10) Increased pituitary FSH content (4–10) No changes in pituitary weight (4–10)
Fowler *et al* 1980[15]	CD	(a) 0-5-25-50 ppm la in drinking water (b) 4-14-25-37 µg/dl* (c) NA	Intra-uterine	Until 6 months of age	None	No effects on epididymal sperm count (5)
		(a) 0-0.5-5-25-50-250 ppm la in drinking water (b) 5-4.5-11-21-26-67 µg/dl* (c) NA	Intra-uterine	Until 9 months of age	None	No effects on testicular weight, nor on epididymal sperm count in any lead exposure group (4–8)
Chowdhury *et al* 1984[16]	NS	(a) 0-0.25-0.5-1.0 g la/l in drinking water (b) 8-54-71-142 µg/dl (c) 0.3-1.7-1.6-5.3 µg/g	Mature	60 days	Present (dose related)	At 71 µg/dl: partial inhibition of spermatogenesis (NS) At 142 µg/dl: testicular atrophy with cellular degeneration (NS)
Sokol *et al* 1985[17]	Wistar	(a) 0-0.1-0.3% la in drinking water (b) <7-34-60 µg/dl (c) NA	52 days	30 days	None	Dose related suppression of spermatogenesis (NS) Dose related decrease of intratesticular and serum testosterone, suppression of FSH (NS)
Chowdhury *et al* 1986[18]	NS	(a) 0-1-2-4-6 mg la/kg/d ip (b) 20-62-87-187-325 µg/dl (c) 0.4-1.6-2.0-2.6-4.3 µg/g	Mature	30 days	Present (dose related)	Dose related decrease of testis weight (15) At 187 µg/dl: degenerative changes in testicular tissues (NS) At 325 µg/dl: degenerative changes and injury of spermatogenic cells; oedematous dissociation in interstitial tissue (NS)
Saxena *et al* 1987[19]	Wistar	(a) 0–8 mg Pb^{2+}/kg/d ip (b) NA (c) NA	21 days	100 days	Present	Disturbed spermatogenesis (10) Leydig cell degeneration (10)
Chowdhury *et al* 1987[20]	Charles Foster	(a) 0-1-2-4-6 mg la/kg/d ip (b) 5-56-91-196-332 µg/dl (c) 0.4-2.0-1.6-2.6-4.3 µg/g	NS	30 days	Present (dose related)	Dose related decrease of testis weight (18) At 56 µg/dl: decline of spermatids (6) At 91 µg/dl: inhibition of postmeiotic spermatogenic cell (6) At 196 µg/dl: Decreased spermatogenic cell count (6) Detachment of germinal cell layers (6) At 332 µg/dl: Decreased spermatogenic cell count (6) Degenerative changes (6) Interstitial oedema (6) Atrophy of Leydig cells (6)
Sokol 1987[21]	Wistar	(a) 0–0.3% la in drinking water (b) <7–30 µg/dl (c) NA	52 days	30 days	None	Hyperresponsiveness to stimulation with both GnRH and LH (10) Blunted response to naloxone stimulation (10)
Boscolo *et al* 1988[22]	Sprague-Dawley	(a) 0–60 mg la/ml in drinking water (b) 4–17 µg/dl (c) <0.07– <0.10 µg/g	Weaning	540 days	None	Increased vacuolisation in Sertoli cells (10) No other ultrastructural modifications (10) No impairment of spermatogenesis (10)
Sokol 1989[23]	Wistar	(a) 0–0.6% la in drinking water (b) <3–43 µg/dl (<4–18 µg/dl after recovery period) (c) NA	27 days	30 days (+30 days recovery)	None	Suppressed intratesticular sperm counts, sperm production rate and serum testosterone in both lead treated groups (10–10) Sperm parameters and serum testosterone normalised at the end of the recovery period in the prepubertal animals (27 days at start) (10), but not in the pubertal animals (52 days at start) (5)
		(a) 0–0.6% la in drinking water (b) <8–58 µg/dl (<8–20 µg/dl after recovery period) (c) NA	52 days	30 days (+30 days recovery)	Present	
Barratt *et al* 1989[24]	Wistar	(a) 0-0.3-33-330 mg la/kg/d in drinking water, by gavage (b) 2-4.5-7-80 µg/dl (c) NA	70 days	63 days	Present (highest dose)	Increased number of abnormal posttesticular sperm in the highest exposure group (8) Reduced number of spermatozoa at exposure of 4.5 µg/dl (8)

Table 1 continued

Reference	*Strain*	*(a) Dosage schedule†* *(b) Corresponding mean blood lead concentration†* *(c) Corresponding testicular lead concentration†*	*Age at start*	*Duration of exposure*	*Signs of systemic intoxication*	*Observed effects (n)*
Sokol 1990[25]	Wistar	(a) 0–0.6% la in drinking water (b) controls : <8 µg/dl at any time exposed: 42-60-58-75 µg/dl after 7, 14, 30 and 60 days respectively (c) NA	52 days	7-14-30-60 days	None	Decreased sperm concentration, sperm production rate and suppressed serum testosterone concentrations after 14 days of exposure; not dose related (NS)
Sokol and Berman 1991[26]	Wistar	(a) 0-0.1-0.3% la in drinking water (b) <7-25-36 µg/dl (c) NA	42 days	30 days	None	Dose related suppression of spermatogenesis (decreased sperm count and sperm production rate) in the exposed rats of the two highest age groups (8–11)
		(a) 0-0.1-0.3% la in drinking water (b) <7-35-60 µg/dl (c) NA	52 days	30 days	None	Dose related suppression of serum testosterone, starting at 35 µg/dl in 52 day old rats (8) and at 37 µg/dl in 70 day old rats (10–11)
		(a) 0-0.1-0.3% la in drinking water (b) <7-37-40 µg/dl (c) NA	70 days	30 days	None	
Murthy *et al* 1991[27]	NS	(a) 0–250 ppm la in drinking water (b) 4.5–20 µg/dl (c) 0.1–0.8 µg/g	Weaning	70 days	None	At 20 µg/dl no impairment of spermatogenesis (5) Vacuolisation of Sertoli cell cytoplasm and increase in number and size of lysosomes (5)
Nathan *et al* 1992[28]	Sprague-Dawley	(a) 0-0.05-0.1-0.5-1% la in drinking water (b) 2.3-40-44-80-124 µg/dl (c) NA	Mature	70 days	Present (highest dosage)	No effects on spermatogenesis in all groups (7–8) At 124 µg/dl: decreased seminal vesicle weight (7) Decreased serum testosterone in the 0.5% group at 10 weeks (8) No effects in the other exposure categories (7–8) No effects on serum FSH, LH, nor pituitary LH content (7–8)
Pinon-Lataillade *et al* 1993[29]	Sprague-Dawley	(a) 0–0.3% la in drinking water (b) <4–58 µg/dl (c) NA	90 days	70 days	None	No changes in testicular and epididymal weights (8) Decreased weight of seminal vesicles in inhalation study (8) No effects on spermatogenesis (4)
		(a) 5 mg/m^3 lo in aerosol, during 6 h a day, 5 days a week (b) <4–51 µg/dl (c) NA	90 days	70 days	None	No effects on epididymal sperm count, spermatozoal motility and morphology (8) No effects on plasma testosterone, LH, and FSH (8) No effects on fertility (8–12) Decrease in epididymal sperm count of progeny of sires of the inhalation group, however without effect on their fertility (24)
Marchlewicz *et al* 1993[30]	Wistar	(a) 0–1% la in drinking water (b) 0.36–1.23 µg/g (c) 0.4–0.6 µg/g (epid 0.5-0.7 µg/g)	90 days	270 days	None	No changes in testicular and epididymal weight (NS) No histological changes in testis and epididymis (NS) Fever spermatozoa in all zones of the epididymis (NS)
Kempinas *et al* 1994[31]	Wistar	(a) 0–1 g la/l in drinking water + 0.1 mg/kg iv every 10 days (b) 10–41 µg/dl (c) NA	50 days	20 days	None	No effects on weight of testis, seminal vesicle, prostate and cauda epididymis (5–10) Increased epididymal spermatozoa concentration, (not significant) (5–10) No effects on plasma LH (5–10)
		(a) 0–1 g la/l in drinking water + 0.1 µg/kg iv every 15 days (b) 8.5–40 µg/dl (c) NA	50 days	270 days	None	Increased plasma and testicular testosterone concentrations (5–10) No effects on testicular weight (5–10) Reduced weight of prostate (5–10) Increased weight of seminal vesicle and seminal secretion (5–10) Increased epididymal spermatozoa concentration (5–10) No effects on plasma LH, plasma and testicular testosterone concentrations (5–10)
Thoreux-Manlay *et al* 1995[32]	Sprague-Dawley	(a) 0–8 mg la/kg ip during 5 days a week (b) <4–1700 µg/dl (c) <0.1 µ/g—0.8 µg/g (epid <0.1 µg/g—7.1 µg/g)	97 days	35 days	Present (severe)	No effects on spermatogenesis (6) Decreased plasma and testicular testosterone by 80% (6–14) Decreased plasma LH by 32% (6–14) Indications for impaired Leydig cell function (5–7) No effects on fertility (8)
Mice:						
Eyden *et al* 1978[33]	Balb/c$^+$	(a) 0-0.1-1% la in food (b) NA (c) NA	90 days	4-8-11 weeks for each group	Present (highest dose)	Increase in spermatozoan abnormalities in the 1% group from 8 weeks on (3–5)
Johansson and Wide 1986[34]	NMRI	(a) 0–1 g lc/l in drinking water (b) <0.5–32 µg/dl (c) Mean difference in lead content between lead treated and controls: testicular 11 µg/g (epididymal 67 µg/g)	63 days	84 days	None	No effects on sperm count (10) No effects on serum testosterone (10) Reduced number of implantations after mating (40)

Table 1 continued

Reference	Strain	(a) Dosage schedule† (b) Corresponding mean blood lead concentration† (c) Corresponding testicular lead concentration†	Age at start	Duration of exposure	Signs of systemic intoxication	Observed effects (n)
Al-Hakkak *et al* 1988[35]	Balb-c albino	(a) 0-25-50 mg lma/kg in chow	Weaning	35–70 days	None	Reduced number of spermatogonia and spermatocytes in the 50 mg group after 70 days (5)
		(b) NA				Reduced number of implantations after mating (after 35 days exposure) (9–10)
		(c) NA				
Rodamilans *et al* 1988[36]	Balb/c+	(a) 0–366 mg la/l in drinking water	63 days	30-60-90-120-150-180 days	None	Reduction of intratesticular testosterone concentrations after 30 days (30)
		(b) 4 to 6—48 to 67 μg/dl				Reduction of androstenedione concentrations after 150 days (30)
		(c) 0.2 to 0.4—0.4 to 0.9 μg/g				No changes in intratesticular progesterone and hydroxy-progesterone (30)
Johansson 1989[37]	NMRI	(a) 0–1 g lc/l in drinking water	63 days	112 days	NS	No effects on frequency of motile spermatozoa, nor on swimming speed (9)
		(b) 0.5–40 μg/dl				Decreased fertilising capacity of the spermatozoa (8)
		(c) NA				Premature acrosome reaction (10)
Pinon-Lataillade *et al* 1995[38]	NMRI	(a) 0–0.5% la in drinking water	Day 1 of gestation	Until 60 days of age	Present	No effects on testicular histology (22), nor on number and morphology of epididymal spermatozoa (22)
		(b) <4–132 μg/dl				No effects on plasma FSH, LH, and testosterone (2–3), nor on testicular testosterone (15)
		(c) NA				Decreased weight of testes, epididymes, seminal vesicles, and ventral prostate (22)
						No effects on fertility (11)
Rabbits:						
Willems *et al* 1982[39]	White New Zealand	(a) 0-0.25-0.50 mg la/kg sc 3 times a week	NS	98 days	NS	No effects on sperm count, morphological abnormalities, testicular histopathology (5)
		(b) 0.32-2.57-2.97 μmol/l				
		(c) NA				
Monkeys:						
Singh *et al* 1993[40]	Cynomolgus	(a) 0–1500 μg la/kg/d in gelatin capsules	Birth	9 years	None	Degeneration of seminiferous epithelium (4–5)
Cullen *et al* 1993[41]		(b)Lifetime group: 3–26 μg/dl at 4–5 years	Birth	400 days	None	Ultrastructural alterations in seminal vesicles, most prominent in infancy and post-infancy groups (4–5)
		Infancy group:				
Foster *et al* 1993[42]		5–36 μg/dl at 100–300 days				No effects on serum testosterone, LH, and FSH (4–5)
		3–3 μg/dl at 4–5 years				Suppressed LH response to GnRH stimulaton in the lifetime group (4)
		Post-infancy group:				
		3–22 μg/dl at 4–5 years				
		(c) NA	300 days	Until 9 years of age	None	

*Median values.
NS=not specified; NA=not assessed; ip=intraperitoneally; sc=subcutaneously; iv=intraveneously; la=lead acetate; lc=lead chloride; lma=lead monoxyde alloy; lo=lead oxide; epid=epididymal lead concentration; n=number of animals per exposure group on which the particular variables have been assessed.
†First figure represents control group.

did not mention the starting age. It is also important to consider the duration of exposure relative to the duration of spermatogenesis and maturation processes of the sperm cells when spermatozoal end points are studied. It is recommended that in subchronic toxicity studies, exposure to a test compound should continue for at least six cycles of the seminiferous epithelium before animals are sacrified and fertility tested. Six cycles should allow sufficient time for the agent to accumulate in tissues, exert its effect, and produce changes in the sperm in the ejaculate.[45 46] Conclusions on subchronic effects should therefore be made with great caution when the test period is <77 days for rats, 53 days for mice, 64 days for rabbits, and 57 for monkeys. Taking this into account, about half of the animal studies can be considered to assess only acute effects.

A major issue that has to be taken into account while interpreting animal studies is the variation in response to Pb between and within species. Already in 1971 the experiments of Schroeder and Mitchener pointed out that mice are more vulnerable to the toxic effects of lead on reproduction than rats.[47] Also on variation within species Der *et al*[12] suggested that gene differences might be an important factor of difference in response to foreign compounds, when they compared their own findings in Sprague-Dawley rats with those of Hilderbrand *et al*[11] on Sesco rats. The Sprague-Dawley rat strain seemed to be more resistant to the toxicity of Pb than the Sesco strain.

The significant differences in vulnerability to Pb toxicity between strains could be due to a different toxicokinetics of Pb accumulation in the testes or to a different functioning of the blood-testis barrier. In particular, Sprague-Dawley rats and NMRI mice seem to be rather resistant to the reproductive effects of Pb. In other strains, exposure to Pb in sexually mature animals caused minor to major signs of impaired spermatogenesis[11 12 16 20 24 37] or of a disturbed endocrinogy[26 36] at PbB concentrations ranging from 30–187 μg/dl. Unfortunately, the great lack of uniformity, whether it concerns age of the animal, duration of exposure, assessment methods for reproductive end points, or internal doses, makes it impossible to draw any strong conclusions on dose-response relations. However, there could be a relation between testicular Pb content and histopathological changes.

Table 2 Studies on semen quality and endocrine function in lead exposed workers

Reference	*Population*	*Subjects (n)*	*Age (y)*	*Duration of exposure (y)*	*Internal dose (PbB, µg/dl)*	*Reported effect/out come*	*Quality evaluation*	
							Laboratory	*Study design*
Wildt *et al* 1983[50]	Battery workers	16	18–61	months–>10 y	33–75 (range)	↓ Sperm chromatin stabily ↓ Secretory function of prostate and seminal vesicle	ID STC	CG D/R S
Fisher-Fishbein *et al* 1987[51]	Firearms instructor	1	41	2	88	↓ Infertility ↓ Sperm count, volume, density After chelation, improvement of semen quality, reversal of infertility		
Lancranjan *et al* 1975[52]	Battery workers, technicians, office workers	150		1–27	23 (14) 41 (12) 52.8 (21) 74.5 (26) (mean (SD) for groups)	Dose response relation for asthenospermia, hypospermia, teratospermia		N D/R CG
Lerda *et al* 1992[53]	Battery workers	38	36 (mean)	11.7 (mean)	48.6 (4.2) 65.9 (1.6) 86.6 (0.6) (mean (SD) for groups)	↓ Sperm volume, count, motility increased sperm deaths, anomalies	STC	N CG D/R S
Braunstein *et al* 1978[54]	Secondary lead smelter	10	38.8 (mean)	2–11	29 (5) to 88.2 (4.0) (range of mean (SD))	↓ Frequency of intercourse ↓ Testosterone concentrations		CG S
Cullen *et al* 1984[55]	Battery, painting, brass foundry workers	7	22–43	1 month–15 y	66–139 (range)	↓ Sperm count, motility ↓ Thyroid function ↓ Glucocorticoid production Slight improvement of semen analysis after chelation	ID STC	
Assennato *et al* 1987[56]	Battery workers	18	41 (mean)	1–10	61 (20) (mean (SD)) Sperm Pb: 79 (36) ppb	↓ Sperm count	ID HA	CG S
Alexander *et al* 1996[57]	Lead-zinc smelter workers	119	39.7 (mean)	17 (mean)	<15, 15–24, 25–39, >40 (groups) mean=28.7	↓ Sperm concentration, total sperm count (inversely related to PbB) No alteration of sperm morphology, motility, hormones	STC	N D/R S CG

ID=quality control for indicator of dose; STC=use of standardized criteria for semen analysis; HA=quality control for hormonal analysis N=adequate number of subjects; CG=presence of control group; D/R=evaluation of dose/response; S=adequate statistical analysis ↓=decrease.

The great variations in hormone concentrations, whether they are circadian, age related, seasonal, individual, or even related to strain make it difficult to draw valid conclusions on hormonal effects.[45 48 49] Again, Sprague-Dawley rats seem to be less vulnerable than other rat strains. Taking this into account there seems to be a trend of suppression of serum testosterone concentrations in animals exposed to lead. In the experiments of Sokol *et al* on Wistar rats, a kind of threshold PbB concentration can be seen between 35 and 40 µg/dl.[17] Still the data are much too few to draw any strong conclusions.

HUMAN STUDIES

Tables 2 and 3 outline the characteristics and summarised findings in one case report and seven cross sectional occupational studies on semen quality and on the endocrine system related to reproductive function in humans.

Table 4 summarises the main features of the studies on the relation between trace elements in seminal fluid or blood and semen quality. These studies mainly evaluated the concentrations of certain elements (Pb, Al, Cd, Cu, Hg, Mg, Mn, Mo, Se, Sn, and Zn) in seminal fluid or blood in healthy, infertile, or azoospermic men, apparently not occupationally exposed to Pb.

A few studies deserve more comments.

The classic study by Lancranjan *et al* performed in Romania first provided some evidence of impaired spermatogenesis for mean PbB >40 µg/dl. The subjects were classified into four groups: men poisoned with Pb (n=23), men with moderate (n=42), slight (n=35), or physiological (n=50) Pb absorption. The major strengths of this study were the dose-response relation for the decrease in sperm count, decrease in sperm motility, and increase in abnormal sperm morphology with increasing Pb absorption, the use of a standardised questionnaire to collect the subjects' data, the relative comparability of controls, and the relatively high number of subjects involved. On the other hand, the dose-response is limited by the fact that some overlapping of exposure groups occurred, control subjects had relatively high PbB, the semen collection included coitus interruptus, no information was given on sperm counts; all these factors limit the interpretation of the results.[52]

Similar findings were reported by Lerda *et al* in Argentina, although no dose-response relation was found. The results should be noted, mainly because selection of subjects and characterisation of exposure to Pb were well conducted as well as the semen and statistical analyses.[53]

Assennato *et al* studied sperm count, concentrations of FSH, luteinising hormone (LH), testosterone, prolactin, and ketosteroids. A significant decrease in sperm count was detected in exposed workers compared with controls whereas no differences were found for hormone concentrations, thus suggesting a direct toxic effect of Pb on sperm production or transport. The main limitation of the study was the low number of subjects. The selection of subjects was well conducted, the role of several confounding factors was well evaluated, and it was also shown that concentrations of Pb in

Table 3 Studies on endocrine function in lead exposed workers

Reference	Population	Subjects (n)	Age (y)	Duration of exposure(y)	External dose (PbA, μg/m³)	Internal dose (PbB, μg/dl)	Reported effect/outcome	Quality evaluation: Laboratory	Quality evaluation: Study design
Raule *et al* 1952[58]	Welders, battery workers, secondary foundry workers	12	21–56	4 months–12 y	—	100–136 (range)	↓ Urinary elimination of FSH, LH		
Govoni *et al* 1987[59]	Pewter workers	76	19–52	—	—	28.2 (7.1) 33.1 (6.7) 49.1 (4.2) 60.3 (19.3) (mean (SD) for groups)	↑ Prolactin in the highest exposure group		N D/R
Rodamilans *et al* 1988[60]	Lead smelter workers	23	21–52	<1– >5 y	>30	66 (22) 73 (24) 76 (11) (mean (SD) for groups)	↓ Testosterone ↑ Steroid binding globulin ↑ LH		D/R CG
Gustafson *et al* 1989[61]	Secondary lead smelter workers	25	36 (mean)	10 months–6.5 y	—	49 (5) (mean (SD))	↓ FSH ↓ LH, cortisol, TSH, in subjects <40 y ↑ TSH	ID	CG S
Mc Gregor *et al* 1990[62]	Non-specified	90	16–60	1–45	>30	11–77 (range)	↓ LH ↑ FSH	ID	N D/R S CG
Ng *et al* 1991[63]	Batterry workers	122	17–54	0.1–19	30–600 (range)	9,6–77.4 (range)	↑ LH-FSH ↓ Testosterone in older workers	ID HA	N CG S D/R
Gennart *et al* 1992[64]	Battery workers	98	22–55	1–28.4	—	40–75 (range)	No correlation of endocrine function with lead exposure		N CG S

ID=quality control for indicator of dose; HA=quality control for hormonal analysis; N=adequate number of subjects; CG=presence of control group; D/R= evaluation of dose/response; S=adequate statistical analysis; ↓=decrease; ↑=increase.

seminal fluid in exposed subjects were significantly higher than in controls.[56]

The cross sectional study by Alexander *et al* in Canada is probably the best published survey available evaluating semen quality and serum concentrations of reproductive hormones in workers exposed to Pb. As with other findings, the research showed that PbB >40 μg/dl may affect spermatogenesis by reducing sperm concentration and total sperm count. No association was found between exposure to Pb and sperm morphology, motility, or reproductive hormones. The strengths of the study were mainly the size and careful selection of the study population, availability of historical Pb monitoring data, the control for all the relevant confounding factors—for example, age, smoking, alcohol consumption, period of abstinence, and blood concentrations of other metals such as Cd and Zn—the statistical analysis, and the validity of seminal analysis. The major limitation of the study was the poor participation rate, as in other studies. This research provided further evidence of a direct toxic effect of Pb on spermatogenesis.[57]

Some of the studies on hormones involved in reproductive function are very old, and others are not very informative.[58 59] The study by Rodamilans *et al* in Spain showed no simple correlation between PbB and endocrine variables. The smelters were divided into three groups according to duration of exposure. The controls were appropriate.The findings were compatible with an initial direct testicular toxic effect of Pb followed by hypothalamic or pituitary disturbance after a longer period of exposures. A main limitation was the small sample size.[60]

Gustafson *et al* carried out a study in Sweden in a group of secondary Pb smelter workers and appropriately selected controls, finding a complex effect on the endocrine system induced by moderate exposure to Pb, possibly mediated by changes at the hypothalamic-pituitary level. It is notable that all the hormone values were within the normal range for the Swedish population. Among the limitations of the study, it should be noted that the number of subjects of the study was low and that duration of exposure was not included in the analysis.[61]

The study by Mc Gregor *et al* suggested that lead may cause subclinical primary toxic damage to the seminiferous tubules in the testes at PbB concentrations >47 μg/dl.[62] The testosterone concentrations were normal, by contrast with the findings of Rodamilans *et al*.[60] This research is of good quality, as the number of subjects was high, the controls were appropriately selected, it had a precise characterisation of exposure to Pb (duration of exposure, PbB, and bone Pb were measured), and included multiple regression analyses.

Ng *et al* carried out a study in Singapore. The number of workers was high, exposure to Pb was well defined, the statistical analyses were good, and confounders were considered; the overall quality is probably the best available in endocrine function studies. An important finding was that LH and FSH showed a moderate increase in relation to PbB in the range of 10–40 μg/dl, thereafter reaching a plateau or declining. An increase in concentrations of LH and FSH, with normal testosterone, was noted in subjects with <10 years of exposure to Pb. The main conclusion was that moderate exposure to Pb was dose related with small changes in endocrine function, reflecting primary and secondary effects of Pb on the testes and hypothalamopituitary axis.[63]

Table 4 Studies on trace elements concentrations in seminal fluid or blood in subjects not occupationally exposed to lead

Reference	*Population*	*Subjects (n)*	*Age (y)*	*Internal dose (PbB, µg/dl or sperm Pb µg/dl)*	*Reported effect/outcome*	*Quality evaluation*	
						Laboratory	*Study design*
Dawson *et al* 1985[65]	Healthy men	64	—	—	Concentrations of seminal Pb inversely related to the percentage of live sperm	—	N D/R S
Dawson *et al* 1985[66]	Azoospermic and normal subjects	30	—	—	Pb seminal concentrations higher in azoospermic subjects		CG S
Umeyama *et al* 1986[67]	Fertile and infertile men	91	26–48	Sperm Pb: 0.18 (0.14) 0.21 (0.19) 0.25 (0.12) 0.28 (0.16) 0.28 (0.18) (µg/l, mean (SD) for groups)	No differences in Pb seminal concentrations between fertile and infertile men	—	N CG (D/R)
Saaranen *et al* 1987[68]	Infertile men	109	22–47	Sperm Pb: 3,6 (3.1) µg/l (mean (SD))	No relation between semen quality and Pb seminal concentrations	ID STC	N CG D/R S
Chia *et al* 1992[69]	Subjects attending andrology clinic for poor sperm parameters	35	37,7 (5.5) (mean (SD))	PbB: 7.2 (mean)	Higher PbB in asthenospermic subjects	STC ID	S
Xu *et al* 1993[70]	Men undergoing screening for infertility	221	24–54	PbB: 7.7 (3.1) Sperm Pb: 12.7 (2.9) µg/l (mean (SD))	No relation between blood and semen Pb concentration and sperm variables	ID STC	N S CG

ID=quality control for indicator of dose; STC=use of standardized criteria for semen analysis; N=adequate number of subjects; CG=presence of control group; D/R=evaluation of dose/response; S=adequate statistical analysis.

Gennart *et al* assessed the thyroid, testes, kidney, and autonomic nerve function in Belgian battery workers, with good characterisation of exposure and of confounding factors. The number of workers was high and controls were well selected. The study did not show any alteration and suggested that compliance with the European Communities Directives on exposure to Pb (health surveillance in workers with PbB >40 µg/dl, and removal from exposure when PbB >70–80 µg/dl) would prevent significant biological changes in most Pb workers.[64]

Information about a possible role of Pb on testicular function may be drawn form studies carried out in non-exposed subjects by measuring the concentrations of several elements in seminal fluid. Generally, higher seminal concentrations of certain elements (and Pb among them) are reported in azoospermic, infertile subjects compared with control subjects.

Asthenospermic subjects had significantly higher blood Pb and Cd than normospermic subjects.[65–69]

The most recent and well conducted study by Xu *et al* in Singapore, however, found no differences in sperm quality (motility, density, morphology, volume, and viability) among men undergoing screening for infertility not occupationally exposed to Pb compared with appropriate controls and no relation between blood and seminal Pb concentration and semen quality.[70]

Two studies, not summarised in the tables, examined the effects of Pb on fertility and so far results are conflicting. A Belgian group compared birth rates through a logistic regression (four age strata) model in 74 battery workers (mean PbB 46.3 µg/dl, mean duration of exposure 10.7 years) with 138 blue collar workers not exposed to Pb (mean PbB 10.4 µg/dl). Within the exposed group, the fertility was significantly reduced during years of exposure compared with years before exposure. Also, the fertility of workers exposed to Pb was 19% higher than that of unexposed workers before the onset of exposure, but 35% lower during exposure to Pb. The study examined few subjects but the data collection was standardised, the selection of subjects was good, and the exposure data were of good quality.[71]

On the other hand, a French cohort study on live births of 229 workers exposed to Pb (mean PbB 46.3 µg/dl) compared with 125 unexposed subjects showed no association between exposure to Pb and fertility, and did not provide clear evidence of the adverse effects of occupational exposure to Pb on male fertility as studied by recording live births. While interpreting this research, it should be noted that it had a small sample size, the possible existence of uncontrolled confounding factors could not be ruled out, and the quality of exposure information was limited.[72]

The studies with appropriate sample size and contrast of exposure across study groups indicate fairly consistently an inverse relation between concentration of PbB and sperm count.[53 57] However, the data are too crude and sparse to identify a possible threshold below which Pb has no detectable effect on the human male reproductive system, and, moreover, there are indications of effect of long term exposure as well as effects related to current concentration of PbB.[57] The reproductive effects were decribed for PbB generally >40 µg/dl; they include decrease of sperm count, volume, density, change in sperm motility and morphology, and possible non-relevant effects on the endocrine profile.

In human studies, the number of workers examined is often limited, which results in low statistical power for some of the seminal variables (sperm density and sperm count). Furthermore, the participation rate in the cross sectional studies are usually <60%–70%. If the

participation rate is related to exposure and semen quality, the findings may be biased. This type of selection bias has been described in occupational sperm studies.

The adequate characterisation of occupational exposure is a common deficiency, in particular for the determination of PbB and its relation with the job title, the period of sample collection in relation to the duration and type of exposure to Pb, and the analytical quality controls are often missing.

In most studies, confounding factors (age, seasonal variation, period of continence before semen collection, some disease states) are not adequately controlled for.

A failure to assess exposures to other occupational factors which may affect reproductive ability—such as exposure to other metals, solvents, and heat is lacking in some studies.

The relation of sperm quality with indicators of exposure to Pb and effect or with time variables such as duration of exposure to Pb is also unclear. It should finally be pointed out that one of the most serious limitations of cross sectional studies for causation analysis is the lack of a time relation between exposure and the health condition.

Conclusions

Several factors limit the ability to extrapolate the animal data to the human situation. Lack of knowledge of the physiological differences among species makes it difficult to compare the findings between different animals.

The difficulties are primarily due to differences in reproductive end points between species, but there are also difficulties when comparing the level of exposure assessment.

Difference in toxicokinetics and dynamics of lead between species are still not very well understood. Even in humans, hypothetical distribution models are reported. Some authors emphasise that reproduction in laboratory animals is not similar to reproduction in humans,[73] and that no simple reproductive end point can serve as an accurate indicator of reproductive risk both in animals and humans.[74]

The combination of all these factors makes it unlikely that any valid conclusion can be drawn by extrapolation from animal data to humans.

One important issue in reproductive toxicity studies is the assessment of the dose of exposure. This includes the selection of the most appropriate indicators and their relations—for example, PbB, Pb in seminal fluid and in its fractions, Pb in air—the time of sample collection and analysis relative to exposure to Pb, the analytical procedures, the identification and measurement of other sources of exposure to Pb or other xenobiotics, both occupational and non-occupational.

In experimental studies PbB at the end of exposure was the elective variable for measuring exposure to Pb.

However, measurement of PbB has been considered to be inadequate to estimate the total absorbed dose of Pb in humans, as it reflects the exposure to Pb during the past 30 days. It also does not provide valid information on the distribution of Pb in tissues.[75–79] The inadequacy of PbB to reflect Pb accumulation in the organs has also been documented in rats.[80] If there were any cumulative effect of Pb on reproduction, relying on blood concentrations alone would be insufficient.

However, with the data on testicular Pb concentration, there seems to be a relation between testicular Pb content and histopathological changes. Unfortunately, outcome variables involving histopathological end points are mostly descriptive and did not seem to lend themselves to fit into a quantitative dose-response relation. However, it could be noted that changes in spermatogenesis tend to be initiated at a concentration of about 2.0 μg Pb/g, indicating the existence of a possible testicular threshold concentration. Furthermore, as the most consistent finding in the hormonal studies is a supression of testosterone, a similar influence of local Pb accumulation on the Leydig cells cannot be excluded. However, as the number of studies with testicular Pb content is very small, the findings cannot be conclusive.

The scarce data on testicular Pb concentrations seem to indicate interstrain differences in the capacity of accumulating Pb in the testes. In the high dose experiment of Thoreux-Manlay *et al* PbB of 1700 μg/dl after 35 days of exposure resulted in a testicular Pb concentration of 0.8 μg/g in Sprague-Dawley rats,[32] whereas the Charles Foster rats, used in the experiment of Chowdhury *et al* in 1987, reached testicular Pb concentrations of 2.0, 1.6, 2.6, and 4.3 μg/g with PbB of 56, 91, 196, and 332 μg/dl respectively after 30 days of exposure.[20] Apparently Sprague-Dawley rats accumulate less Pb in their testicles than other rat strains. Unfortunately, few studies have focused their assessment of exposure on the cumulative capacity of Pb.

Different Pb accumulations in the testes and the possible effects related to it could explain the discrepancies between the negative findings in histopathological end points of the investigators when Sprague-Dawley rats were used[12 22 28 29 32] and the positive findings of investigators who used other rat strains.[11 16 17 19 23–26]

Use of testicular Pb content as an exposure variable for Pb when studying reproductive toxicity, can only be applicable in an experimental setting. The content of Pb in seminal fluid has never been assessed in experimental studies, probably as collection of animal semen is either impossible or very impractical.

On the other hand, human semen, which can easily be collected, can provide a good medium for Pb measurement. If seminal Pb concentration reflected testicular Pb content, this could provide a promising assessment of exposure for the reproductive toxic effects of Pb.

Blood lead >40 μg/dl seems to be associated with a decrease in sperm count, volume, motility, and morphological alterations, and possibly a modest effect on endocrine profile.

Most of the studies, however, did not consider the question of the dose-response curve, which is of importance especially when a biological limit value has to be established. The

cohorts of workers so far evaluated had mean PbB generally >40 μg/dl and often had very broad ranges of exposures. This fact makes it difficult to determine a no effect level and an accurate dose-response characterisation.

The question of the biological mechanisms of Pb damage has also to be clarified. It is not yet clear whether the possible mechanism is a direct effect of Pb on reproductive organs, or on the endocrine control of reproduction, or both. It is also difficult to say if the deposition of Pb in the tubules of the testis is important or not or if it is the localisation or distribution of Pb in spermatozoa that determines the possible adverse effect. A theory about the blood-testis barrier (functionally very similar to the blood-brain barrier) suggests that the germinal epithelium is divided into two compartments: the basal compartment, related to spermatogonia, and the adluminal compartment, mainly related to the more differentiated cells. The different substances (nutritional or toxic) reach the first compartment more easily and seem to be excluded from the second by occlusive junctions, located in the lateral surfaces of Sertoli cells and immediately above the spermatogonia layer. Therefore, the interaction between Pb and germinal cells is easier for spermatogonia than for differentiated cells.

To overcome the difficulties in interpetation and to reach strong conclusions, future studies on human male reproductive effects of lead should consider several methodological problems—such as the standardisation of analytical procedures with strict quality controls within and between laboratories—the adoption of well defined terms of the outcome to be measured, the use of concurrent control subjects, the analysis of semen with respect to the time variables, the adoption of standardised criteria for information and motivation of the participants, and exposure assessment. It is also important to study large cohorts to evaluate possible effects at the Pb concentrations currently encountered in occupationally exposed populations and to design such studies to characterise the dose-response relation and to evaluate change of values over time. This will also enable establishment of a no effect level and proper biological limit values.

The question of the indicator of exposure should also be clarified, taking into account that possible indicators—such as seminal Pb—may be more useful in fertility studies than the indicators of recent exposure.

It should be underlined that interactions with other occupational and non-occupational exposures must be considered and every possible confounding factor should be controlled for.

In this respect, integrated studies should be promoted which will evaluate not only seminal and endocrine end points, but also other aspects such as the time to pregnancy.[81 82]

Appendix

The Asclepios project is a European Union concerted action research project coordinated by The Steno Institute of Public Health, University of Aarhus, Denmark, with the following participants: Belgium, Gent (P Kiss, A Mahmoud, M Vanhoorne, H Verstraclen); Denmark, Aarhus (A Abell, JP Bonde, S Brixen Larsen, G Danscher, E Ernst, H Kolstad), Copenhagen (A Giwercman); England, London (A Dale, M Joffe, N Shah); Finland, Helsinki (ML Lindbohm, H Taskinen, M Sallmen), Turku (J Ladhetie); France, Paris (P Jouannet, P Thonneau), Strasbourg (A Clavert); Germany, Erlangen (KH Schaller, W Zschiesche); Italy, Brescia (P Apostoli, S Porru), Milano (L Bisanti), Pietrasanta (L Lastrucci), Rome (M Spano'); The Netherlands, Nijmegen (N Rocleveld, H Thuis, GA Zielhuis), Zeist (W de Kort); Poland, Lodz (K Sitarek). The Asclepios project is funded by the BIOMED programme of the European Union and by several complementary national sources of funding.

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