

Lead and its inorganic compounds (inhalable fraction)

MAK value	–
Peak limitation	–
Absorption through the skin	–
Sensitization	–
Carcinogenicity (2006)	Category 2
Prenatal toxicity	–
Germ cell mutagenicity (2004)	Germ cell mutagen category 3A
BLW (2005) *	400 µg/l blood for women > 45 years and for men 100 µg/l blood for women < 45 years
Chemical name (CAS):	lead
CAS number:	7439-92-1

The 2000 MAK documentation for lead and its inorganic compounds was published in Volume 17 of the present series. Since this publication, new information has become available on the mechanism of action, on genotoxicity and for the evaluation of carcinogenicity.

In 2006, the IARC undertook an assessment of lead and inorganic lead compounds on the basis of limited evidence for humans and sufficient evidence in animal experiments and classified them as probably carcinogenic for humans (Category 2A). In the case of lead metal dust, the data are not sufficient for a clear assessment of carcinogenicity (inadequate evidence) in animal experiments (IARC 2006).

* **Biologischer Leit-Wert:** The BLW is the amount of a chemical substance or its metabolites or the deviation from the norm of biological parameters induced by the substance in exposed humans which serves as an indicator for necessary protective measures.

1 Mechanism of Action

It can be concluded from integrating the relevant toxicological data that the release of lead ions is responsible for the toxic effects of all inorganic forms of lead.

1.1 Mutagenicity

Up to now, it has been assumed that a genotoxic effect from lead and its inorganic compounds does not occur at low doses relevant for humans ("Lead and its inorganic compounds", Volume 17, present series). However, following are a number of mechanisms which are considered to be inducers of a genotoxic effect already produced by lead compounds at low concentrations. The increased formation of reactive oxygen species induced by lead ions is indicated by *in vitro* tests with isolated DNA (Roy and Rossman 1992), by the influence of different radical scavengers on mutation frequency and, finally, by a mutation spectrum typical for reactive oxygen species (Ariza and Williams 1999; Ariza *et al.* 1998). The causes for an increased production of reactive oxygen species induced by lead ions may be found in reactions similar to those of the Fenton type, or in an interference with detoxication reactions of endogenously formed reactive oxygen species, or a deregulation of protein kinase C (PKC) (see below).

A further cause for the genotoxic effects of inorganic lead compounds may exist in the inhibition of DNA repair processes (Hartwig 1994). As a result of endogenous processes the DNA is permanently damaged; delayed or restricted repair results in the accumulation of premutagenic DNA lesions. One possible mechanism for this could be the substitution of zinc ions by lead ions in so-called zinc finger proteins, such as have been demonstrated for several transcription factors in subcellular test systems (Hanas *et al.* 1999; Hartwig 1994; Petering *et al.* 2000). However, the activity of the zinc finger repair protein *Xeroderma Pigmentosum A* was not inhibited in the presence of lead acetate (Asmuss *et al.* 2000).

To summarize, this means that the mechanisms described are based on interactions of lead ions with proteins (binding to SH groups), or on competition with essential metal ions, and not on the interaction of lead ions with the DNA. In spite of this, they can still induce a disturbance in repair and, by fixation of the mutations, lead to irreversible genetic damage.

1.2 Influence on protein kinases C (PKCs)

The mechanism of lead-induced carcinogenesis is not completely clarified. Apart from the promotion of mutation formation at the molecular level, a large number of individual effects produced by lead have been observed, which are well able to induce cancer. Some of these mechanisms are summarized in Figure 1.

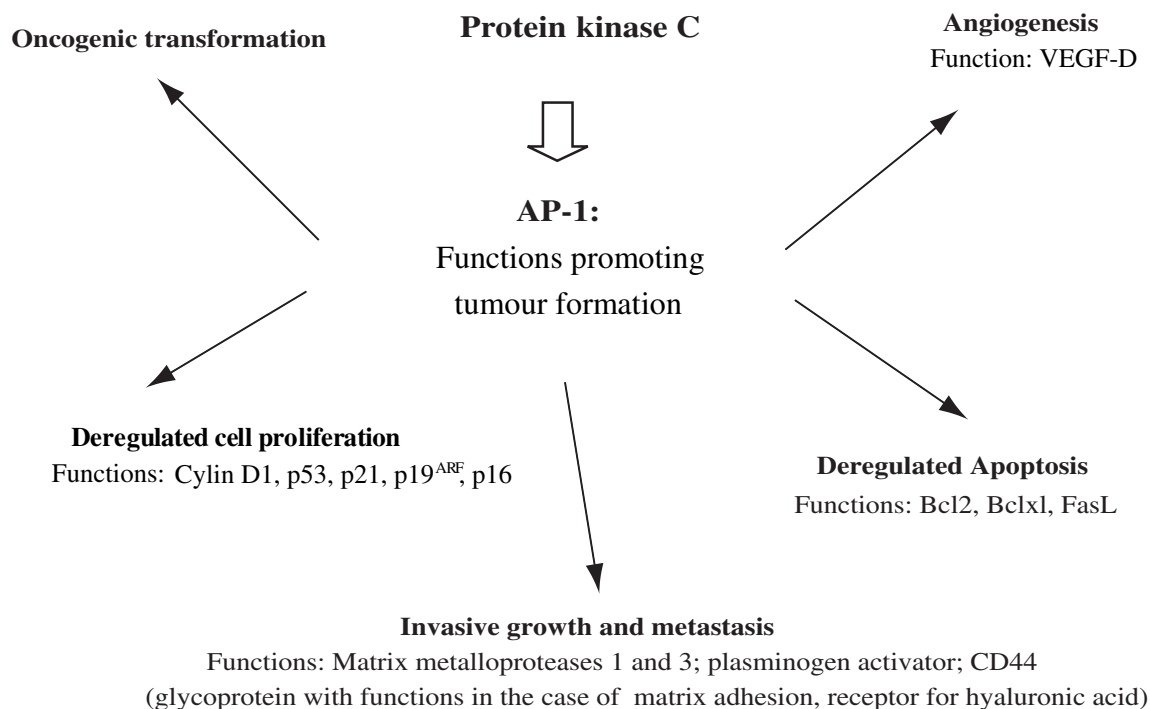


Figure1. Effects of the stimulation of AP-1 transcription factors modulated by protein kinases C (according to Jochum *et al.* 2001)

In principle, lead ions are able to disturb all regulatory and catalytic mechanisms occurring physiologically with the involvement of calcium and zinc ions. The reason for this is that lead ions can replace the physiological calcium and zinc ions in a large number of cell types, whereby they do not simply neutralise their effect but induce abnormal effects themselves. The critical displacement processes occur with calcium or zinc at the coordination sites of the proteins. Typical amino acid sequences in the calcium or zinc binding domains, for example the zinc finger structure, are known. These are found in many transcription factors and enzymes (see above).

One example for the displacement of zinc ions (cofactors) by lead ions is found in replicative DNA polymerases. The replacement of zinc by lead ions reduces the copying accuracy of the enzymes. As a consequence, the primarily reversible ion exchange can result in irreversible replication errors (Zakour *et al.* 1981).

Which calcium or zinc regulated mechanism is first disturbed by the lead ions depends mainly on how much greater the binding affinity of the lead ions is to the coordination sites relative to that of the calcium or zinc ions involved.

In the case of protein kinase C α in the rat brain, the displacement of calcium by lead ions is especially marked. Thus, lead was observed to activate the enzyme even at picomolar concentrations. The potency of lead was greater than that of calcium by a factor of six, that is, lead was activating at a micromolar concentration. (Markovac and Goldstein 1988). In pheochromocytoma cells an increase in PKC activity as well as the release of reactive oxygen radicals was demonstrated at 10 nM (Jadhav *et al.* 2000). In principle, therefore, the possibility exists that the toxic effects of inorganic lead are produced by displacement processes of this type (Nihei *et al.* 2001).

Investigations with recombinant PKC isozymes have shown that only the group of conventional PKCs (isozymes α , β I, β II and γ , called cPKCs), which possess the regulatory binding domains for calcium, respond to lead ions with an activity change. Activation takes place in the concentration range of 10^{-11} to 10^{-8} M (Long *et al.* 1994). Consequently, for the conventional PKCs, lead is a high-affinity partial calcium agonist. At higher lead concentrations (10^{-8} to 10^{-5} M), however, this agonist can become an inhibitor, namely when it additionally blocks the phosphorylating activity of the enzymes. The reason is that lead also displaces calcium from its coordination site in the catalytic domain of protein kinase C α , itself developing a new – but inhibiting – effect (Sun *et al.* 1999). After their activation, conventional PKCs initiate the following processes, which are capable of favouring carcinogenic growth:

- Induction of immediate-early response genes, including *c-fos*, *c-jun*, *erg-1*, and thus increased formation of proteins JunD and Fra-2, according to whose homo- or heterodimerization the activator protein-1 (AP-1) transcription factor is formed (Chakraborti *et al.* 1999). As a result of the forced expression of AP-1-dependent genes, tumour growth is promoted at several stages (see Figure 1). The increase of cyclin D1, a positive regulator of cell proliferation, is especially important.
- Stimulation of the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF-1) (Hossain and Laterra 2000).
- Formation of reactive oxygen radicals in leukocytes (Nishizuka 1986) and formation of hydrogen peroxide in CHO cells (a cell line from Chinese hamster ovary) which causes, for example, mutations in the *gpt* gene. The number of mutations is lower in the presence of allopurinol, a xanthine oxidase inhibitor. Copper-zinc superoxide dismutase is also activated by lead ions to form hydrogen peroxide (Ariza *et al.* 1998).
- Activation of nuclear transcription factor- κ B (NF- κ B) and an increased expression of NF- κ B-dependent genes (Ramesh *et al.* 1999).
- Upregulation of mitogen-activated protein kinase (MEK) and of kinase JNK, which can be activated by stress. Altogether, the transcription factors NF- κ B and AP-1 (see above) as well as the protein kinases MEK and JNK may be important mediators of lead-induced signalling in gene expression (Ramesh *et al.* 1999).
- The inhibition of gap junctions, for example in fibroblasts, epidermal cells and hepatocytes (Nishizuka 1986).

1.3 Amplification of gene expression

By means of the microarray technique, a large number of genes has been identified in rat astrocytes, which show a partly increased and partly attenuated expression after incubation of these cells in a medium containing lead (Bouton *et al.* 2001). The following changes in gene expression are among those up-regulated or down-regulated to a greater extent:

- Stress response genes: especially microsomal glutathione *S*-transferase and the heat shock proteins p10 and p70 were all up-regulated.

- Genes coded for calcium-binding proteins or enzymes: calcium/calmodulin-activated adenylyl cyclase, annexin A5 and phospholipase A2, which catalyze an increase in arachidonic acid level in subjects exposed to lead, were up-regulated; annexin A1 was down-regulated.
- Genes whose products influence angiogenesis and the blood-brain barrier: the expression of VEGF and its receptor is up-regulated and that of thrombospondin 2 (the antagonist of VEGF) is down-regulated. A large number of genes for coding collagen and genes for collagen biosynthesis as well as the functional elements in calcium-dependent signal paths (including specific PKCs and EGF), which participate in controlling collagen biosynthesis, are practically down-regulated.
- Genes for amino acid synthesis and tRNA synthesis: all of these are up-regulated, together with exportin-t, the major transport protein for tRNAs. Exportin-t is the major nuclear exporter of tRNA molecules into the cytoplasm. The initially inexplicable increase in protein and tRNA synthesis becomes understandable in the light of a particular (destructive) property of lead: lead ions are capable of cleaving RNA, especially tRNA through nucleophilic attack to fragments, which experience a further breakdown in the cells. Apparently, the stimulated protein and tRNA synthesis mechanisms compensate the lead-induced loss of RNA.

Altogether, the gene expression analyses have demonstrated that the endogenous substitution of calcium and zinc by lead constitutes a central mechanism in lead toxicity.

2 Genotoxicity

2.1 Effects in humans

A large number of studies on the genotoxicity of lead are summarized in the documentation on lead and its inorganic compounds published in 2000 (see Volume 17, present series), the majority of which report a clastogenic effect. As the validity of many studies is very limited due to methodological shortcomings, the genotoxic potential of lead in humans could not be evaluated conclusively.

As regards the possible genotoxic effect of heavy metals in workers occupationally exposed to cadmium, cobalt and lead, a field study found that the results of a comet assay (single strand breaks in mononuclear blood cells) correlated with the exposures to cadmium and cobalt but not with those to lead. Possible interactions between these heavy metals were discussed in this study (Hengstler *et al.* 2003).

In another occupational medical field study, the induction of micronuclei in the peripheral blood lymphocytes of 103 workers exposed to lead (78 controls) was investigated after cultivation with cytochalasin B. The volunteers were divided into four groups according to their blood lead levels. The micronucleus frequency was significantly increased ($p < 0.05$) in the second group (1.2–1.91 μM corresponding to 248–369 μg lead/l blood) compared with the group with the lowest lead level

(< 1.2 μM). However, a considerable variability in micronucleus frequencies depending on body burden (lead level in the blood) was found, which makes interpretation difficult. Altogether, the linear dose-effect relationship determined in this study appears to be convincing, though it cannot be excluded that the amount of the lead exposure has been underestimated in the past (Vaglenov *et al.* 2001). If a linear dose-effect curve ($Y = a + bX$) between the determined control values and the three exposure groups is calculated from the values cited in the publication of Vaglenov *et al.* (2001), the value $Y = 12.62 + 9.94 X$ is obtained. From this, a doubling dose $DD = a/b$ of 1.27 μM (263 μg lead/l blood) is calculated. Taking the individual control values as a basis, $a = 10.94$ and $b = 10.55$, the doubling dose for the micronucleus frequency is 1.04 μM (215 μg lead/l blood).

In an Iraqi study of workers at a battery factory, an increase in achromatic lesions ($p < 0.001$) and structural chromosomal aberrations ($p < 0.001$ for chromatid breaks and $p < 0.05$ for chromosome type aberrations) was detected in the lymphocytes of blood samples from 19 workers (9 male control persons) (Al-Hakkak *et al.* 1986).

2.2 Animal experiments and *in vitro* studies

2.2.1 *In vitro*

The data on the genotoxicity of lead have been summarized in several review articles (Hartwig 1994; IARC 1980, 1987; Silbergeld *et al.* 2000). Whereas lead salts (with the exception of lead chromate) are generally non-mutagenic in bacterial test systems, mutagenicity tests in mammalian cells show predominantly positive effects. However, the extent of the mutagenic effects and the applied concentrations vary considerably and are dependent both on the cell line and on incubation conditions. Only weak mutagenic effects were observed for the most part in classical mutagenicity tests (HPRT test with V79 or CHO cells). On the other hand, more recent studies with AS52 cells show a marked concentration-dependent mutagenicity even at very low, submicromolar concentrations (0.1–1 μM) (Ariza and Williams 1996, 1999; Ariza *et al.* 1998). Whereas principally base pair substitutions were induced in the lower concentration range between 0.1 and 0.5 μM , deletions predominantly occurred between 0.5 and 1 μM . The most important difference to earlier studies is found in the test system: AS52 cells were used, in which the inherent *hprt* gene was inactivated and a bacterial *gpt* gene integrated. This test system also records larger deletions especially well, which in classical test systems result in cell death via a loss of vital genes closely related to the *hprt* gene. The AS52 cells were thus found to be especially sensitive to substances causing mutagenicity via reactive oxygen species, including a large number of metal compounds.

In addition, lead ions > 0.05 μM caused the induction of micronuclei (Thier *et al.* 2003) and co-mutagenic effects in combination with UV radiation in the submicromolar range (Hartwig *et al.* 1990).

2.2.2 *In vivo* DNA strand breaks

In Swiss mice, the induction of single strand breaks was investigated using the comet assay. After a single oral dose of 0.7 to 89.6 mg lead nitrate/kg body weight, blood samples were taken from the orbital plexus of mice between 24 hours and 2 weeks after administration and the tail length of DNA comets was determined in the comet assay. Although the number of DNA strand breaks was significantly increased, no dose-effect relationship could be demonstrated (Devi *et al.* 2000).

The frequency of DNA single strand breaks and alkali-labile sites in nasal epithelium, lung, blood, liver, kidney, bone marrow, brain and testes was investigated in male CD mice with the help of the comet assay after inhalation of lead acetate (single and repeated; twice per week, for up to 4 weeks) for 60 minutes. The exposures were carried out in a whole-body chamber at a nominal concentration of 0.0068 µg/ml (6.8 mg/m³) and a particle size of < 1 µm, whereby details on the techniques used to measure exposure are completely absent in the publication. No DNA single strand breaks were found in the testes under these conditions. There were in some cases significantly increased DNA single strand breaks in the remaining organs and in the leukocytes at different sampling times (Valverde *et al.* 2002).

These studies are thus of little value due to methodological shortcomings.

2.2.3 Chromosomal aberrations

The induction of chromosomal aberrations in bone marrow cells and spermatocytes was investigated in Swiss mice after administration of lead acetate doses of 200 or 400 mg/kg diet (about 19.1 or 38.2 mg lead/kg body weight) for five days. An increased number of chromatid aberrations (gaps, deletions, fragments and breaks) as well as Robertsonian translocations were found in the bone marrow cells 6, 24 and 48 hours after the end of treatment. The systematics applied in listing the observed aberrant forms appear to be highly unconventional. Starting two weeks after termination of exposure to lead acetate, the oral administration of calcium chloride doses of 40 or 80 mg/kg body weight on three subsequent days reduced the frequency of observed aberration types to control level. This test design makes the study appear questionable. An increased number of structural chromosomal aberrations were found in the spermatocytes in all treated mice 24 and 48 hours after termination of exposure, and an increased incidence of sperm head abnormalities was observed (Aboul-Ela 2002). On account of the unconventional evaluation criteria and non-validated study methods, the data of this publication are not very meaningful.

2.2.4 Micronuclei

The induction of micronuclei was investigated in the bone marrow of Swiss mice 12, 24 and 36 hours after a single intraperitoneal injection of 0.625 to 80 mg lead nitrate/kg body weight. The micronucleus frequencies were described as being significantly

increased. The induction of micronuclei was more pronounced in male mice than in the females. No dose-effect relationship could be found due to the wide range of fluctuation of the results (Jagetia and Aruna 1998).

3 Carcinogenicity

3.1 Effects in humans

In earlier documentation (2000; Volume 17, present series), increases in risk which were found in several studies of collectives exposed to lead were discussed regarding tumours of the stomach, the lung and the bladder. Additional material has been summarized by Steenland and Bofetta (2000) and by IARC (2006). In this connection, studies with workers subject to high-level lead exposure in lead mines, lead foundries and in the production of lead batteries are of particular interest. These more recent data are described below.

3.1.1 Lead battery production

The observation period of the study of lead battery plant workers in the USA already described by Cooper and Gaffey (1975) was extended (Wong and Harris 2000). This cohort consisted of 4518 men from ten companies, who were involved in the production of lead batteries for at least one year between 1946 and 1970. The employment history could be determined only until 1970, and the occupational status only until 1981. The vital status was ascertained between 1947 and 1995. Individual exposure data were not completely available. Therefore, the data on exposure were reconstructed from the available biomonitoring data, with emphasis on the years after 1960. The mean lead concentration in the blood was 627 µg/l and the mean concentration in the urine was 129.7 µg/l. The standardized mortality ratio (SMR) was calculated using the US male population as reference. In the cohort, a total of 2613 workers had died by the end of 1995 (SMR = 1.07; 95 % confidence interval (CI): 1.03–1.11). The total mortality was thus only slightly higher than in the reference population. Mortality from stomach carcinoma was significantly increased (45 observed cases, SMR = 1.53; 95 % CI: 1.12–2.05). Mortality from lung cancer was only marginally increased with an SMR of 1.14 (210 observed cases, 95 % CI: 0.99–1.30). Mortality from cancer of the bladder (SMR = 0.49; 95 % CI: 0.23–0.90) and kidney cancer (SMR = 0.50; 95 % CI: 0.20–1.03) was reduced (Wong and Harris 2000).

In a nested case-control study in the largest of the ten companies with 30 stomach cancer cases and 120 age-matched controls, no significant trend in favour of mortality was found, either for the duration of employment at the company or for the duration of

employment in intermediate or high exposure areas or for the weighted cumulative exposure to lead (Wong and Harris 2000).

3.1.2 Lead smelters

Wong and Harris (2000) describe the more recent data from a cohort study of 2300 workers from six lead smelters, who had been employed for at least one year between 1946 and 1970 and whose vital status was ascertained between 1947 and 1995. The study, as with the cohort study from lead battery production, was presented for the first time by Cooper and Gaffey (1975).

The mean lead concentration in blood was, at 797 µg/l, higher than in the battery makers, the mean lead concentration in urine was 173.2 µg/l. As regards lung cancer, however, cadmium and arsenic must be considered as potential confounders. The mortality in the US male population was used as reference for the SMR. Among the 2300 exposed persons, a total of 1100 workers had died by the end of 1995 (SMR = 1.00; 95 % CI: 0.94–1.06). The mortality from stomach cancer (15 observed cases, SMR = 1.33; 95 % CI: 0.75–2.20) and lung cancer (SMR = 1.22; 95 % CI: 1.00–1.45) was increased. Both stomach cancer and lung cancer mortality showed no positive trend in dependence on the length of employment (Wong and Harris 2000).

Carta *et al.* (2003, 2005) provide data on a cohort of 918 workers at a lead foundry in Sardinia. Four cases with stomach carcinoma (SMR = 1.22; 95 % CI: 0.46–3.23) and 18 cases with lung carcinoma (SMR = 1.21; 95 % CI: 0.76–1.92) were described. A significant increase in relative risk depending on exposure ($p < 0.05$) was observed for lung cancer, for which reason the workers were divided into 4 exposure categories (not exposed: SMR = 0.27; slight exposure: SMR = 1.12; medium exposure: SMR = 1.43; high exposure: SMR = 1.96) (Carta *et al.* 2003, 2005).

Englyst *et al.* (2001) extended the observation of 3979 Swedish workers in lead foundries conducted by Lundström *et al.* (1997). They formed two subcohorts: subcohort 1 consisted of 710 workers employed in the lead departments; subcohort 2 was a part of subcohort 1 and consisted of 383 workers who had always been employed in the lead departments, but never in the arsenic or nickel departments or in the smelting and/or machinery areas. Standardized incidence rates (SIR) were calculated using regional rates for the period between 1958 and 1987. In both subcohorts, the SIR for lung cancer was statistically significantly increased (subcohort 1: SIR = 2.4; 95 % CI: 1.2–4.5; subcohort 2: SIR = 3.6; 95 % CI: 1.2–8.3). The total tumour incidence was not increased. A subsequent investigation of company documents showed that, in spite of the exclusion criteria described above, nine of the ten lung cancer cases in subcohort 1 and four of the five lung cancer cases in subcohort 2 had also been exposed to arsenic. However, those who had not developed tumours had not been examined in the context of an exposure to arsenic.

Van Wijngaarden and Dosemeci (2006) describe results on the relative risk of brain tumours in workers occupationally exposed to lead, for which the National Longitudinal Mortality Study (USA) was taken as basis. The evaluation included a total of 317968 individuals, for whom information was available on the occupation at the time of

reporting or on the last activity performed during the preceding 5 years in the case of non-employed persons. Exposure was estimated using the job exposure matrix developed by Cocco *et al.* (1999b). The mortality of the cohort was compared with that of the US population. In addition, further analyses were carried out using Cox and Poisson regressions. In comparison with non-exposed persons, a hazard ratio of 1.5 (95 % CI: 0.9–2.3) adjusted according to age and sex was found in occupations with a potential lead exposure. Comparison with the population yielded an SMR of 1.11 (95 % CI: 0.74–1.59). On subdividing the exposure according to probability or intensity of exposure, the following hazard ratios adjusted in relation to age, sex, place of residence, education, ethnic origin and family status were obtained: probability low 0.7 (95 % CI: 0.2–2.2), medium 1.4 (95 % CI: 0.8–2.5), high 2.2 (95 % CI: 1.2–4.0); intensity low 1.2 (95 % CI: 0.7–2.1), medium or high 1.9 (95 % CI: 1.0–3.4); probability and intensity high 2.3 (95 % CI: 1.3–4.2). The authors commented their results to the effect that their findings support the hypothesis of an association between occupational exposure to lead and brain cancer mortality (van Wijngaarden and Dosemeci 2006). The fact that the term “brain cancer” does not represent a uniform disease entity and that, in this study, the exact level of lead exposure of the workers had not been measured, must be taken into account in the assessment.

3.1.3 Case-control studies

The occupation-relevant causes of stomach cancer ($n = 41957$), tumours of the heart ($n = 1056$), brain ($n = 27060$) and nervous system ($n = 12980$) were investigated (Cocco *et al.* 1998a, 1998b, 1999a, 1999b) in a number of population-based, case-control studies in the USA using death certificates. Two controls, adapted to region, ethnic origin, sex and age, were selected for every case of death from tumours. Death certificates in the USA include details on the principal occupation and the branch of industry concerned. The probability and intensity of an exposure to lead using a job exposure matrix on the basis of occupation and industry were estimated according to the information found on the death certificate.

In the case of stomach carcinomas, no increase in relative risk dependent on an exposure to lead could be found. The same applies to malignant neoplasms of the central nervous system. A comprehensive population-based, case-control study with 935 cases (renal cell carcinoma) and 4298 controls was performed in Germany to assess occupational risk factors. Exposure to lead was estimated using a job exposure matrix. Accordingly, in the case of renal cell cancer, an odds ratio of 1.5 (95 % CI: 1.0–2.3) was found for substantial occupational lead exposure in men and of 2.6 (95 % CI: 1.2–5.5) in women (Pesch *et al.* 2000).

3.1.4 Meta-analysis of the studies

A meta-analysis was carried out with the available data in analogy to Steenland and Bofetta (2000). To provide a summary of all data, a so-called random effect model was used, in which the differences between the studies were taken into account. Where the results of the individual studies are homogeneous, the results from fixed and random effect models differ only slightly.

The results for the five tumour localizations under consideration (stomach, lung, bladder, kidney, brain/nervous system) are shown in the Figures 2 to 6.

Carcinoma of the stomach (Figure 2): all studies together reveal an increased relative risk of 1.31 (95 % CI: 1.08–1.58).

Carcinoma of the lungs (Figure 3): the total relative risk is 1.24 (95 % CI: 1.02–1.50). The markedly increased relative risk in the Swedish study by Lundström *et al.* (1997) is conspicuous. The test for heterogeneity is also statistically significant ($p < 0.0001$). If the study by Lundström *et al.* is excluded, there is no significant difference between the studies, and the relative risk is reduced to 1.14 (95 % CI: 1.04–1.24).

Carcinoma of the kidneys (Figure 4): all studies together show a relative risk of 1.14 (95 % CI: 0.82–1.59). Statistically, the relative risk is thus not significantly increased.

Carcinoma of the bladder (Figure 5): the studies show in total a relative risk of 1.15 (95 % CI: 0.78–1.71). Statistically, the relative risk is not significantly increased.

Tumours of the brain or nervous system (Figure 6): a relative risk of 1.09 (95 % CI: 0.84–1.40) was calculated from all studies. Statistically therefore, the relative risk is not significantly increased.

End point: Carcinoma of the stomach

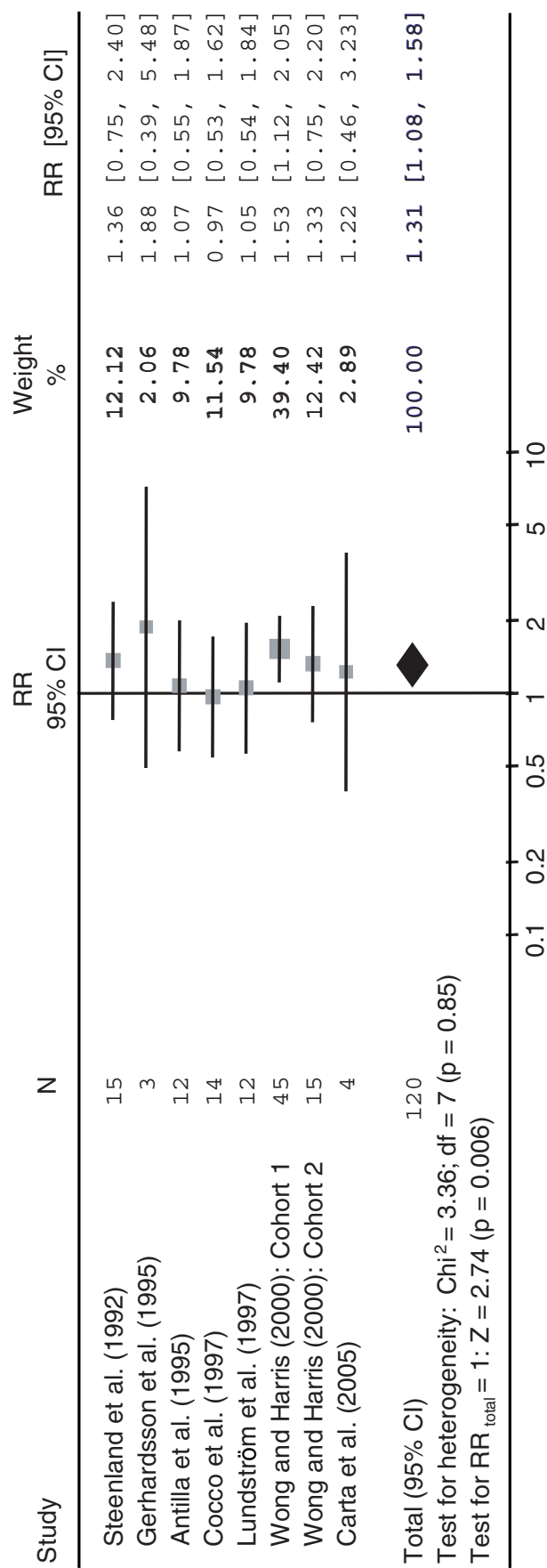


Figure 2. Relative risk (RR) for workers exposed to lead in comparison to the general population or to non-exposed workers (see Higgins and Green 2006)

End point: Carcinoma of the lung

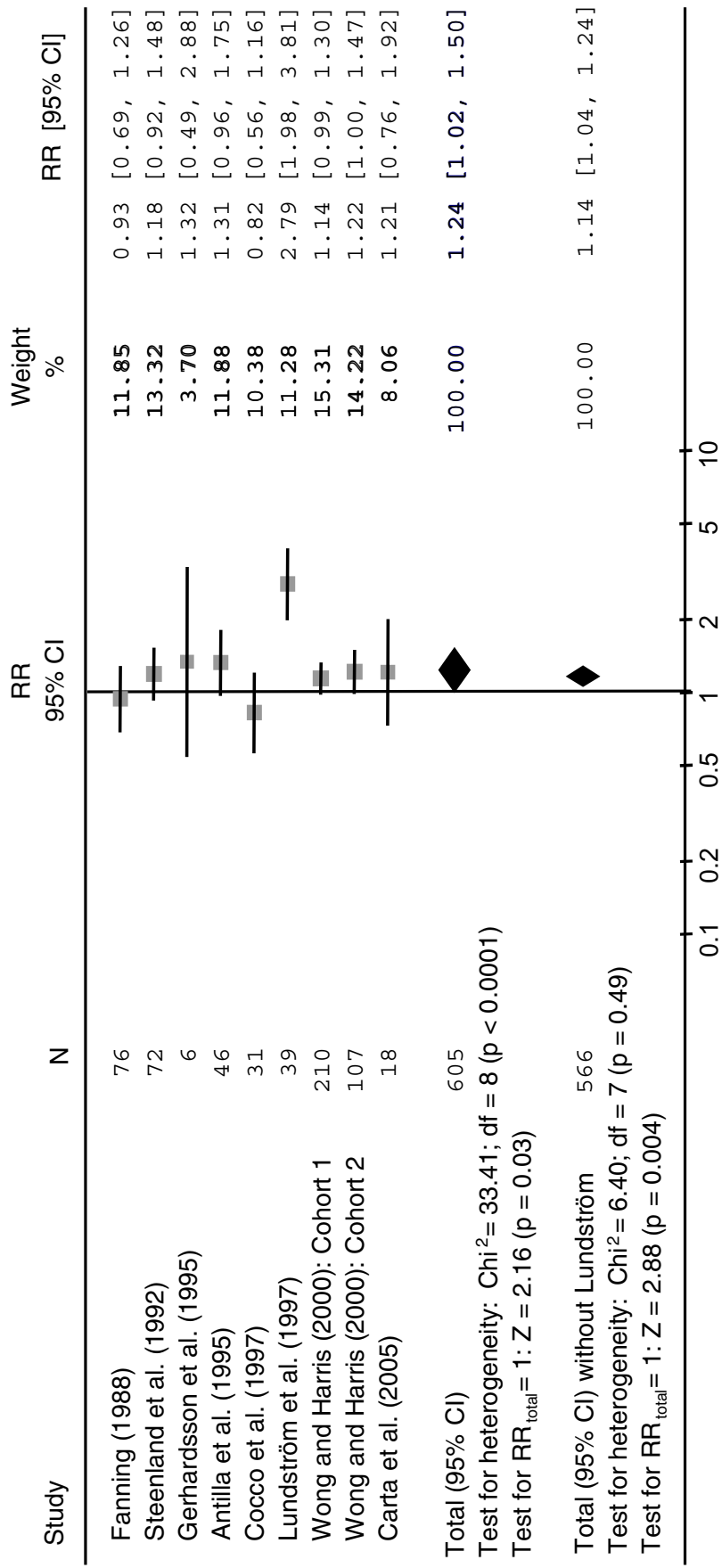


Figure 3. Relative risk (RR) for workers exposed to lead in comparison to the general population or to non-exposed workers (see Higgins and Green 2006)

End point: Carcinoma of the kidney

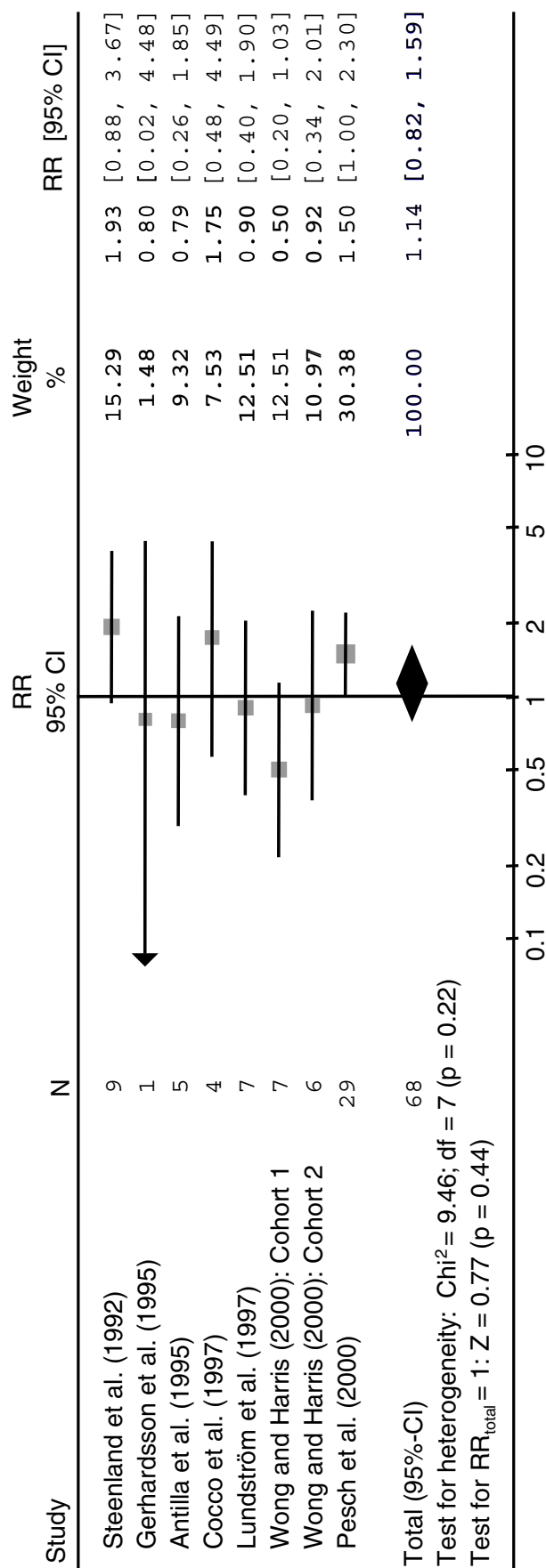


Figure 4. Relative risk (RR) for workers exposed to lead in comparison to the general population or to non-exposed workers (see Higgins and Green 2006)

End point: Carcinoma of the urinary bladder

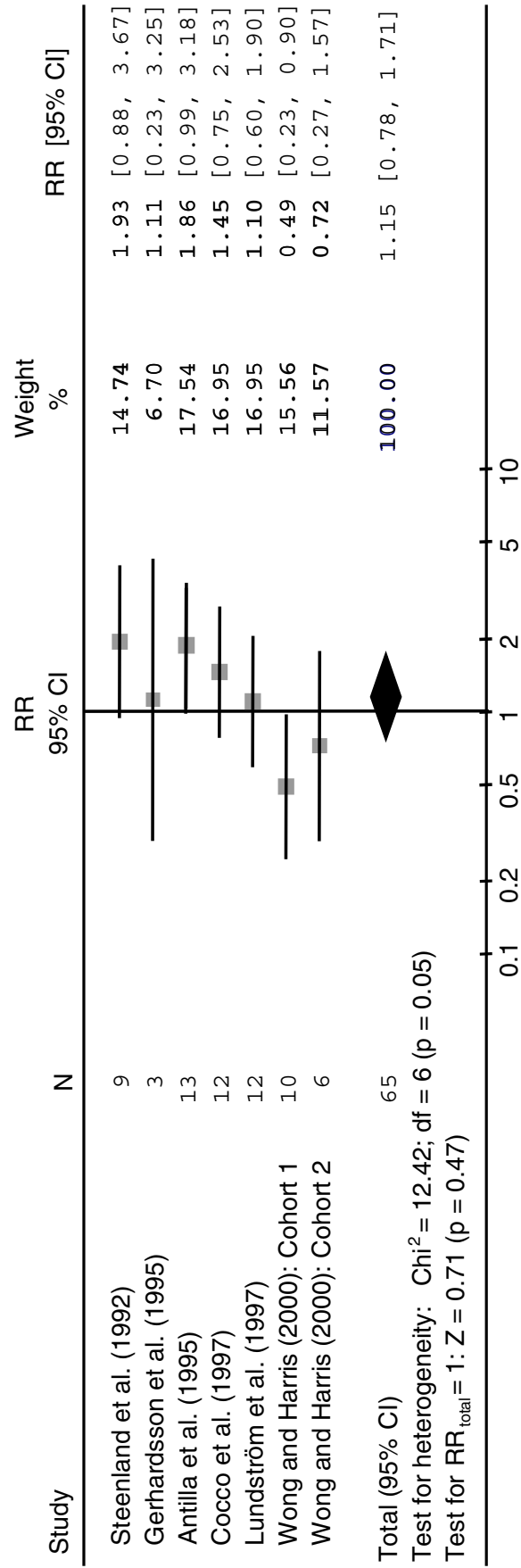


Figure 5. Relative risk (RR) for workers exposed to lead in comparison to the general population or to non-exposed workers (see Higgins and Green 2006)

End point: Tumours of the brain or nervous system

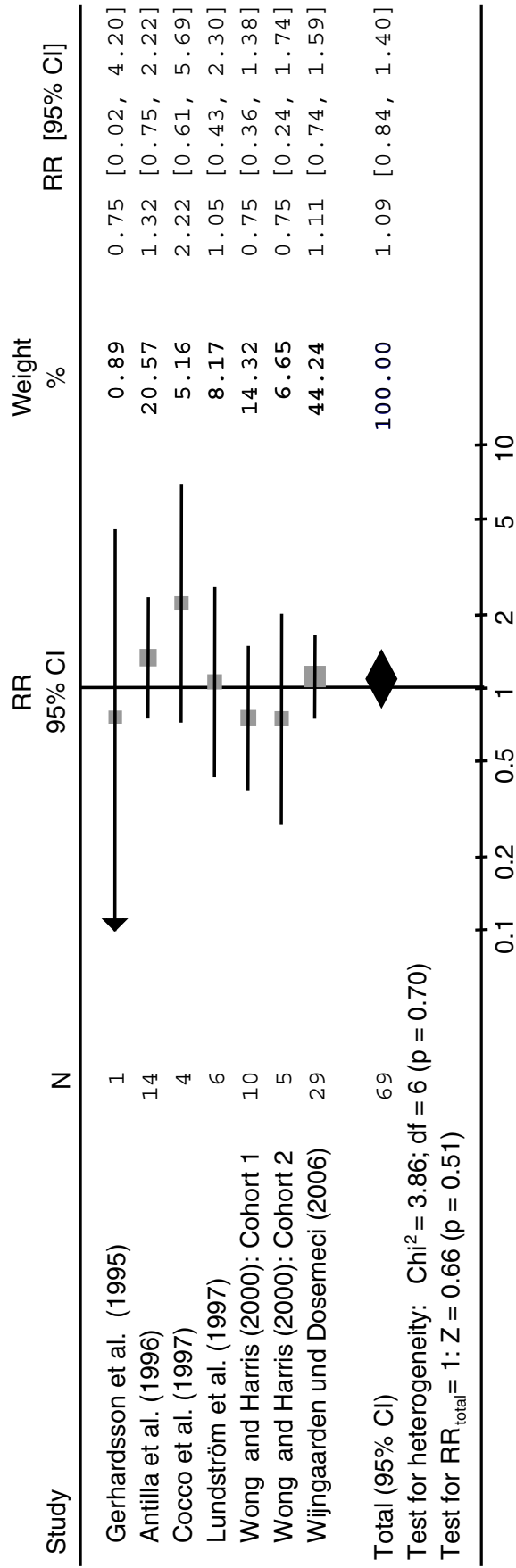


Figure 6. Relative risk (RR) for workers exposed to lead in comparison to the general population or to non-exposed workers (see Higgins and Green 2006)

3.1.5 Assessment

Carcinoma of the stomach: The different studies are in agreement in showing an increased relative risk situated about 30 % above that of the corresponding reference population. No exposure-effect relationship for stomach carcinoma could be demonstrated in the study with workers from lead foundries. Factors such as ethnic effects, dietary behaviour, frequency of *Helicobacter pylori* infection or socioeconomic status may have participated in causing the increased incidence of stomach cancer (Wong and Harris 2000).

Carcinoma of the lung: With the exception of the Swedish cohort, all studies revealed a moderately increased relative risk, which can also be explained by smoking as a factor. Details on smoking habits were not included in most of the studies. The markedly increased relative risk in the Swedish study (Lundström *et al.* 1997) could be explained by an exposure to arsenic. There remains the markedly increased relative risk in subcohort 2, in which it was assumed that exposure to arsenic was excluded, which argues against this hypothesis. However, this subcohort consists of only a few cases. Subsequent investigation has revealed that a number of cancer patients were indeed exposed to arsenic as well (Englyst *et al.* 2001).

Carcinoma of the bladder and kidney: Altogether, no increased relative risk can be observed.

Tumours of the brain or nervous system: Here, too, an increased relative risk is not present. In the Finnish investigation (Antilla *et al.* 1996), a case-control study was performed for this localization. A statistically significant dose-effect relationship between the lead concentration in blood and the relative risk for gliomas was demonstrated. It must be noted, however, that exposures were markedly higher and no increased relative risks were observed in the study by Wong and Harris (2000) in lead battery and lead smelter workers. Based on the different results, no causal relationship can be derived between lead and the development of brain tumours.

3.1.6 Summary

The data from epidemiological studies available at present give some indications of a possible carcinogenic effect of lead. However, they are not sufficient to classify lead as being carcinogenic to humans.

3.2 Results of animal studies

In the documentation published in 2000 (see “Lead and its inorganic compounds”, Volume 17, present series) an increased tumour incidence in the kidney after oral, subcutaneous or intraperitoneal administration of lead acetate, lead subacetate [basic lead acetate, $\text{Pb}_3(\text{CH}_3\text{COO})_2(\text{OH})_4$] and lead phosphate to rats or mice was discussed in particular. The fact that kidney tumours occurred only at dose ranges in which a marked nephrotoxicity had already developed was considered. In the present documentation, also

in the light of new data on the mechanism of action and on genotoxicity, further studies are presented in greater detail in which kidney tumours or other types of tumours were produced even at non-toxic doses.

3.2.1 Lead acetate

The carcinogenicity of lead was investigated in offspring whose dams had been exposed to lead during the gestation and lactation periods. For this purpose, 10 to 15 female C57Bl/6NCr mice were mated with male C3H/HeNCr mice to breed B6C3F1 offspring. The dams were given drinking water *ad libitum* with lead acetate concentrations of 0, 500, 750 or 1000 mg/l (no information on purity; about 0, 100, 150 or 200 mg lead/kg body weight and day) from day 12 of gestation up to weaning the offspring four weeks after birth. The offspring were divided according to sex into groups of 23 to 25 animals. Some of these groups were subsequently observed for periods of different length, up to a maximum of 112 weeks postpartum, others were given 500 mg sodium barbital/l (about 0.05 mg/kg body weight) as renal tumour promoter for a maximum of 112 weeks. Exposure to lead acetate did not affect litter size, the number of offspring per litter or the body weight of the offspring. Viability was not adversely affected in any of the groups. A complete necropsy was performed on each animal. In the male offspring lead acetate alone (without additional sodium barbital) produced a dose-dependent and significant increase ($p = 0.0006$; trend test according to Cochran-Armitage) in hyperplasias (including atypical tubular-cell hyperplasia) and a significant increase in the incidence of renal adenomas ($p < 0.05$; Fisher's exact test) in the kidney. In the female offspring, two tubular adenomas and one adenocarcinoma occurred. The proliferative lesions originated exclusively from the tubular cells. Sodium barbital caused no further proliferative changes and also no increase in the incidence of tumours induced by lead acetate. No tumours were induced in other organs. The incidences of hyperplasias and neoplasms are shown in Table 1.

The tumours induced by lead acetate occurred in the absence of a nephrotoxicity otherwise found to be typical in adult rodents. It was shown that the individual tubuli continued to be recognizable as distinct entities without degenerative secondary features within the hyperplasias, and that these tubuli contained dysplastic basophilic cells which presumably represented a preliminary stage of hyperproliferation. In addition, the formation of degenerative changes such as cystic tubular dilatations, intranuclear lead inclusions or renal fibrosis, were absent. The kidney tissue surrounding the hyperproliferative areas was unchanged. Occasional age-related dystrophies corresponded to those of the control group.

The authors reached the conclusion that, in this test design, the formation of hyperplasias, adenomas and adenocarcinomas were independent occurrences and not associated with degenerative changes. The special test situation of prenatal or postnatal exposure to lead resulted in the formation of kidney tumours in the juvenile animals, which was not associated pathogenetically with an increased cell turnover due to cell damage and subsequently increased regenerative proliferation (Waalkes *et al.* 1995). It was imperative that the authors derive these conclusions from the results obtained.

Table 1. Study by Waalkes *et al.* (1995) on the carcinogenicity of lead acetate in mice

Author:	Waalkes <i>et al.</i> 1995				
Substance:	lead acetate (no details on purity)				
Species:	10 to 15 female C57Bl/6NCr mice				
Administration:	orally with the drinking water				
Concentration/dose:	0, 500, 750 or 1000 mg lead acetate/l drinking water (0, 100, 150 or 200 mg/kg body weight and day)				
Duration:	dams from day 12 of gestation up to weaning of the B6C3F1 offspring postnatal observation of the young up to maximum of 112 weeks				
Toxicity:	rarely slight nephrotoxicity in the form of cytomegalic tubular cells (no further details)				
Tumours:					
		Concentration in the drinking water (mg/l)			
		0	500	750	1000
Kidney:					
Tubular hyperplasia	♂	1/23 (4%)	3/25 (12%)	5/25 (20%)	7/25 (28%) [#]
	♀	0/25 (0%)	0/24 (0%)	0/25 (0%)	4/25 (16%)
Tubular adenomas	♂	0/23 (0%)	0/25 (0%)	0/25 (0%)	5/25 (20%)*
Tubular adenomas and carcinomas	♂	0/23 (0%)	1/25 (4%)	1/25 (4%)	5/25 (20%)*

* Significant at $p < 0.05$ (Fisher's exact test); [#] significant at $p < 0.01$ (trend test according to Cochran-Armitage)

The study by Zawirska and Medras (1968) briefly mentioned in the documentation published in 2000 is presented here in greater detail in the light of the new data. 94 male and 32 female Wistar rats were given 3 mg lead acetate per animal and day with the feed for two months, followed by 4 mg lead acetate per animal and day for 16 months (no information on purity). As untreated controls, 19 males and 13 females were used. At the end of the exposure period, 27 males and 13 females which had been exposed to lead were killed, while the remaining animals were allowed to live to their natural death.

All treated rats were very thin and apathetic before their deaths. Their fur frequently had a rusty discolouration. No details on the number of surviving rats were given. All animals were subjected to a thorough histological examination. In general, the dysplasias observed in most organs were produced by "diffuse proliferations" or adenomas. Especially in the kidneys, lungs and testes, the blood vessels were thickened and extensive calcium deposits were found. Due to a large number of cysts and tumour-like changes, the kidneys in particular were markedly enlarged. Neoplastic changes were observed first in the epithelia of the uriniferous tubules, collecting or connecting tubules and especially in the proximal section of the convoluted tubules (*tubuli contorti*). Mostly cysts and reticulohistiocytic infiltrations occurred in the renal cortex. Fibrous changes were found in the rats that survived the longest. As exposure continued, a thickening of the walls occurred at the junction of the renal artery, the *vasa afferentia* and the *vasa*

effferentia. In the lumina of a large number of tubuli, hyaline cylinders or concentric deposits, which probably contained lead compounds, were found. The fatty degeneration in the liver involved hepatocytes and Kupffer cells and was accompanied by venous congestion. In the early stages of lead poisoning, necrotic foci developed, surrounded by inflammatory infiltrates. In the later stages, a large number of scattered growths of the focal type consisting of enlarged liver cells with lighter-coloured cytoplasm and hyperchromic cell nuclei were found. In most rats, the testes were atrophic. There were calcium deposits in the tunica and the walls of the seminiferous tubules. The in part complete loss of spermatogenesis occurred. Hyperkeratoses at different levels of formation were observed in the forestomach. The spleen and lymph glands contained deposits of haemosiderin.

In the controls, one adenoma and one carcinoma in the mammary gland were diagnosed. No spontaneous tumours were found in the kidneys or in the endocrine glands. The following tumours were found in the 94 male rats treated with lead acetate: 58 kidney tumours (43 adenomas, 15 carcinomas); 23 adrenal gland tumours (22 adenomas, one carcinoma); 23 tumours of the interstitial cells of the testes; 22 prostatic tumours (21 adenomas, one carcinoma); 10 lung tumours (8 adenomas, 2 carcinomas); 4 adenomas of the pituitary gland; 3 liver tumours; 3 brain gliomas; 3 thyroid adenomas; 4 spermatid duct tumours (2 neurinomas, 2 carcinomas); 1 leukaemia and 1 sarcoma (no further details). The incidences of kidney, adrenal gland, testes and prostatic tumours were significantly increased compared with the control group ($p < 0.05$; Fisher's exact test). Among the 32 female rats exposed to lead acetate there were 14 kidney tumours (12 adenomas, 2 carcinomas); 9 adenomas of the adrenal glands; 5 lung tumours (4 adenomas, one carcinoma); 3 mammary gland tumours; 2 liver tumours; 2 thyroid tumours; 1 adenoma of the pituitary gland; 1 carcinoma of the oesophagus; 1 leukaemia and 2 sarcomas (no further details). The tumour incidences are shown in Table 2. According to the authors, only the incidences of kidney and adrenal gland tumours were significantly increased in relation to the control group ($p < 0.05$; Fisher's exact test) (Zawirska and Medras 1968), although the data in Table 2 express significant tumour yields for other organs.

Table 2. Study by Zawirska and Medras (1968) on the carcinogenicity of lead acetate in rats

Author:	Zawirska and Medras 1968		
Substance:	lead acetate (no information on purity)		
Species:	94 male and 32 female Wistar rats; 19 males and 13 females as controls		
Administration:	orally with the feed		
Dose/duration:	3 mg lead acetate/animal and day for 2 months, then 4 mg lead acetate/animal and day for 16 months		
Toxicity:	Enlargements of most organs; thickening of blood vessels especially in kidneys, lungs, testes; in the kidneys: cysts, inflammation, lead deposits; in the liver: fatty degeneration, venous congestion, necroses; in the testes: atrophies; in the forestomach: hyperkeratoses; in spleen and lymph nodes: haemosiderin deposits		
Tumours:			
		♂	♀
Controls*			
Mamma	adenoma	—	1/13 (7.6%)
	carcinoma	—	1/13 (7.6%)
Animals treated with lead acetate			
Kidney	adenoma	43/67 (64.2%)**	12/19 (63.2%)**
	carcinoma	15/67 (22.4%)**	2/19 (10.5%)
Adrenal gland	adenoma	22/67 (32.8%)**	9/19 (47.4%)**
	carcinoma	1/67 (1.5%)	0/19 (0%)
Prostate	adenoma	21/67 (31.3%)**	—
	carcinoma	1/67 (1.5%)	—
Spermatic duct	neurinoma	2/67 (3.0%)	—
	carcinoma	2/67 (3.0%)	—
Testes	interstitial cell adenoma	23/67 (34.3%)**	—
Lung	adenoma	8/67 (11.9%)	4/19 (21.1%)
	carcinoma	2/67 (3.0%)	1/19 (5.3%)
Pituitary	adenoma	4/67 (6.0%)	1/19 (5.3%)
Liver	adenoma	2/67 (3.0%)	1/19 (5.3%)
	carcinoma	1/67 (1.5%)	1/19 (5.3%)
Thyroid gland	adenoma	3/67 (4.5%)	1/19 (5.3%)
	carcinoma	0/67 (0%)	1/19 (5.3%)
Oesophagus	carcinoma	0/67 (0%)	1/19 (5.3%)
Forestomach	adenoma	0/67 (0%)	1/19 (5.3%)
Mammary gland	adenoma	0/67 (0%)	1/19 (5.3%)
	carcinoma	—	2/19 (10.5%)
Brain	glioma	3/67 (4.5%)	0/19 (0%)
Haematopoietic system	leukaemia	1/67 (1.5%)	1/19 (5.3%)
	sarcoma	1/67 (1.5%)	2/19 (10.5%)

* The occurrence of one adenoma and one carcinoma in the controls was reported, though without details on localization or sex. ** $p < 0.01$ (Fisher's exact test)

From week 31 postpartum, 47 male and female Wistar rats were given about 3 mg lead acetate (no information on purity) per animal and day with their diet for 60 to 504 days. In the treated animals, the observation time varied between 60 days and the time of natural death (maximum 572 days). The number of controls was cited in one publication as 31 male and female animals, and in the other as 47 male and female animals. No accurate details are given on survival time. All rats were examined histologically. The porphyrin concentrations in urine, faeces, bone marrow and kidneys were significantly increased in the rats which had received lead acetate for 307 or 504 days. This was more marked in the males than in the females. The deposits in the cell nuclei of the tubular epithelium characteristic for lead poisoning were found in the kidneys of all treated animals. Diffuse reticulohistiocytic infiltrates in the stroma occurred. The lumina of the proximal tubuli were distended and assumed the form of cysts, the epithelium of which showed papillary proliferations and adenomas. In the liver, necroses surrounded by inflammatory infiltrations and fatty degenerations and, later on, preneoplastic foci were found, which usually preceded the formation of hepatomas. Marked erythrocytic regeneration and immature leukocytes were observed in the bone marrow. No hyperplasias or tumours occurred in the animals of the control group. Details on tumour incidence and an assignment of the tumours to male or female animals were generally absent in the study. The 94 animals exposed to lead acetate were found to have 102 tumours: 12 renal adenomas, 15 lung adenomas, 17 pituitary adenomas, 10 brain gliomas, 11 thyroid adenomas, 5 parathyroid adenomas, 11 prostate adenomas, 8 mammary gland adenomas and 13 adenomas of the adrenal cortex. With the exception of the adenomas of the adrenal cortex, all tumour incidences were significantly increased in relation to the control group ($p < 0.05$; Fisher's exact test). The frequency of kidney tumours was dependent on treatment duration (Zawirska and Medras 1972; Zawirska 1981).

Groups of 15 to 20 male and 19 to 26 female Wistar rats (aged 2 to 2.5 months) were treated with lead acetate alone, or sulfathiazole alone (which causes kidney damage) or lead acetate plus sulfathiazole in their diet or normal feed for 18 months. They were observed after the end of exposure for another 7 months. The doses were 3 mg lead acetate per animal and day and 54 mg sulfathiazole per animal and day, respectively. A number of animals died during the observation period (no further details). Histological examination showed that lead acetate damages the renal cortex and sulfathiazole is toxic to the renal pelvis and the renal medulla. In 42 surviving male and female animals, lead acetate induced 14 renal tumours, including 5 carcinomas in males and one carcinoma in one female. In 43 surviving male and female animals, the simultaneous administration of lead acetate and sulfathiazole caused 17 renal tumours, including one carcinoma. Untreated animals and animals that had received only sulfathiazole developed no kidney tumours (Waszynski 1977).

In the context of a behavioural study, lead was administered orally to 12 rhesus monkeys (*Macaca mulatta*) for 12 or 24 months (Laughlin *et al.* 1999). One female monkey developed a chronic myelocytic leukaemia after she had been exposed to lead acetate for a total of 2 years, so that her blood lead level was continuously around 350 μg lead/l. The exposure started on day 8 postpartum and was continued for 6 months

using a commercial milk formula. Following this, the lead acetate was mixed into a fruit-flavoured diet for 1.5 years. The lead concentration in the blood was regularly investigated. The first symptoms of a haematopoietic disease were found at the age of 25 months. Monthly blood samples showed an increased number of white blood cells and immature neutrophils. An increased number of neutrophil precursor cells were also found in the bone marrow. The animal was killed 4 months later after unsuccessful chemotherapy. The authors point out that myelocytic leukaemia is extremely rare in non-human primates. Serum investigations for different retroviruses that have been associated with lymphoid neoplasms in these primates yielded negative results (Krugner-Higby *et al.* 2001).

3.2.2 Lead subacetate (basic lead acetate)

A total of 20 intraperitoneal injections containing lead subacetate ("reagent grade", dissolved in tricaprillin) were administered to 30 male and 30 female Strain A/Strong mice aged 6 to 8 weeks for up to 30 weeks. The total dose, which was also taken as the MTD, was 0.8 mmol/kg body weight (corresponding to 445 mg/kg body weight). A further 30 test animals of each sex (no other details) simultaneously received calcium acetate (20 injections) or magnesium acetate (9 injections) at 1:1, 3:1 or 10:1 molar ratios with lead subacetate. Calcium acetate or magnesium acetate were admixed with the lead subacetate prior to injection.

Survival of mice to the end of the study was 70 % to 87 % in controls, 67 % in the animals treated with lead subacetate alone, 53 % to 77 % in animals treated with combined calcium and lead subacetate, and 43 % to 60 % of those receiving lead subacetate combined with magnesium acetate at 3:1 or 10:1 molar ratios. Only one animal survived at a ratio of 1:1. Only the lungs were investigated at the end of the study. Lead subacetate induced a significant increase ($p < 0.05$; Student t-test) in lung tumours per animal (0.86 ± 0.20 ; control: 0.32 ± 0.12). The simultaneous injection of lead subacetate and calcium acetate or magnesium acetate significantly reduced the number of lung tumours induced by lead subacetate ($p < 0.05$). For lead subacetate and calcium acetate in the molar ratios 1:1, 1:3 and 1:10 the following numbers of lung tumours per animal were reported: 0.21 ± 0.10 , 0.22 ± 0.10 and 0.13 ± 0.09 , respectively. For lead subacetate and magnesium acetate in a 1:1 molar ratio, only one animal survived (no other details); at ratios of 1:3 and 1:10, 0.08 ± 0.08 and 0.28 ± 0.11 lung tumours per animal occurred (Poirier *et al.* 1984).

In an investigation with 16 male and 16 female Strain A/J mice, 6 to 8 weeks old, which were injected intraperitoneally for 24 weeks with lead subacetate (purity not specified) dissolved in water at a total dose of 38, 95 or 190 mg/kg body weight, 81 % to 100 % of the mice in both low dose groups survived. In the high dose group, 3 animals (19 %) survived. Only the lungs were investigated for adenomas at the end of the study. In the male mice, the mean number of lung tumours per animal was significantly increased ($p < 0.05$; Wilcoxon test) versus controls (0.07 ± 0.07), with 0.5 ± 0.18 at

38 mg/kg body weight and with 0.67 ± 0.33 at 190 mg/kg body weight, but not at 95 mg/kg body weight with 0.20 ± 0.11 (Stoner *et al.* 1986).

Male Sprague-Dawley rats, 5 to 8 weeks old, were given feed into which had been mixed 1.0 % lead subacetate (no information on purity) or 1.0 % lead subacetate together with 1.6 % indole for 12 to 17 months. Indole was used because it had prolonged the survival time of 2-AAF-treated rats in preceding investigations. The average survival time was 53 to 69 weeks. All animals were examined histologically.

In the rats fed with lead subacetate only, cerebral gliomas occurred in 2 of 17 animals ($p < 0.01$), and in 3 of 41 ($p < 0.01$) with additional exposure to indole. One malignant glioma was observed in 325 controls. The incidence of tumours of the renal cortex was 13 of 17 in the rats treated with lead subacetate and 25 of 41 in those subjected to mixed exposure. Only in the rats treated with indole were there no tumours of the renal cortex. Further details on tumour incidences in the 325 controls are lacking. No tumours were induced by lead subacetate in other organs (Oyasu *et al.* 1970).

4 Manifesto (MAK value, classification)

The cytogenetic investigations in exposed workers do not enable the establishment of a direct relationship between the extent of lead exposure and the frequency of DNA damage. A significant increase in micronucleus frequency was observed in workers with lead concentrations of 248 μg to 369 $\mu\text{g/l}$ blood (Vaglenov *et al.* 2001). In an earlier study as well, an increased occurrence of chromosomal aberrations was described at a lead concentration of 250 $\mu\text{g/l}$ blood (Nordenson *et al.* 1978). In addition, indications of a clastogenic effect of lead (DNA single strand breaks) were found in mice (Devi *et al.* 2000; Valverde *et al.* 2002). The genotoxicity of lead and its inorganic compounds is based on complex mechanisms. It is not possible to derive a no observed adverse effect level for the clastogenic effect of lead and its inorganic compounds, either for these mechanisms, or from experience with humans, or from the experimental investigations with animals. Therefore, no threshold value can be established. The current MAK value, which had been derived on the basis of the neurotoxic effects, is therefore withdrawn.

The data available at present from epidemiological studies provide evidence for a possible carcinogenic effect of lead. They are, however, not sufficient to classify lead as a human carcinogen.

Carcinogenicity studies in experimental animals which meet present requirements are not available. However, the investigations with lead acetate in mice show that kidney tumours also occur without concomitant kidney toxicity. Lead acetate is a pluripotent carcinogen in rats. It caused tumours in the kidney, adrenal gland, testes, prostate, lung, liver, pituitary, thyroid and mammary gland as well as leukaemias, sarcomas of the haematopoietic system and cerebral gliomas. Cerebral glioma is a type of tumour rarely occurring spontaneously.

As the release of lead ions is responsible for the toxic effects of all forms of lead, and genotoxicity has been demonstrated both for metallic lead as well as for its inorganic

compounds, a carcinogenic effect of lead itself and its inorganic compounds must be assumed. Therefore, lead and its inorganic compounds are classified in Carcinogen category 2.

As a result of the positive findings on the clastogenicity of lead in exposed workers and the bioavailability of lead in germ cells, lead and its inorganic compounds are classified in Category 3A for germ cell mutagens.

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