Review of recent animal and human data on the effects of inorganic Lead to reproduction

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1 INTRODUCTION

A major challenge in studies of the reproductive and developmental toxicity is how to define the dose. Without an appropriate definition of dose, it is difficult to assess the risk. In the human studies on lead exposure the dose is defined in terms of the lead level in blood (PbB) of man. In animal experimental studies the target tissues themselves are accessible for analysis, but in human studies one has to apply biological monitoring.
Since lead is known to be a potent systemic toxicant it should be considered that systemic maternal toxicity may indirectly affect the reproductive function and the offspring.

2 ANIMAL DATA

In 1980 IARC, Gerber et al., and Chang et al. published reviews of the reproductive hazards of lead in experimental animals.
Table 1 summarizes these data together with more recent data, which will be discussed more extensively. IARC concludes, that lead has been demonstrated to express teratogenic effects. A reduction in fertility, retardation of fetal and post-natal growth, neurological damage and an increased rate of fetal and post-natal death are reported. Lead can induce malformations in the skeleton and their incidence is increased when the animals suffer from a deficiency of calcium. Lead is capable of crossing the placenta. The effects of lead on development seem to be caused by the toxic action on the embryo, perhaps except the failure of implantation where maternal factors may be involved. A toxic action of lead is also shown by the biochemical and morphological alterations seen in exposed embryo.

In rats, lead can be transferred from the mother to the fetus at various stages of gestation; transport is rapid, with the result that the fetus is in equilibrium with the mother 24 hours after i.p. injection (5 mg/kg of lead acetate). Substantial amounts of lead are transferred via the milk to suckling rats as already measured one week after a single administration.

The concentration of lead is higher in the head of these suckling rats than in other parts of the body (Green & Gruener, 1974).

Dencker et al. (1983) reported that inorganic lead [administered intravenously at various days of gestation to mice as ^{203}Pb-acetate (8.8×10^6 dpm/g body weight)] was found in embryonic and fetal tissues at all stages of gestation, although more in late (day 13, 15, 16, or 18) than in early stages. This probably depends in part on the marked accumulation in fetal skeleton, which was observed from day 13 and onwards. Apart from the skeleton, high concentrations (as shown by autoradiograpic resolution) of lead in the fetus were seen in the blood and in the liver. Lead also accumulates at these sites (blood, liver and skeleton) in adults. The fetal blood concentration was only slightly lower than the maternal blood concentration. In this study the fetal kidney, however, did not show any specific accumulation of lead.

Gerber et al. (1978) administered 0, 0.25 or 0.5% of lead (as lead acetate) in the diet to mice starting on day 7 of gestation. Placental blood supply measured by injected radioactive microspheres (crosslinked dextrans of 15 μm diameter, labelled with ^{144}Ce) was of the order of 23% of the cardiac output (calculated as the percentage of activity in a given organ) for a litter of eight mice on day 18 of pregnancy. In lead-treated animals not only placental blood flow per embryo but also that per embryo weight was reduced.

Grobler et al. (1985) dosed pregnant rats with 0, 3 or 10 ppm lead (as acetate) in the drinking water during pregnancy and during lactation until 21 days post partum. The litters were killed at the age of 21 days and the teeth analysed for lead by atomic absorption spectrophotometry. In both treatment groups there was a significant increase in lead content of teeth in litters of rats having received lead.

Hackett and Kelman (1983) investigated the contribution of different routes of exposure to fetal body burdens of lead (^{210}Pb) in the rat by comparing the fetoplacental unit burdens following intravenous (3.1 and 15.6 mg/kg lead), intragastric (312 mg/kg lead) and inhalation exposures

(1.11 mg/kg lead, calculated from initial body burden following 60 minutes nose-only exposure) of the dam. Treatment was on day 9, 15 or 19 of gestation. Fetal effects were observed only following intravenous administration of the high-lead dose (which produced not more than 20% maternal mortality). According to the authors it appears unlikely that acute exposure would be teratogenic in the rat under environmental conditions.

2.1 Risks of exposure of female animals with respect to the reproductive organs, endocrine system and fertility

Delayed vaginal opening was observed in female rats exposed from weaning to maturity and successively during pregnancy to lead in drinking water at concentrations of 5 mg Pb/kg/ day and higher (Kimmel et al., 1980, details of the study on page 10). This phenomenon was also observed in their female offspring exposed in utero (Grant et al., 1980, details of the study on page 15). Doses of 0.9 mg Pb/kg/day (approximately 8 µg/dl) and lower had no effect on time of vaginal opening or other reproductive parameters.

Laughlin et al. (1987) reported altered female reproductive physiology in rhesus monkeys exposed to 3.6 to 8.1 mg/kg/day. Blood lead levels reported in this study ranged from 44 to 89 µg/dl.

2.2 Risks of exposure of female animals with respect to gestation and prenatal development

Table 1 summarizes the data mentioned by Gerber et al. (1980), and Chang et al. (1980), IARC (1980), together with studies discussed below. See also risks of exposure of female animals with respect to post natal development (Laughlin, 1986: page 14)

2.2.1 Oral administration

Wardell et al. (1982) treated rats orally by gavage on days 6-18 of gestation with 0 (N=10), 10 (N=6), 50 (N=2), 100 (N=4) or 150 (N=2) mg lead/kg/day. Embryonic death, number of external-, and internal malformations, length of tibia, fetal weight and length were comparable

with controls. In this study the NAEL with respect to embryolethality, embryotoxicity and teratogenicity is higher than 150 mg lead/kg/day. However, the sample size is considered too low for a reliable evaluation of the data. Therefore, the observed NAEL is only of limited value.

Miller et al. (1982) administered lead acetate to female rats by gavage 3 weeks before mating and until day 20 of gestation at daily dosages of 0 (N=9), 50 (N=7), 75 (N=9) or 100 (N=8) mg of lead/kg body weight/day. Lead produced a significant reduction in conceptus weight in the high dosage (100 mg/kg) group. No teratogenic effects were observed. Blood sampling indicated that lead can cross the placenta, as shown by fetal kidney values (values up to 5.93 ppm of lead, i.e. 5.93 mg of lead/kg). In this study the NAEL with respect to embryotoxicity is higher than 75 mg lead/kg/day.

Lögdberg et al. (1987) exposed squirrel monkeys (N=11) to lead acetate by oral administration during the latter two-third of pregnancy [from around 9 weeks of gestation until the end of pregnancy (week 21)]. About half of the daily dose was dissolved in 50 ml distilled water. The monkeys had access to this solution for about 8 hours. The other half of the dose was applied on a freshly cut surface of pieces of apple, which were given to the monkeys twice daily- just before and just after the 8 hours access to the water dose. For the remaining 16 hours tap water with Folacin was given ad libitum. To maintain the individual lead blood levels at 0.55-1.0 µg/ml [mean lead value in the study was 0.54 µg/ml (range 0.39-0.82 µg/ml)] daily doses of around 50-100 mg of lead/kg were given. There were no maternal toxic symptoms. During pregnancy or on the first day after birth 45% of the developing offspring died. Cerebral lead levels in these animals were between 0.1-0.7 µg/g. In nonexposed offspring the mortality was 7-8% in the same period. The mean birth weight of the lead-exposed offspring was significantly reduced. Significantly reduced cerebral weight was seen in the deceased lead-exposed newborns. The reduction of the head circumference of the surviving offspring was not statistically significant. The observed gross and microscopic cerebral lesions (multiple characteristic petechiae, pericapillary haemorrhages, endothelial changes, massive oedema with gross pallor, flattening of the

gyri, and microscopic vacuolation of the neurophil) had, according to the author, features in common with those of encephalopathy seen in human infants. Since the 50-100 mg dose revealed various effects, the NAEL is less than 50-100 mg of lead/kg/day.

Carpenter (1982) -not mentioned in table 1- exposed hamsters to drinking water containing 0.05% or 0.1% lead acetate (respectively 274 and 547 ppm of lead), and a calcium or iron deficient diet 4 weeks prior to and/or during pregnancy. The groups are summarized in table 2. The combined exposures induced marked increases in blood lead levels over lead-exposed controls fed normal diets; the blood-lead levels were significantly higher in calcium-deprived dams than in iron-deprived dams. Prenatal mortality and the number of abnormal fetuses in the litters were significantly increased in lead-exposed, mineral-deprived dams. Abnormalities observed consisted of cleft palate and tail malformations. Toxic effects in dams and offspring were most pronounced in the group that received a calcium-deficient diet, which is in agreement with the higher blood lead levels in this group. The results indicate that there is a certain critical threshold concentration of lead in the maternal blood for the development of teratogenic effects. In the case of the hamster, this threshold blood level (in combination with diets deficient in minerals) appears to be between 100-150 μg/dl. Whether or not the mineral deprivation attributes to the induction of teratogenic effects is not clear from this study. Therefore, the significance of the so-called critical blood lead level of 100-150 μg/dl is obscure.

In a study of Dilts and Ahokas (1979) rats were exposed to 0 (N=7), 10 (N=7), 50 (N=7), 100 (N=8), 200 (N=7) or 500 (N=7) mg Pb/l (as lead acetate) in their drinking water throughout pregnancy. According to the authors, daily intake ranged from 1 to 50 mg of lead/kg (based on 300 g average weight and 30 ml/day average water intake). Maternal weight gain, and level of food intake were significantly reduced in animals exposed to 50 mg/l or higher. Food efficiency for each dam was significantly reduced at 500 mg/l level. Placental weight was not significantly changed at any dose. Average fetal weight and average live litter weight were already significantly reduced at the 50 mg/l level and above. Therefore, in the

study, the NAEL for both maternal and embryotoxic effects was 10 mg Pb/l day (1 mg/kg/day).

Kimmel et al. (1980) administered lead (as acetate) in the drinking water to female rats 6-7 weeks before breeding, and until day 21 or 22 of gestation. The dosages were 0, 0.5, 5, 25, 50 or 250 ppm Pb. These concentrations corresponded to mean intake values of approximately 0, 0.1, 0.9, 5, 9 and 45 mg Pb/kg/day. Number of animals in control and Pb groups ranged from 60 to 148 during the prepregnancy period, and from 24 to 75 during pregnancy. There was a considerable replicate variability in food consumption of the parent females per gram body weight, but no apparent dose-related patterns were noted. Parent females in the 50- and 250- ppm groups exhibited significant growth retardation within 1 to 3 weeks after the start of exposure. Vaginal opening was significantly delayed in the offspring of the 50- and 250- ppm groups, and to a lesser extent in the 25-ppm group. The percentage of malformed fetuses, resorptions, and preweaning mortalities were unaffected by Pb exposure. Only the day 1 mean body length of female offspring (control, 5.3 cm) was significantly altered by exposure to 250 ppm (4.9 cm). With respect to maternal toxicity the LED was 25 ppm (5 mg Pb/kg/day), i.e. blood Pb concentrations of more than 20 μg/dl, and a NAEL of 5 ppm Pb (0.9 mg Pb/kg/day). The NAEL for maternal and fetal toxicity is 25 ppm Pb (5 mg Pb/kg/day).

Rabe et al. (1983, abstract) exposed female rats to lead acetate in their drinking water (5000 mg lead acetate/l, i.e. 280 mg of lead/kg). The exposures were: prenatal throughout gestation (N=14); postnatal to day 15 (N=8); pre and postnatal (N=6). The exact exposure periods were not mentioned in the abstract. Combined pre- and postnatal exposure to lead reduced the body weight of the pups by 20%. However, the available data are very limited, therefore a NAEL could not be established.

Hayashi (1983) administered lead acetate to pregnant rats (N=5 per group) in the drinking water at dose levels of 0 or 500 ppm (0.5 mg/kg/day) lead from day 1-18 or 1-21 of gestation. Only one dose level was used, sample size was low and effects on maternal growth were not determined. Fetal weights in the test group were lower than controls on day 18 but not on

day 21. There were no differences in number of implantations, rate of resorption, placenta weight and external malformations in living or dead fetuses. Erythrocyte ALAD activity in treated dams and fetuses was decreased on day 18 and 21. The RBC count, Ht and Hb decreased in the lead-treated dams as gestation progressed, while an increase of these variables was found in fetal blood. The lead concentration in maternal and fetal blood [respectively 28.54 (control 3.98) and 62.20 (control 20.20) µg/dl on day 18 of pregnancy; 34.24 (control 3.72) and 42.57 (control 4.10) µg/dl on day 21 of pregnancy], liver (respectively 1.16 (control 0.19) and 0.78 (control 0.16) µg/g on day 18 of pregnancy; 1.59 (control 0.18) and 0.61 (control 0.06) µg/g on day 21 of pregnancy], and in the placenta [respectively 0.23 (control 0.06) to 0.29 (control 0.06) µg/g on day 18 and 21 of pregnancy] were reported to be increased in the lead-treated group; the lead concentration in amniotic fluid was not increased.

The ILSI commented in a report (1988) the lower fetal weights observed at 0.5 mg/kg/day were in contrast with other studies and might be explained by:

(1) a considerable variation in litter size between the control and treatment groups with the lead-treated group having larger litters,

(2) the statistical method used (t-test) which did not consider the litter to be the experimental unit, as is generally done in teratology studies, but the fetus,

(3) the reported blood-levels of lead-treated versus control animals also suggest that the apparent fetal weight reduction is an artefact, since maternal blood-lead levels in control and lead-treated groups were not significantly different.

Therefore the study is considered inadequate and a NAEL cannot be established.

2.2.2 Intravenous administration

Kamimura et al. (1982, abstract) treated mice (N=?) with 100 mg/kg lead acetate i.v. on day 11 of gestation. On day 18 of gestation cleft palate was present in 38.2% of the fetuses. Also higher incidences of embryonic death, reduced length of live embryos and a delay of their palatal development was observed from day 14 of gestation and onwards as compared

to normal fetuses. The data obtained from this study are insufficient for establishing an NAEL.

Hackett and Kelman (1983) investigated the contribution of different routes of exposure to fetal body burdens of lead (^{210}Pb) in the rat by comparing the fetoplacental unit burdens following intravenous (3.1 and 15.6 mg/kg lead), intragastric (312 mg/kg lead) and inhalation exposures (1.11 mg/kg lead, calculated from initial body burden following 60 minutes nose-only exposure) of the dam. Treatment was on day 9, 15 or 19 of gestation. Fetal effects were observed only following intravenous administration of the high-lead dose (which produced not more than 20% maternal mortality). According to the authors it appears unlikely that acute exposure would be teratogenic in the rat under environmental conditions.

2.2.3 Intraperitoneal administration

Edwards and Beatson (1984) studied the effects of lead on the early embryonic development of the central nervous system of guinea pigs. They were given a single dose of 0 (N=12), 6 (N=6), 12.5 (N=7) or 25 (N=7) mg/kg body weight as an i.p. injection of a solution of 0.5% lead acetate on day 20 or 21 of pregnancy. These doses are considered equivalent to 4, 8 and 16 mg of lead/kg body weight, respectively. The mean body weights of newborns of the treated Pb groups did not differ from that of the control group. The mean brain weight of newborns in the 8 and 16 mg Pb group were significantly reduced when compared with the control. The percentages of microencephaly in the control, 4, 8 and 16 mg Pb/kg body weight group were respectively 0%, 0%, 11% and 5%, which may indicate a treatment-related effect without dose-response-relationship. In the same study it was also shown that the effect of the brain retardation could be increased by hyperthermia (induced in an electric forced-draft egg-incubator set at 42.5-43.5°C on two occasions at 11 A.M. on days 20 and 21, or 21 and 22 of pregnancy). However, group sizes are considered too small for a reliable estimate of a NAEL. Therefore, the significance of the observed NAEL of 4 mg Pb/kg body weight/day is very limited.

2.2.4 Intramuscular administration

Tachon et al. (1983) administered lead acetate to monkeys every day during pregnancy and/or lactation at levels of 1 or 5 mg Pb/kg/day (a total of 22 female monkeys, average group size 2 to 5 monkeys). After three months the dose level of 5 mg Pb /kg/day was lethal for all 4 pregnant females. In the 1 mg/kg group, at the end of gestation, at sacrifice or at delivery, the blood lead concentration was 1160.6 µg/l (control 50.5) and 1386.7 µg/l (control 47.0) for respectively mother and new born. In the low dose group the mean weight of fetuses removed just before the delivery, and of the new-borns, was comparable with the control group. In the low-dose group erythrodiapedesis in cerebral matter of the new-borns at birth, after the mothers's treatment during pregnancy was observed. This alteration was not found in pups only exposed via breast-milk during lactation. In this group the blood lead level of the young monkeys increased from 59 µg/l (control 57) to 300 µg/l (control 96) at 150 days after birth.

2.2.5 Discussion and conclusions

Oral administration of lead was shown to be embryotoxic, with a primary effect on delayed fetal growth; however, this effect may be associated with maternal toxicity. Some studies did not report maternal parameters and are therefore considered inadequate. High doses, up to 100 mg/kg did not give rise to teratogenic effects, but could induce cerebral lesions. The NAEL for mother and fetus is 1 mg/kg/day (Dilts and Ahokas, 1979)

Intraperitoneal administration at doses of 8 mg/kg and higher gave rise to embryolethal lesions, embryotoxic and teratogenic effects (litter resorption, reduced mean brain weight, abnormally shaped heads and microencephaly).

Intravenous administration at dose levels higher than 32 mg/kg body weight gave rise to embryotoxic or teratogenic effects in prairie voles (table 1, IARC studies quoted by Gerber et al. 1980). In this study the NAEL was 16 mg/kg body weight/day.

2.3 Risks of exposure of female animals with respect to post natal development

In 1986 Laughlin has published a detailed review of animal models measuring behavioral effects of early lead exposure. The scope of this review (according to Laughlin) has been broadly defined to include effects resulting from lead exposure that occurred early in development, although not necessarily limited to the prenatal period, and that was discontinued before testing for the behavioral effect. It was concluded that "lead exposure during early development" has effects on the behavior of experimental animals that persist beyond the period of actual lead intake and perhaps, even beyond the time that lead is present in the body. The effects appear to be widespread, affecting various types of behaviour. Lead-induced alterations in emotionality or stress responses as well as attentional deficits may underlie many of the effects. Whether these can be designated as true teratological effects is yet to be determined. Few studies of lead effects in experimental animals have exposure limited exclusively to the prenatal period. In studies in which lead is administered in water or food, consumption often is not measured, so that actual lead intake cannot be established. Finally, growth retardation can result from perinatal lead exposure and may have to be considered as confounding factor in the studies mentioned.

2.3.1 Oral administration

Grant et al. (1980), Fowler et al. (1980) administered lead (as acetate) in the drinking water to female rats 6-7 weeks before breeding, and until day 21 or 22 of gestation. The dosages were 0, 0.5, 5, 25, 50 or 250 ppm Pb. (approximately 0, 0.1, 0.9, 5, 9 and 45 mg Pb/kg/day, see also Kimmel et al., 1980: risks of exposure of female animals with respect to gestation and prenatal development, page 8). At 21 days of age, offspring were weaned onto the same concentration their mothers had been given, and exposure continued until sacrifice at 6 or 9 months of age. Significant depressions in body weight were seen at most time points for offspring exposed to 50-, and 250 ppm. Clinical signs of respiratory infection, as poor fur condition, tail-tip necrosis, and sialodacryoadenitis were noted to occur at 250 ppm. A highly significant delay in the occurrence of

vaginal opening was noted in 25-, 50-, and 250 ppm females. Surface righting and air righting were delayed in 50,- and 250 ppm animals. The Lowest Effect Dose (LED) for Pb using chronic exposure in the Grant et al. study was 25 ppm (Pb blood level 20-40 µg/dl), a level associated with alterations in reproductive development. In the study of Fowler et al. (1980) immune function and performance of an operant task in adult offspring were altered at 25 ppm, and renal morphology after 9 months of exposure was altered at 5 ppm (Pb blood level 10-16 µg/dl). Both studies are considered as well-conducted and provide an appropriate basis for the determination of a NAEL for developmental effects.

Winneke et al. (1982) exposed a total of 24 female rats and their progeny to dietary lead as lead acetate in four concentrations: 0, 80, 250 and 750 ppm, in order to test neurobehavioral effects of lead exposure. Analytical control gave the following average Pb values: ?, 82, 236 and 749 ppm. According to the authors these diets have found (in the parental females) to give rise to blood lead-levels of <5, 11, 18 and 31 µg/dl after 130 days of feeding. After 50 days of feeding the animals were mated; the male remained with the females for 60 days, and their offspring were weaned at 21 days after birth. All the offspring were kept on their dam's diet until testing. Average litter size and body weight of the animals at the different experimental stages did not reveal pronounced systematic changes across conditions. The treatment resulted in erythrocyte ALAD-inhibition of 40, 73 and 83% in the female offspring at 90 days postnatum. Male and female offspring were first tested at 70-100 days postnatal in a 2-way active avoidance-task, and the females at 190-250 days postnatum in a visual discrimination-task. Lead-exposure was associated with impaired performance in the discrimination-task, which was significant ($P<0.001$) already at the 250 ppm-exposure level. In addition significant ($P<0.05$) difference in the performance of the avoidance-task was also observed at that dose level. With respect to task dependent neurobehavioral effects the NAEL is 80 ppm of lead, which is equivalent to Pb blood level of 11 µg/dl. The study is considered adequately performed. Therefore, the observed NAEL is of significance for the safety evalution of Pb.

Bornhausen and Hagen (1984) administered Pb (0.2, 0.7 or 2.0 mg/ml) in drinking water of rats during pregnancy until day 21 after birth. For the operant behavior test the females were tested in lever-boxes in three steps of the conditioning program Differential Reinforcement of High rate. There was a learning deficit after prenatal treatment in mid and low Pb dose, but not in the high Pb dose. The NAEL is considered to be 0.2 mg/ml (0.02 mg/kg/day). However, since there was no clear dose-response relationship, the relevance of the NAEL is obscure.

Rabe et al. (1983, abstract) exposed female rats to lead acetate in their drinking water (5000 mg lead acetate/l, i.e. 280 mg Pb/kg/day). The exposures were: prenatal throughout gestation (N=14); postnatal to day 15 (N=8); pre-and postnatal (N=6). The exact exposure periods are not known. Prenatal exposure to lead had no effect on any of the behavioral measures in the offspring. Postnatal treatment increased the number of head dips and ambulation scores in pups. Combined pre- and postnatal exposure to lead revealed behavioral changes (impaired left-right discrimination and its reversal) in the pups. In all three groups the lead levels were similar: about 100 mg/dl (remark: this level is probably not correct in the abstract, and should be 100 μg/dl) in blood, and 90 mg/100 g dry brain weight (remark: this level is probably not correct in the abstract, and should be 90 μg/100 g). The control values were less than 10. The authors concluded that the effects of early exposure to lead on behavior also depend on factors other than the level of lead in blood and brain.

Taylor et al. (1982) exposed rats to either 400 or 200 mg/l of lead acetate from 14 days prior to breeding until the pups were weaned. On day 10 after birth two male pups from each litter were removed from their dams. Ten hours after isolation the pups were used in behavioral tests [25 acquisition trials (400 mg/l, N=4; 200 mg/l, N= 16; controls N=4 and 12, respectively) followed by extinction trials]. No significant differences were observed between the control pups and the treated pups with respect to acquisition rates, but there were significant differences between the control group and the two lead-treated groups with respect to extinction rates. With respect to learning deficits the NAEL was less than 200 mg/l of lead acetate ($\pm$ 11 mg Pb/kg/day).

Kitchen et al., 1984 administered lead at 300 and 1000 ppm in maternal (N=2) drinking water of rats from conception to weaning. Treatment impaired the antinociceptive activity (measured by using the tail immersion test) of morphine in 10-day-old, but not in 21- and 30-day-old neonatal rats (N=4, and 8 observations per time point). Blood lead levels in the 10-day-old animals were below 50 μg/100 ml in the high lead dose group and below 35 μg/100 ml in the low lead dose group (control value $\pm$ 1 μg/100 ml). Neonate and maternal weight gain was not impaired by the lead treatment. It is suggested that lead disrupts the development of opioid receptor systems in the central nervous system and that this disruption occurs already early in development. With respect to antinociceptive activity the NAEL is less than 300 ppm (i.e. 30 mg/kg/day). Since the number of animals was very low the significance of the observed NAEL is only of relative value, and should be noted with reservation.

Bailey and Kitchen (1986) administered 0, 100, 300 or 1000 ppm lead (as lead acetate) in the maternal drinking water from conception to weaning at 21 days post partum. At 10, 21 and 30 days after birth, the pups were killed and blood and brains were collected. The highest Pb blood levels (n.d., 34.7, 39.8, 68.9 μg/100 ml, respectively) were observed at 21 days after birth, the time at which exposure via the mother was ceased. There were no significant differences in the striatal levels of dopamine, noradrenaline or adrenaline between normal and lead exposed rats at the ages studied. For every determination at each dose level and time point, measurements were the mean of 6 observations taken from 3 litters. At 10 days of age, the highest dose of lead produced a significant reduction of 47% in striatal y-aminobutyric acid (GABA) levels. With respect to ontogeny of catecholamine and GABA levels the NAEL is more than 300 ppm (i.e. 30 mg/kg/day), but less than 1000 ppm (i.e. 100 mg/kg/day).

2.3.2 Discussion and conclusions

In several studies the neurodevelopmental effect of lead was studied; most of these studies are based on post-weaning or combined pre- and post-weaning models. Neurobehavioural effects on reflex development, locomotor activity, learning ability and social behaviour have been observed, but

some effects occurred only at high exposures. The effective dose varies greatly between studies and parameters. The most complete study, carried out by Kimmel et al, 1980 and Grant et al 1980, revealed delays in surface righting reflex and air righting reflex development at 9 mg/kg (Pb blood level 20-40 μg/dl) and higher.

2.4 Risks of exposure of male animals with respect to the reproductive organs, endocrine system and fertility

2.4.1 Oral administration

Fowler et al. (1980) administered lead (as acetate) in the drinking water to female rats 6-7 weeks before breeding, and until day 21 or 22 of gestation. The dosages were 0, 0.5, 5, 25, 50 or 250 ppm Pb. (see also Kimmel et al., 1980: risks of exposure female animals with respect to gestation and prenatal development, page 6). At 21 days of age, offspring were weaned onto the same concentration their mothers had been given, and exposure continued until sacrifice at 6 or 9 months of age. Sperm morphology and sperm count were not altered in males exposed chronically to up to 250 ppm lead in drinking water from conception through nine months of age. Microscopic examination revealed no effects on the testicles, epididymides, seminal vesicles, or prostate of the exposed males. The NAEL with respect to male reproductive effects is equal to or higher than 250 ppm (approximately 25 mg/kg/day; mean Pb blood level 67 μg/dl). As mentioned earlier this study is adequately conducted and the results are therefore reliable for the assessment of a NAEL for male reproductive effects.

Chowdhury et al. (1984) administered lead acetate to male rats at concentrations of 0.25, 0.50 and 1.0 g/l for 60 days (correponding to estimated lead doses of 14, 28 and 56 mg/kg/day, respectively). Body weight reduction, increase in testicular cholesterol and a decrease in ascorbic acid was seen in all treatment groups. Premeiotic spermatogenic inhibition and degeneration of spermatocytes and spermatids were observed in the mid-dose animals. More severe effects, including disintegration of the cellular pattern of the seminiferous tubules, were observed in the high-dose group. In this study the NAEL of 14 mg Pb/kg/day (Pb blood level of 54 μg/dl) was observed for effects on the male reproductive system.

Sokol et al. (1985) exposed 52-day-old male rats to drinking water containing lead acetate [0%, 0.1% and 0.3% (i.e. 0, ±5, ±16 mg/kg/day); each treatment group consisted of eight rats] for 30 days prior to killing. Whole blood lead levels were below detection (<7 µg/dl) in the control animals, 34 ± 3 µg/dl in the 0.1% group and 60 ± 4 µg/dl in the 0.3% group (P<0.001). Although the 0.3%-treated rats lost weight, no significant correlations were found between body weight and serum testosterone or body weight and intratesticular testosterone. As the level of lead exposure increased, intratesticular sperm counts decreased significantly. No significant changes in serum luteinizing hormone values were found, but spermfollicle-stimulating hormone values were significantly suppressed after lead treatment. There was a significant decrease in ventral prostate weight, but no differences in testicular or seminal vesicle weights.

Johansson and Wide (1986) exposed 9-week old male mice to water containing 1 g/l lead chloride [corresponding to about 3.6 mmole of Pb/liter (i.e. ca. 74 mg/kg/day)], and the duration of the exposure was 12 weeks. After the exposure period concentration of lead in the blood, hypothalamus and male reproductive organs were measured (in 10 treated males, and to 10 controls). Examination for plasma levels of testosterone was also conducted in 10 treated males, and to 10 controls. Males (40 treated animals, 10 controls) were allowed to mate each with three virgin females for a maximum period of 15 days for of fertility evaluation (see for the results also risks of exposure of male animals with respect to gestation of the partner and the neonatal development, page 21).
The mean concentration of lead in the blood of the lead-exposed males was 32 ± 2.8 (SEM) µg/100 ml, which was significantly higher than that of the control males (below detection limit of 5 µg/liter). The difference in lead content (µg/g, mean ± SEM) between lead-treated and control mice was 56 ± 17 (P<0.01) in the hypothalamus, 11 ± 3.6 (P<0.01) in the testis, 67 ± 10.8 (P<0.001) in the epididymis, 10 ± 3.2 (P<0.01) in the seminal vesicle, and 45 ± 18 (P<0.05) in the ventral prostate. Neither the mean body weight of the lead-exposed males nor the mean weights of the male reproductive organs of these animals differed significantly from those of the control group. No significant difference in the mean level of

testosterone was found between the two groups. The number of epididymal spermatozoa in lead-exposed males did not differ significantly from that in the control males. A blood level concentration of 32 µg/dl did not have effects on the male reproductive system of the mouse.

Boscolo et al. (1988) divided 20 male rats into two equal groups, and fed a standard laboratory diet with a content of lead ranging from 0.2 to 1.7 µg/g. The animals of one group received 60 µg/ml of lead (as acetate) for 18 months starting from weaning, while the others were kept as control group. The daily dose of lead of each exposed rat was about 2 mg and the cumulative dose about 1 g. Throughout the experiment, body weight and general appearance of the animals were not affected by the lead treatment. Both weight and diameters of the testis did not differ from the control group. In the exposed rats, the examination by light microscopy did not evidence alterations in the testis. Ultrastructural examination of the testis with both transmission and scanning electron microscopy did not evidence modifications in the external part of the seminiferous tubules, in the spermatogenic cells and in the connective tissue including the Leydig cells; only Sertoli cells presented an increased size of lysosomes. Lead content of blood, kidney and brain of exposed rats, respectively 16.7 µg/dl, 2.70 µg/g and 0.85 µg/g were significantly higher when compared with the control rats, being 3.9 µg/dl, 0.08 µg/g and <0.03 µg/g, respectively.

2.4.2 Intraperitoneal administration

Ghelberg and Bordas (1981) induced lead intoxication in rats by i.p. administration of lead acetate in doses of 8.5 mg/kg; eight weekly doses and another seven doses at 14-day intervals (a total of 15 doses in 154 days were given). The testes of all lead-intoxicated rats demonstrated marked morphologic changes manifested by alteration of the structure of seminiferous tubules with a reduction in their size, detachment of the hypocellular germinal layer from the basal membrane, spermatocyte and spermatid injury as well as slight oedematous dissociation in the interstitium. Administration of sodium aspartate (5 ml/kg/day, i.p.) on the last 21 days immediately prior to sacrifice of the rats revealed that in 75% of the lead-intoxicated rats the histological aspect of these testes was similar to that seen in control groups.

2.4.3 Discussion and conclusions

With respect to effects on male reproductive organs after oral administration of lead acetate, the most appropriate studies were from Chowdhury et al. (1984) and Fowler et al. (1980). In the study by Chowdbury et al. effects were observed in the testicles at dose levels of 25 mg/kg/day and higher. The NAEL of 14 mg Pb/kg/day (Pb blood level of 54 µg/dl) was observed for effects on the male reproductive system. In the study of Fowler the NAEL with respect to male reproductive effects was approximately 25 mg/kg/day (mean Pb blood level 67 µg/dl).

2.5 Risks of exposure of male animals with respect to gestation of the partner and post natal development

Johansson and Wide (1986) exposed sexually mature male mice to lead in drinking water [1 g $PbCl_2$/l, corresponding to about 3.6 mmole of Pb/liter (i.e. ±74 mg/kg/day)] for 3 months. They attained a mean blood level of 32 µg/100 ml. In control mice blood lead levels were <0.5 µg/100 ml (the detection limit). At the end of the exposure period each male mouse was allowed to mate with three females. The ability of the males to mate was not affected by exposure to lead. However, a significantly increased number of mated females without signs of implantations were found in the lead-exposed group. According to the authors it cannot be decided whether the decrease in the number of pregnant females was due to a reduction in the ability of spermatozoa from the lead-exposed males to fertilize or to a preimplantation loss of fertilized ova. Examination of the uteri of pregnant females fertilized by males from the lead-exposed and the control groups did not reveal any significant differences in litter size, resorption frequency, or fetal weight between the two groups. No differences in soft tissue abnormalities, fetal skeletal ossification or skeletal malformations were observed in any of the fetuses observed.

Johansson (1989) exposed nine-week-old NMRI male mice to lead chloride in drinking water (1 g/l). The duration of the exposure was 16 weeks, after which time the mean concentration of lead in the blood was about 40 µg/100 ml. In control male mice the lead concentration in the blood was about 0.5 µg/100 ml (= the detection limit). After this period spermatozoa were

collected. Oocytes were obtained from virgin, mature (9-10 weeks old) not-treated NMRI mice. Lead-exposed male mice were found to show a significantly increased frequency of acrosome-reacted spermatozoa. The number of spermatozoa bound to the zona pellucida of denuded mouse eggs was also significantly increased, probably as a result of an acceleration of the processess involved in the capacitation of the spermatozoa and thereby of the acrosome reaction. However, the ability of spermatozoa from the lead-exposed mice to penetrate the zona pellucida and the plasma membrane of the oocyte, was significantly reduced. No significant differences were detected in the frequency of motile spermatozoa or in the swimming speed between spermatozoa from the lead-exposed and the control group of mice. According to the author these results indicate that the observed reduction in fertility after exposure to lead, may be due to a disturbed interaction of the spermatozoa with the oocyte.

2.6 Risks of exposure of both male and female animals (mating partners) with respect to the gestation, and post natal development

No data available

2.7 Risks of exposure of animals after birth with respect to their postnatal development

Angell and Weiss (1982) exposed rats to lead before or after weaning (through day 133). For preweaning exposures, the nursing dams received 0.2% Pb acetate [1090 ppm Pb (i.e. $\pm$ 109 mg Pb/kg/day)] in drinking water. Offspring treated after weaning were exposed to the same concentration in their drinking water. Tapwater served as the control fluid. Pre- and post weaning treatments were distributed among four experimental groups of 10 rats each: tapwater-tapwater, Pb-tapwater, tapwater-Pb, Pb-Pb. Operant behavior training began on postnatal day 58. Animals performed on a multiple reinforcement schedule of food presentation consisting of fixed-interval, fixed ratio, and time out components. Statistical analysis of experimental sessions 4 to 11 (postnatal days 72 to 79) revealed that postweaning exposure significantly increased the median interval

(interresponse time) between consecutive responses on both the interval and ratio schedules. The tapwater-Pb and Pb-Pb group did not differ from each other. Preweaning exposure alone tended to produce a decrease of the median interresponse time. The significance of these contradictory findings is obscure.

Rice and Gilbert (1985) dosed cynomolgus monkeys 5 days/week from day 1 post partum onward with the equivalent of 0, 50, or 100 μg/kg/day of lead as lead acetate in a milk substitute formula. The formula was withdrawn at 200 days of age. This treatment resulted in blood lead concentrations of 3, 15 or 25 μg/dl, respectively, and steady-state concentrations of 3, 11, or 13 μg/dl after withdrawal of the milk formula. At approximately 3 years of age, monkeys were tested on an intermittent schedule, with differential reinforcements of low rate. This schedule required the monkey to withhold responding for a specific time in order to be reinforced. The performance of the treated monkeys did not improve as rapidly as of controls as was measured by an increase in the reinforced responses and a decrease in the nonreinforced responses during initial sessions. In addition, the treated monkeys exhibited a greater between-session variability during terminal sessions. These effects were dose related.

Rossouw et al. (1987) divided rats (males and females combined?) in three groups. The first group received a solution of 0.2% lead acetate in drinking water, 7 days prior to conception and continued until parturition. In the second group the tap water was replaced with a solution containing 0.2% lead acetate on the day of parturition and continued for a period of 21 days, while the rats were lactating. During this period both the dams and their suckling litters had free access to the lead acetate solution. In the third group tap water was maintained during gestation and during lactation. Immediately after weaning the animals received 0.2% lead acetate for a period of 21 days. On day 64 postpartum radioligand binding studies were performed to determine apparent receptor densities and affinities in brain membranes. Mean blood lead levels in the offspring achieved immediately after exposure were 0.18-0.26, 33.15, 62.64 and 35.67 μg/100 ml in groups control, I, II, and III, respectively. Mean brain lead levels achieved immediately after exposure were 0.17-0.24,

1.07, 0.84 and 0.44 $\mu g/g$ in groups control, I, II, and III, respectively. Lead exposure during the neonatal phase (group II) resulted in significant increases of the apparent densities of forebrain $\alpha_1$adrenoceptors (by 92%), cortical β-adrenoceptors (by 116%), and striatal D-2dopamine receptors (by 133%). Lead exposure also resulted in a significant decrease of the apparent density of striatal muscarinic receptors in group 1 (by 43%) and group II (by 25%) and an increase in the apparent density of hippocampal S_1 receptors in group II (by 78%). Lead exposure after weaning did not alter the apparent densities of any of the receptors investigated. Other receptor affinity changes, which were not identical in the three groups of rats, were also induced by lead exposure. The developmental phase during which exposure occurs appears to be a determinant of the type of neurotransmitter receptor changes induced by lead.

2.8 Discussion animal data

2.8.1 Quality of the data

Although many studies have been carried out to investigate the reported reproductive effects of lead in experimental animals, the most comprehensive studies, carried out according to EPA guidelines, have been conducted by Kimmel and coworkers, reported in a series of articles (Kimmel et al. 1980, Grant et al. 1980 and Fowler et al. 1980). In these studies there was a large sample size, a chronic dosing regimen, multiple exposure levels, teratology endpoints and appropriate statistical analysis. Together with the study of Dilts and Akohas (1979) the most appropriate basis for determining the NAEL with respect to reproduction in female animals is provided from these studies.
For establishing a NAEL with respect to effects on male reproduction the studies of Chowdbury at al.(1984) and Fowler et al.(1980) are most significant.

2.8.2 Route of exposure

The experimental studies in laboratory rodents have demonstrated the potent embryotoxic and teratogenic effects of inorganic lead salts when administered via intravenous or intraperitoneal route to mothers in single doses during early stages of gestation. However, efforts to produce

similar effects in rodents via oral administration of lead (in drinking water, solid diet or by gavage) were in general negative. No data were found on embryotoxic and teratogenic effects by inhalation.

2.8.3 Placental transfer and levels in tissues

After intravenous administration, lead can be tranferred across the placenta and rapidly accumulates in the teeth, skeleton, and liver.

2.8.4 Blood lead levels and correlation with the dose

Mean blood lead levels are reported by various authors, but there is little consistency in the data. In different studies mean blood levels were found of 30, 26 and 54 μg/dl, after oral administration of doses ranging from 0.22, 5 or 14 mg/kg/day respectively. Interspecies differences may be even more striking, rats treated orally with 14 mg/kg for 60 days revealed a blood lead level of 54 μg/dl (Chowdbury et al. 1984) and mice treated with 149 mg/kg during 84 days showed a level of 32 μg/dl (Johansson and Wide, 1986)

2.8.5 Blood lead levels with respect to the NAEL

In the Kimmel et al. (1980) study, blood lead levels in animals exposed at the NAEL of 0.9 mg Pb/kg/day ranged from 9 to 37 μg/dl with respect to female reproductive aspects.

2.8.6 Conclusion and Evaluation

Lead can be transferred across the placenta in experimental animals,and can accumulate in fetal tissues such as teeth, skeleton and liver.
With respect to female reproductive organs, endocrine system and fertility the only available study reported delayed vaginal opening at orally administered concentrations of 5 mg Pb /kg/day and higher. The NAEL was approximately 0.9 mg Pb/kg/day (PbB lead level 10-16 μg/100 ml).
With respect to gestation and prenatal development, oral administration proved to be embryotoxic with a primary effect on fetal growth, often associated with maternal toxicity. The NAEL corresponds to approximately 0.9 mg Pb/kg/day). IP or IV exposure gave rise to embryotoxic and teratogenic effects at doses of 12.5 mg/kg and 32 mg/kg respectively.
Teratogenic effects comprised exencephaly, microencephaly, cleft palate, spina bifida etc. A NAEL could not be established.

With respect to post-natal development, neurobehavioural effects on reflex development, locomotor activity, learning ability and social behaviour have been observed, often at high doses. Delayed surface righting and air righting were observed at 9 mg/kg/day (Pb blood level 20-40 μg/dl). A NAEL is not established because of the complexity in determining a NAEL from behavioural effects.
With respect to effects on reproductive organs, endocrine system and fertility in males, abnormalities in the spermatogenesis were observed. An oral NAEL value of 14 mg/Pb/kg/day (Pb blood level 54 μg/100 ml) was established.
The oral NAEL for any effects on fertility and/or reproduction is considered to be less than 1 mg of Pb/kg/ body weight (maternal effects).

3 HUMAN DATA

3.1 Risk of exposure of women with respect to reproductive organs, fertility and endocrine system

3.1.1 Gynaecological disorders and fertility

Rom (1976) presented a historical survey on the effects of lead on female reproduction. During the late 19th and early 20th century women in the pottery and white lead industries considered lead to be an abortifacient. From the early statistics on women in the English potteries and white lead plants it was reported that sterility occurred more frequently if compared with women not employed in such plants; moreover an increased incidence of abortions was reported.

In that period of time no data were available to relate biological monitoring data with the prevalence of gynaecological disorders and infertility in women. Most studies should be considered with reservation. The possibility of systemic poisoning by lead should certainly be recognized because excessive exposure certainly occurred. However, the general conditions at and off work were usually poor for occupationally exposed workers, not only with respect to lead exposure. This may have contributed to an unknown extent to the induction of reproductive

endpoints. The studies performed at that time are considered to provide low level human causal evidence.

Zielhuis et al. (1984) reviewed a few studies from Bulgaria, which suggested adverse effects on menstruation in female workers; the human causal evidence has to be considered low level.

3.1.2 Effects on the endocrine systems (see also 4.2)

Numerous data are available from experimental animals. Only a few human studies have been found. Lead has been reported to alter the thyroid function, and this might influence the pituitary regulation of the gonads (Cooper et al., 1986).

Sandstead et al. (1969, USA) observed a decrease of the 24-hour J^{131} uptake in 24 male patients judged to have lead intoxication on the basis of their urinary lead excretion following an infusion of EDTA. The author did not mention participation in an analytical quality assurance program. Thyroid Stimulating Hormone stimulation was followed by a striking increase in J^{131} uptake in all but three subjects. The observations described are consistent with injury of the thyroid trapping and concentrating mechanism for iodine. The baseline lead levels in urine of the patients ranged from 10 to 480 µg/l, but the PbB levels were not determined. Therefore no exposure-dependent effect could be established.

Jhaveri et al. (1977, USA) reported an inverse relationship between serum thyroxine and the PbB levels in 61 children, aged 1 to 6 years. All children were referred to a special lead clinic, because the PbB levels were higher than 400 µg/l. It is questionable whether these findings are applicable to adults; still they certainly warrant further studies wich respect to the thyroid function in relation to the blood lead levels. No data were presented on the quality of the analysis of the PbB levels.

Robins et al. (1983, USA) studied the thyroid function of 12 probably male patients examined for lead intoxication in the Occupational Health Program, Yale University. The PbB levels ranged from 440 to 1170 µg/l and ZPP from 120 to 3100 units/l (upper limit of normal range 220 units/l).

The authors did not mention whether they participated in an analytical quality assurance program. Seven patients had lower estimated free thyroxine and total serum thyroxine levels than the established lower limits for the laboratory. No subject showed myxoedema. The total serum triodothyronine and basal TSH levels were normal in all patients. The authors claimed that these data were compatible with a central depression of the thyroid axis or an alteration in thyroxine metabolism or a binding to proteins. In a cross-sectional study of 47 production and supervisory male personnel working at the shop floor of a brass foundry with average PbB levels of 160 to 1270 μg/l and ZPP levels of 70 to 1870 units/l, they observed a moderate, negative relationship between PbB and the estimated free thyroxine concentrations. 16 Men had levels lower than the borderline thyroid indices. There were differences with respect to race, white men having higher levels than black men; moreover, the social economic status was not taken into account.

The data on the effect of lead on the thyroid function can be classified as low level human causal evidence. The working mechanism is still not known. No data are available at present on the effect of lead exposure on the state of sexual hormones in female workers; it has been assumed that the effects on the thyroid function in male workers may also occur in female workers.

3.1.3 Cancer of the reproductive organs and breast

No data are available on the induction of cancer in the reproductive organs and the breast in women exposed to lead.

3.2 Risk of exposure of women with respect to pregnancy and prenatal development

3.2.1 Spontaneous abortion, stillbirth and perinatal mortality

Wilson (1956, quoted by Rom 1976) followed the course of pregnancy in 72 non-occupationally exposed women who lived in an area in Scotland with soft, lead-solvent drinking water (> 50 μg Pb/l) for two years. The excretion of coproporphyrin in urine was increased in 31 % of women (35 pregnancies), who did not take any preventive measure, but in only 1 %

of women (40 pregnancies) who consumed as little water as possible and drank large quantities of milk. In the first group four complications occurred (stillbirth, congenital abnormalities, premature delivery). The PbB level ranged from 310 to 720 µg/l.

Fahim et al. (1976) examined the course of pregnancy in 253 women living in the "lead belt" area in Missouri, USA and in 249 women from a different region as controls. The authors reported an increased incidence of premature delivery (13 %) and of early rupture of the membranes (17 %) in the first group compared with 3 % and 0.4 % respectively in the controls. However, these events should not be ascribed to lead, because the PbB levels of the mothers and neonates were similar in both groups, albeit somewhat higher in women with complicated pregnancies. No quality assurance program on lead analysis had been performed. The reported PbB levels of the neonates were very much lower than those of the mothers, which finding does not correspond with most data in literature; moreover, the first group also had increased exposure to cadmium. This study does not provide any evidence for adverse effects of lead on the pregnancy in non-occupationally exposed women.

Bryce-Smith et al. (1977, UK) studied the levels of lead and cadmium in samples of rib and/or vertebrae, obtained from stillborn children in Birmingham hospitals. The lead levels of the ribs ranged from 0.4-24.2 µg/g wet weight (n=26) and that of the vertebrae 0.2-13.2 µg/g wet weight (n=42). Necropsy samples of 6 infants, who died at the age of 6 weeks to 10 months, showed bone lead levels ranging from 0.2 to 0.6 µg/kg wet weight. For assessment of the analytical quality, additional repeated checks for the recovery of standard additions of trace elements were carried out. The Pb levels in bone tissue of stillbirths were about 5 to 10 times the levels in the infants with postnatal mortality; 80 % of the levels exceeded 1 µg/g wet weight.

Nordström et al. (1978, Sweden) studied the frequency of spontaneous abortion in the population located at different distances from a lead smelter. Exposure to arsenic, lead and SO_2 may have contributed to the incidence of abortion. On the basis of the data presented a causal role of

exposure to lead and/or arsenic can not be established; further studies are needed, with due emphasis on individual indices of exposure.

Angell et al. (1982, USA) measured PbB levels in 635 specimens of umbilical cord blood collected at delivery at Louisville General Hospital. No information was presented on the quality control of the PbB analysis. The incidences of complications studied were 9.0 % for premature rupture of fetal membranes, 11.8 % for preterm delivery, 5.0 % for preecclampsia and 19.4 % for meconium staining. They found no relation between the PbB levels in cord blood and the observed incidences. Although the authors suggest a treshold concentration for lead for meconium staining and for preterm delivery, the data do not support this. The highest PbB levels for the mother, the child and cord-blood were about the same, about 250 μg/l. This means that the critical level for induction of these complications should be higher than 250 μg/l maternal blood.

Siegers et al. (1983, FRG) studied the lead (and cadmium) levels in amniotic fluid of 155 pregnant non-occupationally exposed women, residing in Lübeck. The samples were taken during early (16-20th week) and late (32-38th week) pregnancy. They did not observe a correlation between the age of the mothers or the week of gestation and the lead levels in amniotic fluid. The mean lead concentrations were 24 μg/l in non-smokers and 22 μg/l in smokers. The same research group reported that the findings neither showed a relationship between the lead levels in the amniotic fluid and pregnancy disorders, nor with the incidence of abortion (Klink et al., 1983). The highest Pb level in amniotic fluid was about 55 μg/l. The paper shows methodological inaccuracies; there is some confusion about the use of units applied for the levels of metals in amniotic fluid. The results should be interpreted with caution.

Recently, Borella et al. (1986, Italy) studied the lead concentration in aborted conceptuses obtained from 50 non-occupationally exposed women following induced abortions at the obstetric and gynaecological department of a Modena Hospital. The authors did not present data on the indications for the abortions. The mean age of the mothers was 27 (15-43) years; 68 % were non-smokers or smoked less than 10 cigarettes per day. The geometric

mean maternal PbB was 103 µg/l (47-397); that of free erythrocyte porphyrin (FEP) 467 µg/l RBC (240-1360). Quality assurance was maintained by using internal and external samples and by participating in quality-control programs of the EC. The geometric mean lead level in abortion materials was 1.27 µg/g dry weight (0.07-5.29). No significant correlation was observed between the parameters measured in mother's blood and the lead content in specimens of the first trimester. The authors concluded that lead did not accumulate in human embryos/fetuses in the first trimester of pregnancy. This conclusion is questionable, because it was based upon a cross-sectional study; this does not permit any conclusion on trends in time. Moreover, no conclusion on reproductive risks can be drawn when the indications for the induced abortions are not known. The human causal evidence with respect to spontaneous abortion, stillbirth and prenatal mortality is inadequate.

3.2.2 Congenital malformations

In recent years a few case studies of women with high PbB and/or lead poisoning during pregnancy have been published. A historical review was published by Rom (1976); this review started in 1881 with a report of a high prevalence of convulsions and a peculiar form of macrocephaly in infants in the German village of Almerode where pottery glazing was a home industry. In an identical situation the same peculiar form of macrocephaly prevailed in a Hungarian village in 1908, which was later confirmed by the British Inspector of Factories.

More recently, Pearl and Boxt (1980, USA) reported in a two-year follow-up study of an infant born with a nonlethal congenital lead poisoning. In the 38th week of pregnancy the 17 year old mother showed signs and symptoms of acute lead poisoning (laboratory findings: positive basophilic stipplings of RBC, increased coproporphyrin levels in urine, PbB 790 µg/l and erythrocyte porphyrin level 2000 µg/l). The lead level in amniotic fluid was even 900 µg/l. The mother went into spontaneous labor and delivered a girl, weighing 2670 g, who appeared to be normal and with Apgar scores of 6 and 8. The PbB was 790 µg/l. In the follow-up period no clinical or laboratory evidence of neurologic, hepatic or renal abnormalities were observed. At the age of two days radiographs revealed a dense skull with

delayed deciduous dental development, multiple sclerotic lines were seen at the distal ends of the long bones and the iliac crest. No ossification of the distal femoral or proximal tibial epiphyses was noted. At 17 days of age the infant was treated with CaEDTA. At three months of age growth and development were normal. An analysis of the child's chromosomes revealed simple breakage in over 40 % of the lymphocytes. The PbB dropped to 200 µg/l after another chelation therapy. At seven months of age the skeletal, mental and dental development was normal.

Qazi et al. (1980, Rumania) examined an infant which mother had lead poisoning; the PbB of the infant was 600 µg/l, that of the mother 860 µg/l and the lead level in amniotic fluid 900 µg/l. At 6 weeks and at 3 months of age the infant showed an increased prevalence of chromosomal and chromatid anomalies, with normalization after 3 months.

Bridbord (1980, USA) mentioned the birth of a probably normal infant of a mother who had been occupationally exposed until 7 weeks before delivery; the maternal PbB was 570 µg/l. At delivery the PbB of the neonatus was 330 µg/l.

Wibberley et al. (1977), UK, observed higher levels of lead in the placentas of perinatally deceased neonates than in those of normal infants; in 13 lethal congenital abnormalities the Pb level in the placenta was 1490 $\pm$ 570 µg/kg (wet weight) compared to 930 $\pm$ 640 µg/kg in 24 normal births. However, the authors emphasized that the high placental lead levels were not necessarily causative, because both the lead levels and the abnormalities might have been the consequence of underlying diseases; moreover, the normal levels were very high in comparison with other literature data. The quality of the trace analysis in this laboratory had been checked by double-blind experiments, and also by assays in other laboratories by alternative procedures. Subsequently Khera et al. (1980, same research group) found average lead levels in the placenta of 320 $\pm$ 40 µg/kg (wet weight) in nine live neonates with congenital malformations, 430 $\pm$ 190 µg/kg in 10 neonates with fetal pathology and about 120 µg/kg in placentas of normal neonates. The latter level is much lower than that found by Wibberley et al. (1977). Although the authors

again did not conclude that occupational lead exposure had been the cause, they nevertheless considered the findings a cause of concern. They advised against exposing pregnant women to lead.

Needleman et al. (1984, USA) reported that lead was associated with an increased risk of minor congenital anomalies. For 5183 non-occupationally exposed mothers giving birth to live born neonates of at least 20 weeks gestational age, 4354 data were available from maternal interviews and PbB levels in cord blood. The lead in blood analysis was verified throughout the study by participation in interlaboratory comparisons every 3 months. They divided the population into 4 groups according to the lead levels in cord blood: 1118 neonates (0-48 µg/l), 1068 mid-low (49-65 µg/l), 1105 mid-high (66-86 µg/l) and 1063 high (87-351 µg/l). The analysis was controlled for confounding factors, e.g. gestational age, birth weight, history of either induced or spontaneous abortion, maternal parity and age. The authors found that low birth weight, short gestation time, low Apgar score, jaundice, blood type and neonate gender were not related to the PbB levels in cord blood. Multiple and major malformations did not reveal a pattern, but the incidence of minor malformation was associated with the PbB level in cord blood. The most common major anomalies found in the total population studied were hemangiomas and lymphangiomas (14/1000 birth) and hydrocele (27.6/1000 male births). Minor anomalies consisted of skintags and papillae (12.2/1000 births) and undescended testicles (11/1000 male births). No data were given on incidences in the general population. No particular type of malformations was associated with lead. This study was not designed to study the impact of lead on fetal death, abortion or other embryotoxic endpoints.

From the compiled data it may be concluded that at present there exists inadequate human causal evidence of induction of congenital malformations, if the maternal PbB during pregnancy does not exceed 800 µg/l. Possible chromosomal aberrations in the infants' lymphocytes, e.g. breakage, appear to be reversible. The skeletal congenital malformation (macrocephaly) as reported in the past probably occurs at much higher levels of exposure and under different conditions of total exposure than are observed at present.

3.2.3 Birth weight/length and prematurity

Ernhart et al. (1986, USA) carried out a cross-sectional study in Cleveland on the possible relation between the PbB levels in maternal (n=185) (non-occupationally exposed) and cord-blood (n=162) with infant's routine measurements. Assessment of the accuracy and precision of the analysis of lead in blood were made using CDC samples of bovine blood as well as on one human and two bovine samples with PbB determined by isotope dilution-mass spectroscopy. The authors did not observe a relation between these exposure variables and infant bodyweight (adjusted for gestational age), body length and head circumference. For further information see also 3.2.4.

The human causal evidence is considered to be inadequate, also when taking into account some studies discussed in 3.1 and 3.2.

3.2.4 Effects on the developing central nervous system

A highly crucial aspect of reproductive risks in low-level lead exposure is the issue of subtle neurologic damage. The human conceptus is maximally susceptible to development of CNS-malformations from week 3 through week 6, but remains vulnerable throughout pregnancy (Mitchell, 1987) and during lactation. This preposition is based on the rapid development of the nervous system during the fetal and the neonatal period and on the evidence of placental transfer of Pb from the maternal system to the fetus.

A case of neonatal lead intoxication and its effects on CNS- development was reported by Ghafour et al. (1984). A Kuwaiti 32 year old woman gave birth at gestational age of 37 weeks to a girl whose birthweight was 2300 grams. The Apgar score was 7 and 9 at one and five minutes; 36 hours after birth the neonate was admitted to the nursery because of convulsions, opisthotonic posture with trismus and stridor and frequent tonic spasms. Examination of other systems revealed no abnormalities. The mother, a housewife, had used lead-glazed utensils for the last 15 years. At the 12th day after birth the analysis of lead in blood showed PbB levels of 760 $\mu g/l$ in maternal blood and 660 $\mu g/l$ in infants blood. Chelation therapy started and the development of the child was closely followed. At the age of 16 months the girl looked clinically normal; however, a basic developmental screening at the age of 2 years showed a poor language development.

Beattie et al. (1975, UK) measured Pb levels in drinking water in homes occupied during the first year of life of 77 mentally retarded children aged 2-5 years and in homes of 77 non-retarded controls, matched for age, sex and geographical location within the city of Glasgow, and also in the homes occupied by their mothers during pregnancy. The mental development was assessed by means of the Griffith and Stanford - Binet test; the quotients were below 70 in all mentally retarded children. The mean lead concentration was 379 $\mu g/l$ for the index group and 223 $\mu g/l$ for the control group. There was a statistically significant association between the lead level in water and the observed mental deficiency. However, in cross-sectional studies it is not possible to prove that the lead exposure caused the mental retardation. In a following study of the same population Moore et al. (1977) measured the PbB levels retrospectively from samples obtained from cards used for testing phenylketonuria in the first two weeks of life. For the analysis of lead, the punched disc technique was used. To upgrade the reliability multiple sampling was carried out: the mean coefficient of variation for standard cards as well as for a series of cards was about 8 %. The 77 cards referred to 41 mentally retarded children and 36 controls. The mean PbB of the index group was 256 $\mu g/l$, which was significantly higher than that of the control group (210 $\mu g/l$). By means of paired analysis 24 mental-retarded/control pairs were studied. Again, a significant difference in the distribution of PbB was found. It should be emphasized that the PbB levels of subjects in the control group appeared to be higher than those generally found in other studies. Moreover, the blood samples used for other purposes do not take into account the possibility of contamination. This may have underestimated the risk.

Winneke et al. (1985, FRG) studied the neuropsychological performance of 114 6-to-7-year-old children living in or near the city of Nordenham (soil pollution by lead). These children represented 30 % of all children born in the district hospital at that time; the group was rather biased with respect to higher social class. The battery of tests referred to intelligence, visual-motor performance, serial reaction performance and cued as well as choice reaction time. Records of PbB levels in cord blood and in maternal blood at delivery were available; the present PbB of the children was also measured. The mean PbB level in cord-blood was 82 $\mu g/l$ (40-300) and the present PbB of the children was 82 $\mu g/l$ (44-238). There

was some discrepancy in the levels reported in this paper which reduced the credibility of the outcome; no information was presented on the quality assurance of the lead in blood analysis. Nevertheless, the authors suggested an association between children's present lead levels in blood and the performance deficit after correction for confounding factors; however, the association did not exist for the lead level in cord blood. The only conclusion of this study may be that lead exposure later in childhood might have a somewhat stronger impact on the neuropsychological development as established with these tests than prenatal lead exposure.

An excellent prospective cohort study was carried out by Bellinger et al. (1984, USA). They studied the possible relationship between the lead levels in cord blood and the early sensory-motor development of 6 months old infants in Boston. The children were divided into three groups according to the PbB levels in cord-blood: 85 with low (< 30 μg/l), 88 with medium (60-70 μg/l) and 76 with high ($\geq$ 100 μg/l) PbB levels; no infant had a level higher than 300 μg/l. The analysis of PbB was performed by Anodic Stripping Voltametry and the mean PbB of the children at the age of 6 months was 62 μg/l (0-489 μ). The quality of the lead in blood analysis was guaranteed by independent internal checks of reliability, blind comparison sponsored by the CDC and repeated analysis of the bovine liver standard furnished by the National Bureau of Standards. At the age of 6 months the Bayley Scales test of Infant Development was applied, which yielded two scores, the mental development index (MDI) and the psychomotor development index (PDI) (Bellinger et al 1985). In the association between the PbB level lead in cord blood and the MDI they found two confounding variables: the total score on HOME (the instrument used to measure the quality of the rearing environment) and the length of gestation. By controlling for these factors, they found that higher PbB levels in cord blood were associated with a lower MDI ($r=0.19$). No relation was found between the present PbB and the MDI. More detailed study of the variables showed that the "fine motor", "visual directed reaching" and "social responsiveness" test results significantly decreased with increasing lead levels in cord blood ($P < 0.05$). On the other hand, no association was found between PbB level in cord blood and the PDI. This study highly suggests that at a PbB level in cord blood > 100 μg/l cord

blood appears to be associated with early developmental disadvantage as assessed with the Mental Developmental Index of the Bayley Scales. It should be pointed out that the population studied belonged to the middle to upper class category; therefore, the data may have underestimated the risks for lower class populations. A MDI score at the age of 6 months reflects largely the child's attentiveness and responsiveness to animate and inanimate stimuli, as well as rudimentary problem-solving.
In 1986, Bellinger et al. reported a follow-up assessment of the same infants at the age of 12 months; in this study the focus was only on the MDI score. The mean PbB level was 77 μg/l (0-360). The results showed that the PbB levels in cord blood were significantly related to the MDI both at 6 and 12 months of age, when adjustments were made for a small set of additional variables. For 6 months old infants this included length of gestation, total score on HOME, weight gain during pregnancy, maternal education and gravidity; for the 12 months old infants length of gestation, HOME, the score of "emotional and verbal responsivity of mothers", the amount of time the infant spent mouthing a standard set of toys during a 10 min observation period and the maternal use of nausea medication during the first trimester of pregnancy. By using multiple regression analysis and trimming for the variables, the adjusted mean MDI scores of the low and high lead infants differed by 5.8 and 7.3 points at 6 and 12 months respectively. The greatest differences were observed for fine motor functions, language and imitation. Infants' postnatal PbB levels were not related to the adjusted MDI scores both at the age of 6 and 12 months. From this follow-up study it may be concluded that assessment at the age of 12 months indicates that the deficit observed at the age of 6 months in the performance of infants with "high" prenatal lead exposure (umbilical cord blood $\geq$ 100 μg Pb/l) persists throughout the end of the first year of life.

In 1987 Bellinger et al. published the results of a follow up of the above mentioned group of children up to 24 months. The differences in the MDI observed at 6 and at 12 months of age appeared to persist. The authors mentioned three reasons to explain the absence of a relation between the postnatal PbB level and the MDI: first, it might have been a statistical artifact. The mean lead level in cord blood of the infants in the high

group exceeded the mean level of infants in the low group by a factor of 8 (146 and 18 μg/l respectively), whereas the postnatal PbB levels were much more comparable at 24 months, 54 and 77 μg/l respectively. This might have limited the ability to detect a small postnatal effect. Secondly, early postnatal PbB levels of 0-250 μg/l may not adversely affect the performance on the Bailey scales. Third, adverse effects associated with postnatal PbB levels may be discernible at later ages or in infants who are at greater risk of a poor outcome on the basis of socio-environmental factors than those in the group studied. It should be kept in mind that the parental socioeconomic status of the studied infants was relatively high. Therefore, the study provided a conservative assessment of the association.
The authors came to the following conclusion: "If replicated in other samples, our findings suggest that the current standard of the Center of Disease Control for acceptable blood lead levels in young children (< 250 μg/l) should not be applied to fetuses".

Dietrich et al. (1986) reported a prospective study on 305 lower socioeconomic status pregnant women residing in Pb contaminated areas of Cincinnati, USA. The mean age of the women at delivery was 22.7 years (range 15-39 years). Infants of less than 35 wk gestation and/or less than 1500 g birth weight were excluded from the study. Further, the recruited infants should have an Apgar score of 6 or greater at 5 minutes, and presented no serious medical conditions such as Down's Syndrome. The infants had a mean birth weight of 3135 g and a mean gestational age of 39.5 weeks. Potential confounders such as tobacco and alcohol use during pregnancy, and SES of family, etc. were taken into account. Assessment of lead exposure was performed by measuring the PbB of the mother at the first visit to the prenatal clinic and PbB of the neonatus at the age of 10 days (corrected for gestational age). Analyses was performed by Anodic Striping Voltametry and the quality was maintained by participation of several quality control programs. The arithmetric mean of maternal prenatal PbB was 80 μg/l (range 10-270) and that of the neonates was 45 μg/l (range 10-220). All PbB's were corrected for hematocrit. Prenatal maternal and neonatal PbB's were weakly correlated (r=0.30). Behavioral assessment was performed at 6 months by means of Mental Development Index (MDI),

Psychomotor Development Index (PDI) and Infant Behavior Record (IBR) of the Bayley Scales of infant development.
Multiple regression analyses of the data revealed a relationship between maternal prenatal PbB and the Bayley development outcomes. Prenatal PbB was not related to 6-months Bayley PDI or the IBR; however, it was inversely related to MDI ($p=0.02$) and there was a significant interaction in PbB by sex ($p=0.02$). It was shown that male infants were apparently more sensitive than female infants to the effects of early fetal Pb exposure. Separate multiple regression by sex of infants showed no such adverse effects in female infants. Multiple regression analyses between neonatal blood and Bayley development outcomes at 6 months showed that PbB was not related to IBR factors of attention, positive mood and activity, and PDI; however, the neonatal PbB was inversely related to MDI ($p=0.003$) and the IBR factor of motor maturity ($p=0.04$). There was also a significant interaction between PbB of the neonates and SES for MDI and motor development. Those infants with lower SES parents had much greater Pb-related deficit in MDI and IBR motor maturity factor. It is important to emphasize that infants with lower SES mothers tended to have higher PbB levels ($p=0.001$). Further statistical analyses by structural equations showed that maternal prenatal PbB was inversely related to birth weight of infants, which in turn was positively associated with 6-months MDI and PDI. Prenatal PbB was also inversely associated with gestational age, which in turn was also positively associated with Bayley developmental outcomes. Therefore, a portion of Pb's adverse effects on behavioral development was "indirect", through its dynamic interaction with fetal growth and maturational variables.

In a more recent publication on the same population, more study data were forwarded (Dietrich et al. 1987). The authors took two more independent exposure variables into account: lead in blood analyses of cord blood and blood of 3 months old infants. All levels were corrected with the mean hematocrit level for the developmental age. The mean PbB in cord blood was 63 $\mu g/l$ (range 10-280) and in the 3 months old infants 59 $\mu g/l$ (range 10-220). Of the 305 cohorts in the samples, 266 prenatal, 96 cord and 302 newborn blood lead values were available for analyses. The variables for behavioral development were also augmented with the Bayley Scales at 3

months old. Multiple regression analyses showed no significant effects of fetal lead exposure on PDI were found, after adjustment for covariates. However, blood indices in utero lead exposure were consistenly related to MDI at 3 months and 6 months of age. Both maternal prenatal and umbilical PbB values were inversely related to MDI at 3 months. On the other hand, none of the assessments in newborn of PbB were associated with 3-months MDI after covariate adjustment. Umbilical cord and 3-months PbB values were also inversely related to 6-months MDI, but were not statistically significant when using a two-tail test.
The results of this study confirmed the earlier report: male infants from the poorest families appeared to be most sensitive to these psychoteratogenic influences. The neurobehavioral deficits appeared to be partly mediated by lead-related reductions in birth weight and gestational age.

A cross-sectional study in Ohio, reported by Ernhart et al. (1986, USA) (see 3.2.3) was of restricted quality. The authors investigated the possible relationship between the PbB levels in maternal or cord-blood with routine newborn assessment variables including minor anomalies, the Brazelton Neonatal Behavioral Assessment (NBAS) and part of the Graham/Rosenblith Behavioral Examination (G/R). Assessment of accuracy and precision of lead in blood analysis was carried out using CDC samples of bovine blood as well as of one human and two bovine samples with PbB determined by isotope dilution-mass spectroscopy. The authors found no relation between the Pb levels in maternal or cord blood with the Apgar scores, birth weight, length, head circumference, neonatal anomalies and seven behavioral scales. Three scales - the NBAS abnormal reflexes, the G/R Neurological Soft Sign and the G/R Muscle Tonus scales - had been studied in relation to either the cord or the maternal lead levels in blood. The mean cord blood lead level was 58 µg/l (26-147) and the mean maternal blood lead level was 65 µg/l (27-118).
Possible confounding factors, which were not taken into account, include the fact that 50 % of the women had histories of alcohol abuse and about 80 % reported smoking of cigarettes; all women were of the lower-class category and 33 % of them were black. This study does neither support nor refute the hypothesis of adverse effects due to rather low-level intrauterine lead exposure.

Bonithon-Kopp et al. (1986, France) reported a significant negative relationship between the degree of in utero exposure to (cadmium and) lead and the motor and perceptual abilities at 6 years of age. As a measure for the in utero exposure they analyzed the lead and cadmium levels in hair samples taken from the infant at birth. The results of this study are questionable, because metal levels in hair may reflect external contamination (Wibowo et al. 1980). Moreover, the relationship between the levels of lead in fetuses in utero and of lead levels in fetal hair has hardly been studied yet.

The human causal evidence with respect to adverse effect on the developing central nervous system at lead levels in cord blood below 250 $\mu g/l$ is considered sufficient, because independent confirmation has taken place.

3.3 Risk of exposure of women with respect to the offspring through lactation

Lead is excreted in breastmilk; the lead concentration is of the same order of magnitude as that in blood plasma, i.e. about 5–10 % of the concentration in whole blood. The additional intake by the infant through breast milk of women who have been occupationally exposed during pregnancy and who are still being exposed during lactation, does increase the body burden of lead in the infant.

Larsson et al. (1981, Sweden) studied the lead contents of 41 human milk samples 3 and 6 months post partum from the same healthy probably non-occupationally exposed mothers, aged 21–35 years, living in Upsala. The median level was 2 $\mu g/kg$ (wet weight) (0.5–0.9). There was no appreciable difference between the levels at 3 and 6 months post partum. It was estimated that the weekly intake of lead by the infants was 1.2 $\mu g/kg$ body weight (0.3 to 6.4).

Huat et al. (1983) also reported no specific pattern of the milk lead levels at different periods of lactation. In their study in Malaysia they found higher lead levels than in other countries. Analysis of lead in milk samples collected from 89 urban and 91 rural mothers showed mean levels of

25.3 µg/l (2.1-58.9) and 21.1 µg/l (4.4-53.8) respectively. The daily average lead levels in air of the urban area ranged from 2.66 to 5.63 $\mu g/m^3$, which can be considered high.

Rockway et al. (1984, USA) found no relationship between the lead levels in maternal blood and breastmilk. They studied 39 lactating women, aged 22-47 years. No information on occupation was presented; in view of the sometimes high PbB levels occupational exposure of some women may be expected. The average PbB was 119 µg/l (32-530); the average lead level in breastmilk was 2.8 µg/l (0.9-10.0). The accuracy of the lead analysis was checked by analysis of bovine liver from the US National Bureau of Standard, but the authors did not report any participation in any quality assurance program. The number of milk samples was much higher than the number of blood samples, which means that an unknown number of samples has been taken from the same mothers; this may have created a selective bias. This study also analyzed lead concentrations in maternal hair; no relation was found with the other exposure variables.

Rabinowitz et al. (1985, USA) reported a good relation between the lead levels in breast milk and the PbB levels in 6 months old infants. The study was performed in 100 breast milk samples from 100 probably non-occupationally exposed mothers who gave births in the Boston lying-In Hospital. The mean lead level in breastmilk was 17 µg/l (0-72) and the mean PbB of 6 months old infants 62 µg/l (0-490). The PbB in cord blood poorly correlated with the lead level in breastmilk. Regression analysis showed that the levels in milk accounted for 10 % of the variance of the PbB of 6 months old infants. The quality of the lead in blood analysis was guaranteed by participation in an interlaboratory comparison program every 3 months.

Sternowsky and Wessolowski (1985, FRG) studied lead levels in breastmilk of 10 women each from the city of Hamburg, and from a rural area at regular intervals for three months after birth. The mean Pb level in the urban Hamburg breastmilk samples decreased non-significantly from colostrum to mature milk from 15.5 $\pm$ 6.1 µg/l to 9.1 $\pm$ 2.5 µg/l and in the rural area from 12.5 $\pm$ 4.1 µg/l to 8.0 $\pm$ 2.1 µg/l. Women over 30 years of age, from

both groups together, excreted a significantly larger amount of lead than those below 30 year of age. The daily intake of lead by the Hamburg infants was estimated to be 1.5–2.3 μg Pb/kg and by rural infants 0.9–1.3 μg Pb/kg (calculated on the basis of an intake of 840 ml breastmilk per day for by an infant of 5.5 kg).

Sartorelli et al. (1986, Italy) found a mean concentration of 3.5 ± 2.03 μg Pb/l in breastmilk (28 mothers) 5 days after delivery.

Ong et al. (1985, Malaysia) noted that transfer of lead from maternal tissue to milk is possible. The mean concentrations of lead in maternal blood was 152 μg/l (75–240) and in milk 48 μg/l (25–106) in 114 mothers 3–5 days after delivery in an urban maternity hospital. This suggests a ratio maternal blood/breastmilk of about 3.

Kovar et al. (1984, UK) reported in a Central London maternity hospital lead levels of < 2 to 9 μg/l in breastmilk of 28 mothers five days post partum; the PbB levels were 60 to 192 μg/l; all pregnancies were medically uncomplicated and went to term.

The kinetic model of lead in breastmilk appears to be complex (Wolff, 1983). Lead is strongly bound to hemoglobin, which may greatly impede the transfer to milk. The plasma levels of lead normally constitute a small proportion of lead in blood (about 5–10 %). Therefore, the partition of lead between the blood compartments may thus determine the transfer to breast milk. Determination of plasma lead (or transferable lead) is important in understanding this phenomenon.
The conclusion is that the human causal evidence that lead is excreted to a certain extent with breastmilk should be considered sufficient. The intake will increase the body burden of lead in the infant. No studies have been reported on the potential risks to infants through lactation. Whether this is reflected in an extra risk in addition to that of the burden due to prenatal occupational exposure has not been studied as far as known.

3.4 Risk of exposure of male workers with respect to reproductive organs, endocrine system, fertility and offspring

3.4.1 Effects on sexual potency, spermatogenesis and fertility

Lancranjan et al. (1975, Rumania) studied male reproductive endpoints in 100 lead-exposed workers and in 50 controls (technicans and office workers); the mean age of the total group was 38,5 years, the mean duration of exposure of the lead workers 8.5 (1-23) years.
The total sample was divided into four groups:

		PbB (μg/l)*	ALA.U (mg/l)
I	exposed workers with lead poisoning (n=23)	745 $\pm$ 260	56,52 $\pm$ 20
II	workers with moderately increased absorption, no lead poisoning (n=42)	528 $\pm$ 210	22.44 $\pm$ 8.8
III	workers with slightly increased absorption (n=35)	410 $\pm$ 120	7.7 $\pm$ 4.2
IV	non-exposed controls, physiologic absorption of lead (n=23)**	230 $\pm$ 140	4.4 $\pm$ 2.2

* when in the paper expressed in μg/100 ml, then adjusted to μg/l
** only for a part of the controls available

The workers were considered to suffer from chronic lead poisoning when at least two symptoms characteristic of lead poisoning were present and when they had at least moderately or severely increased PbB levels, according to the criteria adopted by the Conference on inorganic lead (1971).

The parameters of sexual potency were distributed as follows:

	n	decreased libido %	pathologic erection %	pathologic ejaculation %	decreased orgasm %
group I	23	21	48	30	30
group II	42	33	33	38	4
group III	35	28	22	40	5
group IV	50	16	14	16	2

These data were derived from a detailed sexual history. The reproducability of the questionnaire was not discussed by the authors.

Sperm was collected after at least three days of abstinence in 89 men selected from 119 volunteers after exclusion of those suffering from varicocele, hydrocele, previous venereal disease or genital tuberculosis, and those with recent febrile disease.

The following results were reported:

group		decreased spermatognesis		asthenospermia		hypospermia		teratospermia	
		n	%	n	%	n	%	n	%
I	lead poisoned (n=16)	15	93	8	50	8	50	14	86
II	moderately increased absorption (n=29)	22	68	15	51	13	44	17	58
III	slightly increased absorption (n=19)	12	63	8	42	8	42	6	31

group		decreased spermatognesis		asthenospermia		hypospermia		teratospermia	
		n	%	n	%	n	%	n	%
IV	physiologic absorption (n=25)	7	28	6	24	7	29	4	31

The numbers per group were considerably smaller than the total group of 100 exposed workers and 50 controls, which may have led to a selective bias.
There apparently exists an increasing percentage of sperm abnormalities with increasing PbB level.
The authors also concluded that lead poisoning and even moderate exposure to lead (PbB 528 $\pm$ 210 μg/l) decreased the fecundity of the workers. This was indirectly derived from the suggested effects of lead on sexual function and on spermatogenesis. According to the authors the data were inconclusive with respect inter alia to the number of normal pregnancies per couple, frequency of spontaneous abortion and induced abortion. Therefore, an impact on fertility could not be established.

The study by Lancranjan et al. was the first study that suggested an effect with respect to the sexual functions in lead exposed workers. As such it has served as an important stimulus for further research. However, the study design was deficient in some important aspects. Participation in an analytical quality assurance program was not mentioned. The relationship between the PbB level in blood and the ALAU level in urine of the controls is not consistent with what may be expected in non-exposed workers.
The PbB levels of the four groups show a considerable overlap. Because the PbB level is the best parameter to estimate the hazard, it would have been more appropriate to treat the whole group as a continuum of internal lead exposure, or to divide the total group into four quartiles with PbB as independent variable. Moreover, in the reference group with physiologic absorption the PbB levels had a 95 % confidence interval of 230 $\pm$ 2 x 140, which corresponds to - 50 up to + 510 μg Pb/l, assuming a Gaussian

distribution. Because the distribution of the PbB-levels is highly skew arithmetic averages should not have been calculated. In non-exposed workers usually a PbB level of less than 200 µg/l is observed; therefore, the reference group may also have had increased levels.
The determination of ALA in urine is beset with many difficulties (Herber, 1980); in non-exposed workers one would expect a level below 5 mg/g creatinine. The average level of 4.4 ± 2.2 mg/l in the reference group again suggests increased lead exposure.
The data on sexual potency were based upon information obtained through a questionnaire; no data were presented on the validity of this method. There was no consistent increase of the percentage of decreased libido and pathological ejaculation with increasing lead absorption in contradiction with the conclusions by the authors. The only consistently increased symptom was that of pathological erection (although this phenomenon was also observed in 14 % of the reference group). No adjustment for age was carried out, nor for consumption of alcohol and tobacco.
Therefore, the relationship between the PbB level and the effects on sexual functions as presented in this study cannot be regarded as valid, although the qualitative evidence of an effect on spermatogenesis is suggestive.

A few other case studies also highly suggest an effect on sexual function. Cullen et al (1984, USA) mentioned symptoms of sexual dysfunction in 3 of 7 patients with lead poisoning. Ruse et al (1977, Rumania) reported symptoms of decreased libido, impotence or ejaculatio praecox amongst several of 37 patients with chronic metal poisoning (Pb, Hg, Cu, Mn), 27 of them with lead poisoning.

The human causal evidence with respect to impaired sexual potency still appears to be low level.

Several studies have been carried out after 1975 with respect to adverse effects on spermatogenesis.

Plechaty et al. (1977, USA) measured the lead level in blood and in semen and determined the sperm count in 21 non-occupationally exposed healthy

medical students and technicians, aged 19-41 yrs. The mean PbB level was 131 µg/l (70-190) and the mean Pb level in semen 59 µg/l (10-110). The mean sperm count was $54x10^6$/ml (13-120) and the mean seminal protein concentration 29 g/l (20-40). In each subject the lead level in semen was less than or equal to that in blood. No significant relationships were found between the lead concentration in blood and semen and between the lead level in semen and the spermcount.
No information on the quality assurance of the lead analysis was given. The study suggests that the average no-effect PbB-level is higher than 130 µg/l in groups of non-occupationally exposed men with respect to effects on spermatogenesis.

Ruse et al. (1977, Rumania) also measured in 17 of the 27 patients with chronic lead intoxication the sperm count ($1-48x10^6$/ml) and the percentage of motile spermatozoa (2-56, mostly $\geq 30x10^6$); the subject with only $1x10^6$ spermatozoa/ml also had the lowest motility percentage (2 %) whereas the subject with the highest motility percentage had a sperm count of $41x10^6$/ml. In four patients with lead intoxication treatment with lympholysized choriogonadotrope hormone (extracted from urine of pregnant women) led to an increase of the sperm count with a factor 2 to 3 and of the percentage of motile spermatozoa with a factor 1.5 to 2. Therefore, the authors concluded that the effect on spermatogenesis was due to an indirect effect on the hypothalamo-pituitary axis. The PbB levels in this study were not measured; therefore, no information on the no-adverse effect level of PbB can be given. The study highly suggests that the effect on spermatogenesis is reversible. The authors did not present information on the reason for excluding 10 patients from the 27 with lead intoxication.

Braunstein et al. (1978, USA) studied 6 workers exposed for 2-11 years to high lead in air levels in a secondary lead smelter. They suffered from lead poisoning (peripheral neuropathy, saturnine gout, encephalopathy and/or recurrent abdominal colic pains); all had a marked decrease of libido and frequency of intercourse. They were removed from exposure three months before the present studies; in that period they recieved EDTA-treatment.

In addition 4 lead exposed workers without evidence of lead poisoning were studied; 3 of them reported some decrease of libido and frequency of intercourse. In addition, 9 male volunteers with a similar socioeconomic background served as controls; 8 of them denied a change in libido or in frequency of intercourse. The PbB levels at the time of testing were 387 $\pm$ 30 μg/l, 290 $\pm$ 50 μg/l and 161 $\pm$ 17 μg/l for the lead-poisoned, lead exposed and controls respectively; the Pb in urine levels after EDTA-provocation were 999 $\pm$ 141, 332 $\pm$ 17 and 224 $\pm$ 31 μg/24 h respectively. No mention was made of participation in an analytical quality assurance program.
The sperm count of 5 lead-poisoned men who were able to produce an ejaculate (the 6th patient was impotent), and of 3 lead-exposed men (one had undergone vasectomy) ranged from normal to severely oligo-spermic.

The sperm counts were $<10^6$/ml to about 10^8/ml, $<10^6$/ml to about 10^9/ml and $>10^7$, in the lead poisoned (5), lead exposed (3) and non-exposed controls respectively. Testicular biopsy was carried out in two of the most severely lead poisoned workers (one with azoospermia, the other with testicular pain). There was an increase of peritubular connective tissue; the Leydig cells were morphologically normal; lipofuchsine bodies were observed in the Sertoli cells, presumably representing degrading lysozomes. This case study confirms previous studies with respect to an effect on libido and spermatogenesis (see also 4.2).

In a case study by Cullen et al. (1984, USA) the sperm quality was examined in six patients with lead poisoning. The sperm count was below normal values in 4 patients and the percentage of motile spermatocytes was decreased in 3 of 4 patients; two patients (PbB 550 and 390 μg Pb/l) were azoospermic. Testis biopsy showed markedly depressed spermatogenesis, Leydig cell hyperplasia and tubular and interstitial fibrosis. After chelation therapy the semen analysis showed a tendency towards improvement in two cases, but deterioration in one case after reexposure to lead.

Rachootin and Olsen (1983, Denmark) studied 1069 infertile case couples and 4305 fertile couples. The Odd's Ratio (OR) for abnormal sperm was 0.9 (95 % conf. interval 0.5-1.6) and for idiopathic infertility 0.8 (0.3-1.8)

in workers exposed to lead, cadmium or mercury. When subgroups of male partners in subfecund couples were compared with fertile controls by reported exposures the OR for delayed conception was 1.3 (0.9-1.8). This study did not indicate any significant effect of occupational exposure to lead, cadmium or mercury on sperm abnormality. However, it also did not exclude such a risk in view of the increased upper 95 % confidence limit. The study was only undertaken to generate hypotheses and not to test a specific hypothesis. No data on the level of exposure to lead were available.

Stanwell-Smith et al. (1983, UK) conducted a comparative study of zinc, copper, cadmium and lead levels in blood of 80 in fertile and 38 fertile non-occupationally exposed men, 20-45 years of age. The PbB levels were almost exactly the same: 0.8 $\pm$ 2.4 μg/l. The sperm count and the motility percentages of the infertile men differed significantly ($P < 0.001$) from those of the fertile men; however, this was not related to the PbB level. The PbB levels in this study are unexpectedley low even for non-occupationally exposed subjects (expected 100 to 200 μg Pb/l). No data on the analytical validity of the PbB-measurement were presented. This study cannot be considered informative, because of the probably poor analytical method.

Butrimovitz et al. (1983, USA) measured the seminal lead concentration in normal and vasectomized urban male subjects without a history of occupational exposure to lead. In normal men (n=15) the level was 38 μg Pb/l versus 41 μg/l in vasectomized men (n=16); the intra-assay coefficient was 2.7 %, with a limit of sensitivity equivalent to 0 $\pm$ 3 μg/l. Participation in a interlaboratory analytical quality assurance program was not mentioned. No significant relation between the Pb levels in semen and the sperm count (78 $\pm$ 58 x 10^6/ml) and sperm motility (82 $\pm$ 12%) was observed. The authors suggested that the main contribution to the lead level in semen came from the prostate and the seminal vesicles.
The authors considered the lead in semen levels to be "extremely low". However, they were rather similar to those reported by Plechaty et al. (1977).

Chowdbury et al (1986, India) examined the semen quality of 10 male workers (average age 30 year) exposed to lead in a printing plant during a period of 10 ± 1.8 years; the average PbB level was 425 µg/l; 10 administrative workers served as controls (similar average age and body weight). No information was presented on the period of abstinence. All data were reported only qualitatively. Blood and semen lead levels appeared to be significantly higher in the exposed groups than in the controls; the same was true for the sperm count and the percentage of motility (levels not presented). Moreover, tail abnormalities in sperm cells were markedly predominant in the exposed workers. The seminal plasma acid phosphatase, succinic dehydrogenase and fructose levels were decreased in the exposed group. The authors suggested the following working mechanism: fructose is the principle nutrient for the survival of spermatozoa in the epididymis; the androgen dependent enzymes, c.q. acid phosphatase and succinic dehydrogenase, contribute to the process of fructolysis; this suggests an indirect effect on spermatogenesis.
The authors considered the workers to be moderately exposed ("almost around the normal values"); they concluded that moderate exposure causes subtile changes in some semen parameters.
The number of subjects examined in this study was small. No quantitative information was presented on the various effect parameters examined; no information on the analytical validity of lead analysis was presented. An average PbB-level of 425 µg/l should not be considered as "almost around the normal values". The paper can be considered to be non-informative, although not in contradiction with the other papers discussed.

Assennato et al. (1986, Italy) examined 39 white male battery workers (age 41 ± 10 years, duration of employement 5 ± 2 years) and 39 cement workers as controls (age 40 ± 10 yrs). The groups did not differ significantly in age, weekly frequency of intercourse and wine, coffee and tobacco consumption. The duration of abstinence was minimally 5 days.
The sperm motility was not examined because a delay of 1.5 to 3 h after ejaculation (masturbation) raised questions with respect to the reliability of the motility analyses. The indices of the lead body burden were as follows:

	units	exposed		non-exposed		
		donors	non-donors	donors	non-donors	p*
blood lead	µg/l	610 ± 200	500 ± 140	180 ± 50	220 ± 70	<0.001
semen lead	µg/l	79 ± 36	**	22 ± 9	**	<0.001
n		18	21	18	21	

* : p, two-tailed t-test (semen donors of battery workers versus cement workers)

** : not analyzed

The laboratory participated in an interlaboratory quality assurance program.

The Pearson correlation coefficient between PbB and sperm count was -0.385 (p=0,10) and between PbB and Pb-semen -0.026 (p=0.440) (factor 7 to 8).

The cumulative frequency distribution of battery worker's sperm count was significantly shifted ($p < 0.025$) in comparison to the controls (45 vs 73 x 10^6 cells/ml); a three fold increase in oligospermia (16.7 vs 5.5 %) was observed.

The participation of semen donors was less than 50%. The study suggests a direct effect on sperm production or transport in occupationally exposed workers with a markedly increased PbB up to average 610 ± 200 µg Pb/l.

Fisher-Fishbein et al. (1987, USA) described a case of lead poisoning of a fire arm instructor who appeared to be infertile, although he had a child from a previous marriage. He was treated with chelation therapy and remained under observation over a period of three years. Before treatment the PbB level was about 900 µg/l and the sperm count 12.5 x 10^6/ml with a motility of 50 % (with a poor intensity), 40 % normal spermatozoa and 30 % spermatozoa with defects. After chelation therapy the PbB level decreased to about 300 µg/l, whereas the sperm count increased up to 110 x 10^6/ml; the percentage of motile spermatozoa did not change, but the intensity of motility improved; the percentage of normal spermatozoa increased; the percentage with head defects decreased. Moreover, his wife conceived a child within a few months after the treatment had started. The improvement of the sperm count coincided well with the decrease of the PbB-level and the

increase of the sperm count. This case study is highly suggestive of a reversible adverse effect of lead exposure on spermatogenesis. Although the authors did not mention participation in an interlaboratory analytical quality assurance program, it is expected that they did so when the hospital where this case was investigated, is taken into account.

Wildt et al. (1983, Sweden) studied the semen quality of lead exposed battery workers. The PbB level was periodically determined since 1978. The The laboratory participated in an analytical quality assurance program. Those with a PbB > 600 $\mu g/l$ were removed from exposure until the PbB had decreased to 400 $\mu g/l$; those with a PbB > 450 $\mu g/l$ were examined monthly; the ZPP-level in blood was also determined.
Two groups of workers were examined: group I, n=31, with lead exposure and having been followed up for at least one year prior to the start of the study; the PbB levels during the last six months exceeded at least once 500 $\mu g/l$, n=31, age 18-61 years; group II, n=31, matched for age, ethnic and social factors; the PbB levels exceeded only occasionally 300 $\mu g/l$ and the ZPP-level never exceeded 300 $\mu g/l$ blood. After the available documented data had been checked, two men were rejected: one with azoospermia because of a prostate operation, the other because of extremely high PbB-levels, even after a long period of exclusion from the plant.
Semen samples were obtained after 5 days of abstinence. Examinations were carried out at two different points of time: in September 1978 (3 months after the period with lowest exposure risk of the year) and in April 1979 (3 months after the period with highest exposure risk). The 3 months interval was selected with respect to the spermatogenic cycle of 74 days. Of group I 28 % (4 of 14) had a low semen volume when compared to 4 % (1 of 23) in group II. Moreover, 43.5 % of group I and 12.5 % of group II showed a decreased function of the accessory genital glands ($p < 0.05$) (parameters: fructose, acid phosphatase, Zn and Mg levels in seminal plasma).
The percentages of semen samples with normal properties according to clinical routine evaluation were 69.6 and 62.5 % (two examinations) in group II, but only 35.7 and 31.3 % in group I. However, after adjustment for the impact of the secretory dysfunction, there was no sign of an effect on sperm count, motility and morphology. The test for stability of

the spermatozoa against SDS (sodium dodecyl sulphate) treatment for evaluation of chromatin stability, showed a lower stability in group I than in group II. The SDS-treatment was regarded to offer a parameter of functional maturation. The study showed that this parameter was already affected without an impact of lead exposure on the sperm count and motility. Moreover, a decreased secretory function of the accessory genital glands was noted more frequently among men with the higher degree of exposure.
This study was very well designed, and sufficient attention was payed to analytical quality assurance.
The authors concluded that moderate occupational exposure to lead of carefully periodically examined workers, may adversely affect the sperm quality, even when the normal clinical parameters are not affected. There was no impact on fertility. This study suggests that in a group of workers with an average PbB level of 450 $\mu g/l$ subtile dysfunction of the spermatogenesis and a decreased function of the accessory genital glands may occur. It is not possible to derive an individual NAEL for lead in blood; however, at group level the NAEL appears to be below 450 $\mu g/l$ (individual range 340 to 670 $\mu g/l$).

The human causal evidence with respect to an adverse effect on spermatogenesis appears to be sufficient.

3.4.2 Effects on the endocrine system (see also 3.2)
Lancranjan et al. (1975, see 4.1) also measured the excretion of 17-ketosteroids and of total gonadotropin in urine. No difference between the four groups examined was observed.

Braunstein et al. (1978, see 4.1) also studied the impact on the hypothalamic-pituitary-testicular axis in all 10 lead exposed workers (6 lead poisoned, 4 lead exposed without poisoning) and 9 non-exposed controls.
The basal testosterone levels in blood were significantly lower in the lead workers than in the controls. The percent increment in testosterone following i.m. injection of human chorionic gonadotropin (hCG) was greater in the 6 lead poisoned men than in the 9 controls and the 4 lead-exposed

men. There was no difference between the three groups in the serum testosterone-estradiol binding globulin activity, the basal estradiol level and the percent increment of serum-estradiol following hCG stimulation. However, both the lead poisoned and the lead exposed men had a substantially decreased rise of serum estradiol following clomiphene citrate administration. There was no difference between the three groups in the basal follicle stimulating hormone (FSH) level and the peak response after clomiphene or gonado-tropin-releasing hormone (GnRH) administration, nor in the basal serum luteinizing hormone (LH) level. However, the lead poisoned men had a significantly decreased increment in serume LH after clomiphene citrate or GnRH administration. There was no difference in the serum prolactin levels between the three groups. The authors suggest that lead may affect the male reproductive system both indirectly at the hypothalamic-pituitary level and directly at the testicular level (see 4.1). However, because the lead poisoned men were treated with EDTA, some of the endocrine abnormalities may have been due to this therapy, although EDTA was not known to have a gonadal or hypothalamic-pituitary toxicity.
This study presents the most elaborate evaluation of the impact of moderate or high exposure to lead on the endocrine system. However, the number of subjects examined was small. Data on the analytical quality of various parameters were not presented.

Assennato et al. (1986, see 4.1), who observed sperm count suppression in lead exposed workers, also examined the levels of FSH, testosterone, prolacton, LH and total neutral 17-ketosteroid levels. No difference was observed between the lead exposed workers and the controls.
Therefore, the authors concluded that lead exerted a direct effect at the testicular level. The results of this study do not correspond with those of Braunstein et al. (1978); however, these authors studied much more parameters then Assennato et al.

The human causal evidence with respect to effects on the endocrine system, particularly on the thyroid axis, has been considered to be of low level (see 1.2). The studies discussed in 4.2 examined the impact on the endocrine system in relation to effects on spermatogenesis in male workers.

The present evidence is partly contradictory. The various studies examined different endocrinic parameters, with only a partial overlap. The human causal evidence with respect to an effect of exposure to lead on the endocrine system, leading to a direct or an indirect effect on the spermatogenesis, has to be considered inadequate.

3.4.3 Congenital malformation

Van Assen (1958, the Netherlands) reported the case of a male worker with occupational lead poisoning, who sired three infants with a lethal congenital malformation, and a 3 month abortion. After change of occupation two normal healthy children were born. The author attributed the paternal lead poisoning as the probable cause of the reproductive failures.
The human causal evidence with respect to congenital malformation when the father suffers from lead poisoning has to be considered inadequate because no independent other studies have been found.

3.5 Risk of exposure of both partners with respect to pregnancy and pre-/postnatal development

Verberne (1988, the Netherlands) reported a case of repetitive spontaneous abortion within 2 years in a couple. The husband suffered from lead poisoning, caused by drinking water containing lead. The wife had already given birth to three healthy children before the spontaneous abortion. The drinking water contained 16 mg Pb/l; in running water 3 mg Pb/l. The lead pipe from the water supply was replaced by a galvanized iron pipe. Thereafter, the woman delivered another 6 healthy children. The author, a former family doctor reported this case 50 years after the event.

This case is no illustration of the consequences of exposure of the man per se, since the mother also will have consumed lead contaminated drinking water.
The human causal evidence with respect to spontaneous abortion in exposure of both partners to lead has to be considered inadequate, because no confirmation by independent other studies has been found.

3.6 Evaluation and conclusion of human data, female reproductive toxicity

3.6.1 Study design

Most studies follow a cross-sectional design; only a few have a retrospective or prospective longitudinal design. The latter offers the best possibilities to detect exposure related reproductive risks. Some studies refer to case studies, which hardly can lead to generalized conclusions.
An important drawback is that several studies do not present information on participation in analytical quality assurance programs, which may lead to questionable PbB levels in maternal and cord blood. Information on the external dose and the duration of exposure at work is often not available.

3.6.2 Assessment of exposure

The transplacental transfer of lead in pregnant women was studied by comparing the PbB level in maternal blood at delivery wich the PbB level in cord blood. A summary of recent data is presented in Table 3. The data permit to conclude that lead easily crosses the placental membrane from mother to fetus. The level in cord blood is about 80 % of the level in maternal blood. At present it is not known whether the rate of transfer of lead through the placental membrane is the same throughout the whole pregnancy, although one may presume that in the early phase it should be greater than in the later phase.
It is known that lead is transported in blood bound to hemoglobin in the red blood cells. It is also known that there is a difference in the red blood cell counts between maternal and cord blood. Ernhart et al. (1985, USA), found an average hematocrit of 37.3 $\pm$ 4.3 % in 140 maternal blood samples of white mothers and of 49.9 $\pm$ 7.1 % in 122 cord blood samples. Kovar et al. (1984, UK) found a hemoglobin concentration of 13.7 $\pm$ 3 g/dl in maternal blood and 16.1 $\pm$ 3.0 in cord blood. It is, as yet, not known whether these differences may have an impact on the transport of lead through the placental barrier. Cavalleri et al. (1978, Italy) avoided these problems by comparing the lead levels in red blood cells and plasma of maternal and cord blood in 75 à terme pregnancies of women living in urban and rural areas in Italy. The difference found between maternal and cord blood appeared to be small; the lead concentration in plasma was about 3 % of that in red blood cells in both cases.

3.6.3 Confounding factors

In the evaluation of epidemiological studies confounding factors should be taken into account. In addition to occupational exposure to inorganic lead at the workplace, there are also other sources of exposure. By using a stepwise regression analysis, Rabinowitz and Needleman (1984B, USA) found a relation between the PbB lead in cord blood and maternal demographic variables , e.g. smoking and alcohol drinking habits, maternal age, parity, race, level of education and the amount of coffee consumed each day. However Siegers et al. (1983), FRG, found no difference in the lead levels of the amniotic fluid between smoking and non-smoking pregnant women in Lübeck. This discrepancy might be explained by the fact that lead is bound to the hemoglobin of the red blood cells, whereas the level in amniotic fluid probably represents more the plasma level than that of whole blood. Ernhart et al. (1985, USA) reported that alcohol consumption during pregnancy was related in a dose-response fashion to maternal and to cord blood lead levels. This may be due to the lead level in alcoholic drinks. Rabinowitz et al. (1984C, USA) also reported a correlation between the PbB levels in cord blood and the lead levels in indoor air and the gasoline lead sales in Boston. However, several reviewed studies did not control for confounding factors.

3.6.4 Reproductive endpoints

Effects on the reproductive system in female adults mainly relate to effects on fertility, early embryonic or fetal loss or menstrual cycle, and to morphological effects on the female gonads. Particularly in the case of intra uterine lead exposure evidence of specific effects on the central nervous system exists, the consequences of which still are present in a later stage of infancy. The human conceptus appears to be maximally susceptible to CNS teratogenesis from week 3 through week 6, but it remains vulnerable throughout pregnancy and even after birth (Mitchell, 1987). The human causal evidence in exposure to lead with respect to gynaecological disorders and infertility in women is inadequate. Since lead is a systemic poison, the possibility of an increased incidence of such disorders may be associated with uncommon excessive exposure. There may also exist an indirect effect of maternal toxicity on reproduction. There is some evidence that lead may affect the thyroid function which might subsequently alter the pituitary regulation of the gonads. However,

the mechanim is still not known. The human causal evidence on the impact on the thyreoid function and its relevance for reproductive units is considered to be inadequate.
No data are available on the effects of lead on female sexual hormones, nor on induction of cancer of the reproductive organs and breast.

Hardly any data have been found on the level of lead exposure which may induce spontaneous abortion. From the past, at the time of the Roman empire, it has been known that lead compounds have been used as an abortificiant. There are indications that the critical level for lead in maternal blood should be higher than 250 $\mu g/l$ to induce complications at parturition, such as meconium staining and preterm delivery. There is inadequate evidence that major congenital malformation may be induced, when the maternal PbB levels during pregnancy do not exceed 800 $\mu g/l$. Possible chromosomal aberrations of the peripheral lymphocytes in the infants seem to be reversible. Skeletal congenital malformations which have been reported previously were associated with much higher levels of exposure and other unfavourable working and living conditions than found at present. The human causal evidence in exposure of women with respect to spontaneous abortion, prematurity, stillbirth, decreased body weight and length and perinatal mortality is inadequate when the PbB level does not exceed PbB 250 $\mu g/l$.

Considerable information is available on the effects of low level lead intrauterine exposure on the developing central nervous system. There is some evidence of effects on the mental development of 2-5 year old children when associated with the lead level in drinking water during pregnancy of their mothers. In an excellent prospective study it was observed that higher cord blood levels were associated with a decreasing mental development index (MDI) determined at 6, 12 and 24 months of age. No association was found between the present PbB in infants blood and the MDI. This indicates that impaired development already started at the embryonal/fetal stadium. From this study it may be concluded that at a cord blood lead level lower than 250 $\mu g/l$, there appears to be an increased risk of early developmental disadvantage as assessed with the Mental Development Index of the Bayley scales. Trimming of the variables

showed that the most important impact on the infants with high PbB levels in cord blood referred to deficits in fine motor function, language and imitation. Because confirmation in independent studies is available, the human causal evidence should be considered sufficient.

3.6.5 Exposure during lactation

Lead is excreted in breastmilk; the lead concentration in breast milk is of the same order of magnitude as that in maternal plasma, i.e. about 5-10 % of the concentration in whole blood. Some authors observed a specific pattern of the lead in milk levels during different periods of lactation; colostrum had higher lead levels than mature milk. In one study a relation was found between the lead content of breastmilk and the lead levels in blood of 6 months old infants who consumed this milk; however, the lead levels in breastmilk accounted for only 10 % of the variance in blood lead levels of 6 months old infants. The suggestion that older women may excrete more lead with lactation compared to younger ones needs further confirmation. There exists sufficient human evidence that lead is transported to the infant through breastmilk. However, it is not known to what extent this oral intake during the period of lactation carries an extra risk in comparison with the impact of the intrauterine exposure during occupational maternal exposure.

3.7 Evaluation and conclusion of human data, male reproductive toxicity

3.7.1 Study design

No group studies have been carried out with a prospective design; only a few studies assessed the internal exposure retrospectively. Some case studies permitted a prospective follow-up of individual workers, which led to interesting data particularly with respect to the reversibility of effects on spermatogenesis and fertility. The response rates in group studies were often poor, probably due to the intrusion on privacy. This particularly refers to data on sexual dysfunction and on spermatogenesis.

3.7.2 Assessment of exposure

The exposure assessment usually refers to parameters of internal exposure to lead, particularly levels of lead in blood and/or semen. A few studies also presented some reasonably agent-specific biological effect parameters (ALA in urine, ZPP in blood). However, several studies did not present information on the analytical quality control. When deriving exposure (dose-)effect/response relationships, one should rely on analytically validated parameters of internal exposure and of effects.

3.7.3 Reproductive endpoints

Three studies and/or case reports suggested an effect on sexual potency (libido, erection, ejaculation, orgasm). However, the data usually were derived from non validated questionnaires. It is questionable whether the data reported represent the true prevalence. Nevertheless, there exists low level causal human evidence of effects on male sexual potency. No exposure-effect relationship could be derived, let alone a no-adverse effect level.

Twelve studies referred to effects on spermatogenesis. No effects were observed in non-occupationally exposed men, with a PbB level of 70-190 $\mu g/l$ and a lead in semen level of 10-110 $\mu g/l$ (Plechaty et al., 1977). No evidence of a significant relationship between the levels in blood and semen has been observed, not even in lead exposed workers.

There exists sufficient human causal evidence that overexposure to lead may affect spermatogenesis. The study by Wildt et al (1983) referred not only to the assessment of normal clinical parameters of spermatogenesis (sperm count, motility, abnormal sperms, semen volume), but also to subtle early functional effects (chromatin stability) and to the function of the accessory genital glands. The two latter effects were observed in occupationally exposed workers with an average PbB of 450 $\mu g/l$ (340-670 $\mu g/l$). Analytical quality assurance studies were regularly carried out.

It is not possible to derive an individual maximum blood lead level, but a group average a PbB level of 350 $\mu g/l$, with a maximal individual level of 400 $\mu g/l$, is suggested. The same maximal individual level has also been recommended by the Dutch Expert Committee for Occupational Exposure Limits (1980).

It is emphasized that the effect on spermatogenesis appears to be reversible.

Whorton (1984) discussed various pitfalls in the assessment of the spermatogenesis in field studies: difficulty to receive a high response rate, resulting in a low power of the study; questionable sensitivity of various effect parameters (in the case of germ cell toxicity sperm morphology is probably not more sensitive than the sperm count, but in the case of an effect on spermatogenesis the morphology may be the most sensitive parameter); a conclusive answer on the health relevance of most clinical parameters cannot yet be given; the sperm motility is temperature dependent; exclusion of subjects with particular medical histories or physical findings who may be more sensitive to lead, may underestimate the actual reproductive risk; large interlaboratory differences exist in the methods for assessment of the sperm count, even up to a factor 10; frequency of intercourse and length of abstinence; a skew distribution of most sperm parameters, although arithmetic averages are calculated; difference in the criteria to assess the morphology. Therefore, comparison of data on spermatogenesis should only be relied upon when both the assessment of sperm quality in exposed workers and in control groups has been carried out by the same laboratory. The quantitative data should be compared only within each study separately.

Effects on the endocrine system have been studied in seven studies. Two studies (Lancranjan et al., 1975; Assennato et al., 1986) did not find any evidence of an effect on the endocrine system, whereas five other studies did. However, the latter studies differed with respect to the various parameters measured. Some studies suggest an indirect effect on spermatogenesis through the hypothalamo-pituitary-adrenal-testis-axis. Another study (Chowdbury et al., 1986) suggested an indirect effect on the endocrine system, which may lead to a direct effect on the spermatogenesis through a decreased fructolysis. Further studies of the potential indirect effect on spermatogenesis through the hypothalamo-pituitary-adrenal-testis axis is needed, taking into account not only the common clinical parameters of spermatogenesis, but also early functional effects. The present human causal evidence for such an indirect endocrine effect should still

be regarded inadequate, because of the variety in design and the potential confounding effects of treatment of lead poisoning or of simultaneous exposure to other chemicals, e.g. alcohol. Such studies should be carried out in a prospective design.

Effects on fertility can only be studied by a follow-up of groups of male workers or of patients with lead poisoning. Some case reports suggest such an effect in lead poisoned subjects. The human causal evidence should be considered limited.

Only two case reports were available, suggesting teratogenic and embryotoxic effects of male lead poisoning; one of these reports refers to increased lead exposure of both partners. The human causal evidence is considered inadequate.

4 INTEGRATED DISCUSSION OF HUMAN AND ANIMAL DATA

4.1 Summary of conclusions

One of the important aspects of lead is its kinetics in the pregnant female. There is sufficient evidence that lead easily passes the human placenta. The level in cord blood is about 80% of the level of lead in maternal blood at term. At present it is not known whether the rate of transport is the same during the entire period of gestation, although it is assumed to be greater in the early phase than in later phases. Animal experiments occasionally support this assumption although the methods of administration, e.g. intravenous or intraperitoneal are questionable. Moreover, no information on kinetics of lead with respect to exposure was available from animal studies.

The human causal evidence with respect to adverse effects on reproductive organs, fertility and endocrine system of women is considered to be inadequate. No data are available on the effects of inorganic lead on the state of sex hormones in female workers. Animal data are too few in number to be conclusive; moreover, only high oral doses induce "delayed vaginal

opening" in female rats. Extrapolation of the effects to humans is not possible.

Substantial human data is available on the risk of exposure of women with respect to pregnancy and pre- and post-natal development. It may be concluded that the human causal evidence with respect to spontaneous abortion, prematurity, stillbirth, decreased body weight and length, and perinatal mortality is inadequate. Although, at levels above 250 $\mu g/l$ some studies are indicative for this type of effects. The magnitude of effects on experimental animals seems to be dependent on the method of administration and species of animals used in the experiment. Oral administration of inorganic lead to pregnant rats has shown to be embryotoxic, with a primary effect of delayed fetal growth: however this effect may be associated with maternal toxicity. High doses of up to 100 mg/kg do not give rise to teratogenic effects and the estimated NAEL for mother and the fetus is 1 mg/kg/day. However, for prairie voles a NAEL of 16 mg/kg/day is estimated when lead is administered by intravenous injection. Doses higher than 32 mg/kg give rise to embryotoxic and teratogenic effects. Extrapolation of these data to humans is difficult since no corresponding lead in blood levels are given.

A highly crucial aspect of reproductive risk in low level inorganic lead exposure is the issue of subtle neurologic damage of the offspring. In this respect the human causal evidence can be considered sufficient at lead levels in cord blood of 100-150 $\mu g/l$. Moreover, animal experiments confirm these findings. In the human studies it is found that intrauterine exposure, as measured by umbilical cord blood lead concentrations, significantly increases the risk of minor malformations and delayed cognitive development in the infant. Data from animal experiments more or less confirm these findings. Neurobehavioral effects on reflex development, locomotion activity, learning ability and social behavior have been observed, although some effects occurred only at high doses. The most complete animal study reported a delay in surface righting reflex and air righting reflex development at lead in blood concentration of 200 - 400 $\mu g/l$ and higher.

The route of exposure to inorganic lead in the offspring may not only by means of transplacental transfer from mother to fetus, but may also occur postnatally by ingestion of the mother's breast milk. There is sufficient human causal evidence that lead is excreted to a certain extent with breast milk. Whether this is reflected in an extra risk in addition to that of the burden due to prenatal exposure has not been studied as far as known. Animal data confirm the transport of lead through mother's milk: a substantial amount is transferred to suckling rats as can already be measured one week after a single administration. There are few data on the risks of exposure of animals after birth with respect to their postnatal development. Effects on specific performances of cynomolgus monkeys tested at the age of 3 years are reported after they had been administered with an equivalent of 50 or 100 μg Pb/kg/day in a milk substituted formula, 5 days/week, from day 1 postpartum until 200 days of age. This treatment resulted in lead in blood concentrations of 150 and 250 μg/l, respectively.

There are numerous data on the risks of exposure of male workers with respect to sexual potency, spermatogenesis and fertility. It may be concluded that there is sufficient causal evidence that exposure to inorganic lead induces adverse effects on the spermatogenesis, although the evidence with respect to impaired sexual potency still appears to be of low level. In a very well designed study it is reported that in groups of workers with an average of lead in blood level of 450 μg/l subtile dysfunction of spermatogenesis and decreased function of the accessory genital glands occurred. It was not possible to establish an individual NAEL for lead in blood; however, at group level the NAEL appears to be below 450 μg Pb/l. The effects on male reproductive function has been confirmed in animal experiments. Effects on the testes have been observed; NAEL's of lead in blood concentrations of 540 μg/l in one study and 670 μg/l in another study are reported.
The human causal evidence with respect to effects of exposure on the endocrine system, leading to direct or indirect effects on the spermatogenesis, has to be considered inadequate because no independent other studies have been found.

The human causal evidence with respect to spontaneous abortion after exposure of both partners to inorganic lead has to be considered inadequate, because no studies that confirm this finding have been found and no data are available from experimental animals.

4.2 Risk assessment of exposure to lead from human and animal data

Since there is poor relationship between the external dose and the lead in blood level, the risk assessment has to be based directly upon the PbB level in maternal blood and indirectly on that in umbilical blood. Sufficient evidence exists that inorganic lead can easily pass the placenta, both in animals and humans. In humans the PbB in umbilical cord blood is approximately 80% of that in mother's blood à terme. To date it is not known whether the rate of transport is similar throughout pregnancy; the rate is assumed to be larger in the early non-placentation phase than in the later trimester.
Several animal studies have been conducted with i.p., i.v. or i.m. administration of (soluble) lead solution. Extrapolation of these studies to humans appears to be questionable. Few animal studies are available with exposure via the respiratory tract, in most studies exposure was by the oral route.
The lowest NAEL in animal studies is 0.9 mg Pb/kg/day, which corresponds to a PbB in maternal blood of 100 - 160 μg/l.
The NAEL's with respect to the various reproductive endpoints have been summarized in table 4. There is sufficient evidence that there are adverse effects on the development of the central nervous system at levels of approximately 100 - 150 μg/l PbB in umbilical cord blood. Therefore, the PbB in maternal blood should not exeed 200 μg/l (PbB in umbilical cord blood is ca. 80% of PbB in maternal blood). When the mothers have already been occupationally exposed prior to conception, the PbB might well be 700 μg/l. Even if a pregnant employee should be transferred to a department without exposure to lead, the lead exposure of the embryo (in the early phase of pregnancy) may still be high. In particular when the mother has been exposed for a long period of time, the PbB will hardly decrease to values below 250 μg/l during pregnancy.

For individual male workers the PbB should not exeed 400 µg/l (average group mean 350 µg/l).

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Table 1

Summary of literature data of IARC (1980), Gerber et al. (1980), and Chang et al. (1980), and supplemented with more recent data.
(ALA-D: aminolaevulinic acid dehydratase activity)
(The references taken from IARC and Gerber et al. are not cited in the reference list)

Species	exposure	dose	NAEL	effects	Reference
ORAL EXPOSURE.					
mouse (N=50 per group)	entire period of pregnancy (evaluation) on day 16-18)	0, 0.125, 0.25, 0.5%, diet (lead acetate)		pregnant females: 26, 28, 11, 8; corpora lutea: 352, 361, 138, 92; live embryos 8.3, 8.3, 8.3, 7.5; dead embryos: 1.2, 1.5, 1.9, 2.4; loss before implantation: 4.0, 3.9, 2.4, 1.6; mean weight of fetuses on day 18: 978, 922, 850, 793mg; no gross malformations in the fetuses	Jacquet et al., 1975 (IARC/Gerber)
mouse rat	day 5-15 of pregnancy day 6-16 of pregnancy	7.14, 71.4 or 714 mg/kg, oral (3.9, 39, 390 mg/ kg bw lead) (lead acetate)	<714 mg/kg	714 mg/kg maternal toxicity (hypoactivity, diarrhoea, reduced body weight, death), forcing discontinuation of treatment after 3 doses; increase in fetal resorptions; no teratogenic effect.	Kennedy et al., 1975 (IARC/Gerber)
cow (N=3)	day 22-90, 45-90, or 51-120 of pregnancy	5 mg/kg/day feed (lead acetate)		one cow died from lead poisoning; normal calves	Shupe et al., 1967 (IARC/Gerber)
sheep (N=12)	entire period of pregnancy	0.5-16 mg/kg diet (blood level 0.4 mg/L) (powdered metallic lead)		abortion, miscarriage and transitory sterility; no malformations	Sharma & Buck, 1976 (IARC/Gerber)
rat	3 weeks before mating, during pregnacy and 3 weeks after delivery	0, 0.1, 1 or 10 mg/L, drinking-water (lead nitrate)	1 mg/l	10mg/L: decrease in ALA-D ensyme activity in blood and kidneys of offspring; increased lead concentrations in blood of mothers and newborn;	Hubermont et al., 1976 (IARC/Gerber)
rat	3-generation	1% lead, diet (lead acetate)	<1%	decreased offspring/litter; decreased birthweight of newborn; no malformed fetuses in F1, F1 or F2	Stowe & Goyer, 1971 (IARC/Gerber)

Table 1 continued (1)

Species	exposure	dose	NAEL	effects	Reference
rat	3-generation	25 mg/L in drinking water plus 0.20 mg/kg in diet		decreased litter size in F1; runts in F1, F2 and F3	Schroeder & Mitchener, 1971; (IARC/Gerber)
mouse	3-generation	(lead salt)		increased interval between the litters in F1; runts in F1; in F2 decreased number of offpring; exposure discontinued before F3 generation	
rat	day 6-18 of pregnancy	4-150 mg Pb/kg oral (lead)	>150 mg/kg/day	no teratogenic effects	Wardell et al., 1982
rat	3 weeks before mating and until day 20 of gestation	0, 50, 75 or 100 mg Pb/kg; gavage (lead acetate)	75 mg/kg/day	no teratogenic effects; blood sampling indicated that lead can cross the placenta, as exemplified by fetal kidney values; at 100 mg/kg reduction of conceptus weight	Miller et al., 1982
squirrel monkey	week 9-21 of pregnancy	50-100 mg Pb/kg perorally (lead acetate)	<50-100 mg/kg/day	45% of offspring died during pregnancy; reduced mean birth weight; reduced cerebral weight in the deceased new borns	Lögdberg et al. (1987)
rat	throughout pregnancy	10, 50, 100, 200, or 500 mg/l of Pb drinking water (lead acetate)	10 mg/l i.e. 1 mg/kg/day	reduced total maternal weight gain, net maternal weight gain, and level of food intake from 50 mg/l and up; reduced feed efficiency at 500 mg/l; placental weight not changed; reduced average fetal weight and average live litter at all dose levels.	Dilts and Ahokas, 1979
rat	6-7 weeks before breeding until day 21 22 of gestation	0, 0.5, 5, 25, 50 or 250 ppm Pb; drinking water (lead acetate)	5 mg/kg/day	50 and 250 ppm groups: females exhibited growth retardation 1 to 3 weeks after exposure began, delayed vaginal opening; hydronephrosis and/or hydroureter percentage malformed fetuses, resorptions and postpartum pup deaths to weaning were unaffected; the day 1 mean body length of female offspring was altered (250 ppm group)	Kimmel et al., 1980

Table 1 continued (2)

Species	exposure	dose	NAEL	effects	Reference
rat	pre- and post-natal to day 15	5000 mg lead acetate; drinking water (lead acetate)	not established	reduced body weight of the pups by 20%	Rabe et al., 1983
rat	day 1-18 or day 1-21 of gestation	0 or 500 ppm; drinking water (lead acetate)	not established	reduced fetal weight on day 18, but not on day 21; no differences in litter, size, number of implantations, rate of resorption and placenta weight; no treatment related malformations; increased lead concentration in maternal and fetal blood, liver and placenta	Hayashi, 1983
INTRAPERITONEAL					
rat	day 9 of pregnancy	25 mg/kg i.p. (lead acetate)		litter resorption; surviving fetuses showed abnormally shaped heads (meningocele) and anophthalmia; tooth defects	Zegarska et al., 1974 (IARC/Gerber)
guinea pig	day 20 or 21	4, 8, 16 mg/kg; i.p.; (lead)		> 12.5mg/kg reduced mean brain weight of newborn, microencephaly;	Edwards and Beatson, 1984
mouse	day 8, 9, 10 or 12 of pregnancy	15 or 35 mg/kg i.p. (lead acetate) with calcium-deficient diets (lead acetate)		increased postimplantation mortality (day 9)and skeletal anomalies (predominantly day 8 or 9) increased the effects ($P<0.01$) plus loss of weight and delayed ossification of the fetuses	Jacquet & Gerber, 1979 (IARC/Gerber)
INTRAVENEOUS					
mouse	day 3, 4 or 6 of pregnancy	40 mg/kg, i.v. (lead chloride)	<40 mg/kg	invasion of uterus by trophoblast giant cells and implantation fails	Wide & Nilsson, 1977; Jacquet, 1977 (IARC/Gerber)
mouse	day 11 of pregnancy	100 mg/kg; i.v. (lead acetate)	<100 mg/kg	from day 14: embryonic death, smaller length of live embryos, delay of palatal development; on day 18: cleft palate	Kamimura et al., 1982
prairie voles	day 7-10 of pregnancy	32 mg/kg, i.v. 64 mg/kg, i.v. 8 or 16 mg/kg, i.v. (lead acetate?)	16 mg/kg	exencephaly; spina bifida total litter resorption no abnormalities	Kruckenberg et al., 1976 (IARC/Gerber)
hamster	day 7, 8 or 9	50 mg/kg, i.v. [lead salts (nitrate, chloride, acetate)]	< 50 mg/kg	no maternal toxicity; tail abnormalities; anophthalmia; fused ribs; spina bifida; exencephaly; fetomortality was 10-18%	Ferm & Carpenter, 1967 (IARC/Gerber)

Table 1 continued (3)

Species	exposure	dose	NAEL	effects	Reference
hamster		50 mg/kg, i.v. (lead acetate)	<50 mg/kg	posterior tail malformations; no sympodia (malformation of lower extremities); resorption rate 38%	Ferm, 1969 (IARC/Gerber)
		50 mg/kg + 2 mg/kg cadmium sulphate i.v.		potentiated posterior tail malformations; sympodia; resorption rate 46% (Cd alone 27%, control 7%)	
hamster	day 8 or 9 of preganancy	25 or 50 mg/kg i.v. (lead nitrate)	<25 mg/kg	malformations of the sacral and tail region; few cases of rib fusion	Ferm & Ferm, 1971 (IARC/Gerber)
hamster	day 8 of pregnancy	50 mg/kg, i.v. (lead nitrate)	<50 mg/kg	hyperplasia and disorientation of neuroepithelial cells of the dorsal region of the caudal neural tube in embryos on day 9; haematomas and extensive necroses in the dorsal region of the compressed neural tube on day 10; abnormal formation and development of sacral vertebrae	Carpenter & Ferm, 1974 (IARC/Gerber)
rat	day 9 of pregnancy	50 or 70 mg/kg i.v. (lead nitrate)	<50 mg/kg	malformations of the urogenital and intestinal tracts; abnormalities of posterior extremities	McClain & Becker, 1975 (IARC/Gerber)
	day 10-15 of pregnancy			fetolethal, but no malformations;	
	day 16 of pregnancy			hydrocephalus and haemorrhage of central nervous system	
	after day 16			fetal mortality declined sharply	
rat	day 9, 15 or 19 of pregnancy	3.1, 15.6 mg/kg i.v. (^{210}Pb)	3.1 mg/kg	fetal effects (comparing fetoplacental unit burdens)	Hackett and Kelman, 1983
INHALATION					
rat	entire period of pregnancy	3 mg Pb/m3 (lead aerosol)		inhibition of ALA-D activity in blood of dam and fetus	Prigge & Greve, 1977 (IARC/Gerber)
rat	day 9, 15 or 19 of pregnancy	1.11 mg/kg inhalation (^{210}Pb)		no fetal effects	Hackett and Kelman, 1983

Table 1 continued (4)

Species	exposure	dose	NAEL	effects	Reference
UNKNOWN					
rat	1) gestation/ nursing; 2) gestation/ nursing/post-weaning; 3) post-weaning	400 or 750 mg/day route? (lead acetate)		histological examination brains: dendritic spine loss, abnormalities in pyramidal and stellate cells, difference in spine count (750mg group)	Murray et al., 1977 (IARC/Gerber)
INTRAMUSCULAR					
monkey	each day during pregnancy and/ or lactation	1, 5 mg Pb^{2+}/kg/day (lead acetate)	<1 mg/kg	5mg: after 3 months lethal for females 1mg: erythrodiapedisis in cerebral matter of the new-born at birth, after the mothers treatment during pregnancy	Tachon et al., 1983

Table 2
(Carpenter, 1982)

ORALLY ADMINISTERED LEAD IN FEMALE HAMSTERS FED DIETS DEFICIENT IN CALCIUM OR IRON

Group 1: Lead only

(A) Drinking water: 0.1% PbAc for a minimum of 4 weeks + days 1-15 of pregnancy
Food: : Standard food
No. of dams : 8
(B) Drinking water: 0.1% PbAc during pregnancy only
Food : Standard food
No. of dams : 3
(C) Drinking water: Standard water
Food : Standard food
No. of dams : 5

Group 2: Lead + calcium deficient diet (c. 40 ppm Ca)

(A) Drinking water: 0.05% or 0.1% PbAc for a minimum of 4 weeks + pregnancy
Food : Low calcium diet, minimum of 2 weeks + pregnancy
No. of dams : 7
(B) Drinking water: 0.05% or 0.1% PbAc for a minimum of 4 weeks + pregnancy
Food: : Standard food prior to mating; low calcium diet during pregnancy
No. of dams : 8
(C) Drinking water: Standard water throughout
Food : Low calcium diet, minimum of 2 weeks + pregnancy
No. of dams : 3
(D) Drinking water: Standard water throughout
Food : Standard food prior to mating; low calcium diet during pregnancy
No. of dams : 3

Group 3: Lead + iron deficient diet (<3 ppm Fe)

(A) Drinking water: 0.05% or 0.1% PbAc for a minimum of 4 weeks + pregnancy
Food : Low iron diet, minimum of 2 weeks + pregnancy
No. of dams : 7
(B) Drinking water: 0.05% or 0.1% PbAc for a minimum of 4 weeks + pregnancy
Food: : Standard food prior to mating; low iron diet during pregnancy
No. of dams : 9
(C) Drinking water: Standard water throughout
Food : Low iron diet, minimum of 2 weeks + pregnancy
No. of dams : 3
(D) Drinking water: Standard water throughout
Food : Standard food prior to mating; low iron diet during pregnancy
No. of dams : 4

Table 3 - Recent data on lead levels in blood of mothers and in cord blood, and their relationship

Authors (country)	mean lead levels in mothers (µg/l)	n	mean lead levels in cord blood (µg/l)	N	Relationship between lead levels in mothers and cord blood	Comments
Angell et al. (1982) (USA)	98.5 ± 44	154	97.3 ± 41	154	r=0.60, P<0.01	Unselected women deliver in hospital, paired samples
Cavalleri et al. (1978) (Italy)	264 ± 45 (µg/l RBC)	75	254 ± 43 (µg/l RBC)	75	r=0.37	Urban and rural women PbB corrected by red blood cells
Ernhart et al. (1986) (USA)	65 (27-118)	185	58 (26-147)	162	r=0.80	All women presenting for antenatal care in Cleveland
Ernhart et al. (1985) (USA)						The same population as reported above
white	62 (30-108)	140	57 (26-108)	122	r=0.80, P<0.0001	
black	68 (39-118)	68	58 (26-147)	56		
Hansen et al. (1984) (Greenland)	25-154	83	20-129	79	r=0.57, P<0.05	Samples taken at local hospitals
Kovar et al. (1984) (U.K.)	100 (50-181)	28	88 (60-131)	28		All pregnancies medically uncomplicated, London maternity hospital. Paired samples
Ong et al. (1985) (Malaysia)	152 (75-240)	114	114 (50-256)	114	r=0.63, P<0.001	Urban women at delivery in maternity hospital. Paired samples
Sartorelli et al. (1986) (Italy)	91±29	28	64±29	28	r=0.55, significant	Paired samples
Sartorelli et al. (1983) (Italy)	129 (70-280)	35	93 (50-240)	35	r=0.89, significant	Paired samples
Takacs et al. (1984) (Hungary)	304±294	104	295±265	104		Paired samples. Mean lead level of placenta=1789 ppb dry weight.
Tsuchiya et al. (1984) (Japan)	78 (17-253)	105	84 (9-519)	95	r=0.40, P<0.01	Women residents at Nagoya city, normal delivery. Highly scewed distribution
Winneke et al. (1985) (Germany)	93 (40-300)	114	82 (40-310)	114	r=0.79	Mothers with deliveries in hospital, Nordenham. Paired samples
Zarembski et al. (1983) (U.K.)	geom. 59.6 (15-212)	1665	geom. 40.7 (7-223)	1665	r=0.81, significant	Paired samples. Population from Dundee city

Note: N = number of mothers
n = number of neonates
The range is given in brackets

Table 4 - No Adverse Effect Levels of PbB in human studies

Reproductive endpoints	NAEL of PbB ($\mu g/l$)		Causal evidence
WOMEN			
Gynaecological disorder	> 250	m	inadequate
Spontaneous abortion	> 250	m	inadequate
Stillborn	> 250	m	inadequate
Decrease body weight and length of offspring	> 250	m	inadequate
Perinatal mortality	> 250	m	inadequate
Development of central nervous system	100-150	c	sufficient
	< 250	m	
MAN			
Spermatogenesis	av. 350, maximal individual level 400		sufficient
Sexual potency	> 700		low level

Note: m = maternal blood; c = cord blood