Recommendation from the Scientific Committee on

Occupational Exposure Limits:

Lead Chromate

8 hour TWA:	100 μg Pb / m^3 (see SCOEL/SUM 83)
STEL (15 min):	not applicable
BLV:	30 μg Pb / 100 ml blood (see SCOEL/SUM 83)
Risk assessment for hexavalent chromium compounds in general: see SCOEL/SUM 86	

Substance and use

Lead chromate is a yellow powder which is used in the production of commercial lead chromate pigments, such as Primrose Chrome Yellow, Light Chrome Yellow and Medium Chrome Yellow, containing 65-89% lead chromate (IARC 1990).

EU classification: Carc. Cat. 3, R33; R40; Repr. Cat. 1: R61; Repr. Cat. 3; R63

In this classification and labelling system, lead chromate (carc. Cat. 3) is categorised differently from other hexavalent chromium compounds (carc. Cat. 2), because of its poor solubility (i.e. 0.06 g/l water) and because of the limited data on carcinogenicity that are available (scientific background: see DFG 1985).

Reference to existing recommendations by SCOEL

SCOEL has already issued Summary Documents on both Lead and Lead Compounds (SCOEL/SUM 83) and Hexavalent Chromium Compounds (SCOEL/SUM 86), to which reference is made. On this basis, the present Summary Document on Lead Chromate is focussed on experimental data on the genotoxicity of lead chromate and on the issue of bioavailibility. Specific data on lead chromate published after the latest IARC documentation in 1990 are described in more detail.

General principles of chromate toxicity and conclusions (see SCOEL/SUM 86)

Hexavalent chromium compounds are considered to be carcinogenic, and the common carcinogenic factor appears to be the chromate anion (CrO_4^{2-}). Differences in carcinogenic activity between different chromates have been related to differences in

solubility and, accordingly, in bioavailability. The principle target of carcinogenicity is the lung tissue.

In contrast to other chromates, lead chromate is only very poorly soluble (0.06 g/l). Like barium chromate, lead chromate is positive in bacterial mutation assays *in vitro* following chemical solubilisation. Moreover, these are positive in some mammalian genotoxicity assays (*v.i.*). An intrabronchial implantation study in rats demonstrated elevated lung cancer incidence with calcium chromate, strontium chromate and zinc chromate, but failed to demonstrate evidence for carcinogenicity of the poorly soluble lead chromate and barium chromate (Levy et al 1986). A number of studies in rats of intramuscular or subcutaneous implantations or injections respectively with lead chromate have induced local sarcomata, however such locally induced tumours are no longer considered evidence of carcinogenic potential *per se.* However, in one such study, a number of rats also developed distant tumours; renal carcinomas (Furst et al 1976). With respect to available epidemiological investigations in chromate pigment production workers, a series of studies based on three U.K. factories provided strong evidence that zinc chromate, but not lead chromate, was associated with lung cancer risks in this industry (Davis 1979, 1984a,b).

An assessment of human lung cancer risk induced by exposure to hexavalent chromium compounds has been issued by SCOEL (SCOEL/SUM 86). As exemptions from this general risk estimate, with the likelihood of a lower risk of genotoxicity and carcinogenicity, the two specific compounds lead chromate and barium chromate have been mentioned, as these require effective solubilisation to elicit positive results in bacterial mutagenicity assays or in mammalian cells. Nevertheless, these poorly soluble compounds also appear positive in some assays, and therefore it was not possible to exclude them from possessing some mutagenic or clastogenic potential.

General principles of lead toxicity and conclusions (see SCOEL/SUM 83)

The toxic underlying principle of inorganic lead compounds is the divalent lead cation (Pb^{2+}). Although experimental and epidemiological studies point to the possibility of lead carcinogenicity, the recommended BLV of 30 µg Pb / 100 ml blood is mainly based on neurobehavioural adverse effects in exposed workers. Other endpoints of lead toxicity, namely to the peripheral nervous system (PNS) and the kidneys, are relevant for exposure levels which are consistently higher. The observed experimental carcinogenicity of lead salts is, in first instance, directed towards the kidneys as the target tissue. Most probably, these effects are to a great extent based on the renal toxicity of high doses of lead. There is considerable uncertainty concerning impairment of human reproductive functions by lead. For males, there are valid indications that only blood Pb levels above 40 µg/dl are connected with impairment of fertility. In females, however, it is relevant that cognitive deficits of the offspring are dose-dependently associated with lead exposure. In this respect, no definite NOAEL can be deduced, which calls for a minimisation of exposure.

Genotoxicity of lead chromate and studies into mechanisms

Both bacterial mutagenicity tests and studies of chromosomal genotoxicity in vitro have led to the conclusion that the chromate ion was responsible for genotoxic properties of lead chromate. The following studies appear especially noteworthy.

Nestmann et al. (1979) studied the mutagenicity of lead chromate using a battery of microbial assays: the *Escherichia coli* PolA+/PolA- survival test; the Salmonella/microsome His+ reversion assay; the E. coli Trp+ reversion test as a plate assay; the E. coli Gal+ forward mutation test; and the *Saccharomyces cerevisiae* assay for mitotic recombination. Lead chromate was mutagenic in *Salmonella* and in *Saccharomyces*. Metabolic activation by rat liver homogenate (S9) was not required for the mutagenic activity. The most statistically significant positive result was found with a supplementary assay, the *E. coli* fluctuation test. To determine whether the lead ion and/or the chromate ion were responsible for the mutagenicity observed, lead chloride and chromium trioxide (chromic acid) were also tested. In the *E. coli* fluctuation test, the ranges of maximal mutagenicity for chromium trioxide and lead chromate overlapped at the concentration 10^{-5}M, whereas lead chloride showed no mutagenicity and little lethality at concentrations up to 10^{-3}M.

Douglas et al. (1980) studied chromosome aberrations and sister-chromatid exchanges in cultured human lymphocytes, and DNA fragmentation as detected by alkaline-sucrose gradient sedimentation in cultured Chinese hamster ovary (CHO) cells. Lead chromate caused dose-related increases in chromosome aberration and sister-chromatid exchange in human lymphocytes. No increase in DNA damage was observed in CHO cells, possibly due to the relative insensitivity of the CHO cells and the limited solubility of lead chromate. The mutagenicity of lead chromate in human lymphocytes appeared to be entirely due to the chromate ion since chromosome aberrations were induced by potassium chromate but not lead chloride.

Sidhu et al. (1991) studied the genotoxicity of $PbCrO_4$, in a human osteosarcoma cell line (HOS, TE 85). Electron microscopic studies showed that $PbCrO_4$ was taken up *via* phagocytosis by the HOS cells and accumulated within the vacuoles in the cytoplasm. Cells assessed following multiple treatment of HOS cells with $PbCrO_4$ were morphologically different from HOS cells, formed anchorage-independent colonies in soft agar and formed quickly regressing small tumour nodules in athymic nude mice. The cellular and secreted plasminogen activator (PA) levels of 5 cell lines isolated after $PbCrO_4$ treatment were increased up to 8 fold and up to 10 fold, respectively, as compared to untreated HOS controls. SDS-PAGE analysis in the presence of copolymerised substrates was consistent with increase in 55 kDa urokinase-type PA (u-PA) and 68 kDa tissue-type PA (t-PA). These results were interpreted to show that $PbCrO_4$ leads to phenotypic transformation of HOS cells.

Cytogenetic effects of lead chromate were further evaluated in vivo by Watanabe et al. (1985). They administered 0.5, 1, 2 and 4 g/kg body weight of lead chromate suspensions in 0.5% gum arabic *i.p.* to C57BL/6N male mice twice, and found the frequencies of micronucleated polychromatic erythrocytes in the femoral bone marrow significantly ($p < 0.01$) different from that in the vehicle control. The ratio of polychromatic to normochromatic erythrocytes decreased in a dose-dependent fashion ($p < 0.05$).

Wise et al. (2002) compared lead chromate with sodium chromate as prototypical particulate and soluble chromates. Both compounds induced concentration-dependent cytotoxicity after a 24h exposure in primary human bronchial fibroblasts. The relative survival was 87, 46, 26 and 2% after exposure to 0.1, 0.5, 1 and 5 $\mu g/cm^2$ $PbCrO_4$, respectively, and 74, 57, 13 and 0% after exposure to 1, 2.5, 5 and 10 μM Na_2CrO_4, respectively. Similarly, the amount of chromosome damage increased with

concentration after 24 h exposure to both compounds. Specifically, 0.1, 0.5 and 1 μg/cm^2 $PbCrO_4$ damaged 15, 34 and 42% of metaphase cells with the total amount of damage reaching 18, 40 and 66 aberrations per 100 metaphases, respectively. $PbCrO_4$, at 5 μg/cm^2, induced such a profound cell cycle delay that no metaphases were found. Overall, the data were taken to indicate that lead chromate was cytotoxic and genotoxic to human lung cells.

In addition, mechanistic studies on lead acetate are available in various experimental systems. For instance, the chromosomal damage induced by lead acetate in cultured mammalian cells has been related to the formation of persistent DNA-protein crosslinks (Xu et al. 1992), and DNA strand breakage by $PbCrO_4$ has been linked with the formation of hydroxy radicals generated by $PbCrO_4$ (Leonard et al. 2002). In addition, induction of apoptosis has been demonstrated by lead chromate in different cell culture systems (Blankenship et al. 1997, Singh et al. 1999), and the theory has been put forward that lead chromate-induced apoptosis might represent a mechanism to eliminate chromium- or lead-damaged DNA (Singh et al. 1999).

Disposition of lead chromate particles

With respect on the local genotoxicicity of lead chromate upon cells of the respiratory tract, it has been shown in various cell culture systems that lead chromate is taken up into intact cells. These systems include Chinese hamster ovary cells, human foreskin fibroblasts (Wise et al. 1992), human lung small airway epithelial cells (Singh et al. 1999) and primary human bronchial fibroblasts (Wise et al. 2002). Specifically, electron microscopic studies using a human osteosarcoma cell line demonstrated that lead chromate particles were taken up by phagocytosis and accumulated within vacuoles of the cytoplasm.

The roles of particle-cell contact, particle dissolution and particle uptake for the clastogenicity of lead chromate were investigated by Wise et al. (1993). Using $Pb^{51}CrO_4$ it was found that lead chromate particles (1.2 microns mean diameter, -28 mV surface charge) were slightly soluble in water. The solubility increased 2-fold when particles were incubated in culture medium, but was not increased further by the addition of serum. The extracellular concentration of the chromate ion increased 7-fold when lead chromate was incubated in the presence of Chinese hamster ovary cells compared with culture medium alone. The intracellular concentration of ionic chromium increased in a dose-dependent manner following exposure of Chinese hamster ovary cells to clastogenic doses of lead chromate reached estimated levels as high as 1.2 mM per cell. Treatment of cells with lead chromate particles in the presence of a non-toxic dose of vitamin C was reported to block the uptake of ionic chromium and eliminated the clastogenic activity of the particles. Lead chromate particles were internalised by Chinese hamster ovary cells in phagocytic vacuoles in as little as 1 h; this internalisation was not affected by co-treatment with vitamin C. Sufficient particle-cell contact was required for lead chromate-induced clastogenesis. Based on the differential effects of vitamin C, these data were interpreted to show that although phagocytic particle uptake occurs, particle-cell contact and extracellular dissolution were decisive factors for the clastogenic activity of lead chromate.

Lead level in persons exposed to lead chromate pigment

The data summarised above have led to the general conclusion that, although the lead chromate pigment is frequently considered practically insoluble, it has a low but distinct solubility in aqueous and biological media that leads to a dissociation of the Pb^{2+} and CrO_4^{2-} ions. Subsequently, these may exert their specific biological effects. The relevant human exposure appears to be by inhalation of lead chromate particles.

As far as workplace exposure to lead chromate is concerned, the opinion has been expressed that this would not lead to Pb^{2+} concentrations in the blood higher than those observed in the general population (Ferguson 1981). However, based on the available experimental studies (*v.s.*) at least some systemic bioavailability of lead, upon exposure to lead chromate, must be expected. This view is consistent with epidemiological studies (Davies 1984a,b). Moreover, it is strongly supported by a case report of a worker exposed to lead chromate, as the only lead compound apparent, in the plastics pigmenting industry in Texas (Anonymous 1992). This person displayed an elevated lead level in blood of 52 μg/dl, *i.e.*, a level well above the BLV currently proposed by SCOEL.

Recommendation

Compared to other chromates, lead chromate has a very low but distinct solubility. As the dissociation of chromates, resulting in formation of the CrO_4^{2+} ion, is considered fundamental for their biological effects, the low solubility of lead chromate is thought to explain its apparently low carcinogenic potential, when compared to that of other chromates. This difference is also underlying to the different EU classifications of lead chromate on one hand, and of other hexavalent chromium compounds on the other hand.

Nevertheless, experimental studies show that lead chromate is mutagenic and clastogenic when it becomes solubilised. Also, under conditions of high occupational exposure to lead chromate the lead concentration in blood may be elevated.

Hexavalent chromium compounds, as well as lead and inorganic lead compounds, have been evaluated by SCOEL in SCOEL/SUM 86 and SCOEL/SUM 83, respectively. Due to lack of data a carcinogenic risk assessment, specifically for lead chromate, is not feasible. However, it may well be concluded that the risk for lead chromate-induced lung tumours must be distinctly lower than that calculated for other chromates in general (SCOEL/SUM 86).

The specific data available for lead chromate allow to conclude that the OEL (100 μg Pb/m^3 ambient air) and BLV (30 μg Pb/dl blood), derived for inorganic lead compounds in general, must also be observed for occupational exposures to lead chromate. Upon exposure to lead chromate only, the OEL of 100 μg Pb/m^3 would be equivalent to a concentration of 25 μg Cr/m^3.

References

Anonymous (1992) Lead chromate exposures and elevated blood lead level in workers in the plastics pigmenting industry – Texas, 1990. MMWR Morb Mortal Wkly Rep 41(17): 304-306 (May 1, 1992)

Blankenship LJ, Carlisle DL, Wise JP, Orenstein JM, Dye LE, Patierno SR (1997) Induction and apoptotic cell death by particulate lead chromate: differential effects of vitamins C and E on genotoxicity and survival. Toxicol Appl Pharmacol 146: 270-280

Davies JM (1979) Lung cancer mortality in workers of chromate pigment manufacture: an epidemiological survey. J Oil Chem Assoc 62: 157-163

Davies JM (1984a) Lung cancer mortality among workers making lead chromate and zinc chromate pigments at three English factories. Br J Ind Med 41: 158-169

Davies JM (1984b) Long term mortality study of pigment workers making lead chromate and zinc chromate at three English factories. Br J Ind Med 41: 170-178

DFG (1985) Chrom und seine Verbindungen / Bleichromat und Zinkchromat. In: Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten (D. Henschler, ed.). pp. 1-9. Verlag Chemie, Weinheim/Germany

Douglas GR, Bell RD, Grant CE, Wytsma JM, Bora KC (1980) Effect of lead chromate on chromosome aberration, sister-chromatid exchange and DNA damage in mammalian cells in vitro. Mutat Res 77: 157-163

Ferguson WS (1981) Lead chromate. Science 214: 4516

Furst A, Schlauder M, Sasmore D P (1976) Tumorigenic activity of lead chromate. Cancer Research 36: 1779-1783

IARC (1990) Chromium and chromium compounds, in: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 46: Chromium, Nickel and Welding, pp. 49-256.

Leonard SS, Vallyathan V, Castranova V, Shi X (2002) Generation of reactive oxygen species in the enzymic reduction of $PbCrO_4$ and related DNA damage. Mol Cell Biochem 234/235: 309-315

Levy LS, Martin PA, Bidstrup PL (1986) Investigation of the potential carcinogenicity of a range of chromium containing materials on rat lung. Br J Ind Med 43: 243-256

Nestmann ER, Matula TI, Douglas GR, Bora KC, Howbel DJ (1979) Detection of the mutagenic activity of lead chromate using a battery of microbial tests. Mutat Res 66: 357-365

Sidhu MK, Fernandez C, Khan MY, Kumar S (1991) Induction of morphological transformation, anchorage-dependent growth and plasminogen activators in non-

tumorigenic human osteosarcoma cells by lead chromate. Anticancer Res 11: 1045-1053

Singh J, Pritchard DE, Carlisle DL, McLean JA, Montaser A, Orenstein JM, Patierno SR (1999) Internalization of carcinogenic lead chromate particles by cultured normal human lung epithelial cells: formation of intracellular lead-inclusion bodies and induction of apoptosis. Toxicol Appl Pharmacol 161: 240-248

Watanabe M, Takayama Y, Koike M, Yamamoto M (1985) In vivo clastogenicity of lead chromate in mice. Tohoku J Exp Med 146: 373-374

Wise JP, Leonard JC, Patierno SR (1992) Clastogenicity of lead chromate particles in hamster and human cells. Mutat Res 278: 69-79

Wise JP, Orenstein JM, Patierno SR (1993) Inhibition of lead chromate clastogenesis by ascorbate: relationship to particle dissolution and uptake. Carcinogenesis 14: 429-434

Wise JP, Wise SS, Little JE (2002) The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human lung cells. Mutat Res 517: 221-219

Xu J, Wise JP, Patierno SR (1992) DNA damage induced by carcinogenic lead chromate particles in cultured mammalian cells. Mutat Res 280: 129-136

Criteria documentations used:

Cross HJ, Faux SP, Sadhra S, Sorahan T, Levy LS, Aw TC, Braithwaite R, McRoy C, Hamilton L, Calvert I (1997) Criteria Document for Hexavalent Chromium. International Chromium Development Association, Paris/France. Document No. SEG/CDO/50

DFG (1985) Chrom und seine Verbindungen / Bleichromat und Zinkchromat. In: Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten (D. Henschler, ed.). pp. 1-9. Verlag Chemie, Weinheim/Germany

DFG (1992) Chromium and its compounds. In: Occupational Toxicants, Volume 3, pp. 101-122. VCH Publishers, Weinheim/Germany

IARC (1990) Chromium and chromium compounds, in: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 46: Chromium, Nickel and Welding, pp. 49-256. International Agency for Research on Cancer, Lyon/France