Auchincloss, J. H., J. L. Abraham, et al. (1992). "Health hazard of poorly regulated exposure during manufacture of cemented tungsten carbides and cobalt." Br J Ind Med **49**(12): 832-6.

Forty two of 125 former workers in a factory in Syracuse, New York, which manufactured hard metal parts from tungsten carbide and cobalt, were studied by chest radiographs, spirometry, and plethysmographically determined lung volumes. The plant was closed in 1982 and the studies were performed in 1983-5. Recorded measurements of carbide dust concentrations were only mildly excessive by modern standards, but deceitful efforts to reduce the apparent concentration of dust were known to have occurred during an inspection by the Occupational Safety and Health Administration. Lung biopsies in four cases in the study and necropsy in one of the 83 cases not studied during life showed giant cell interstitial pneumonia and appreciable concentrations of tungsten carbide. This information indicates that exposure was substantial. Four workers had evidence of pulmonary fibrosis by chest radiographs; two of these workers had normal pulmonary function. Fourteen had abnormal pulmonary function, five of whom had a restrictive pattern, eight a pattern of air trapping, and one a combined pattern. Thus radiographic, or functional abnormalities, or both occurred in 16 of the 42 cases studied. No correlation with duration of exposure was established. Progressive clinically important disease (one fatal) has been found in four ex-workers, two in each of the restrictive and air trapping groups. These findings suggest that poorly regulated dust concentrations in a hard metals factory possibly cause pulmonary abnormalities and sometimes severe illness.

Bartl, F. and M. E. Lichtenstein (1976). "Tungsten carbide pulmonary fibrosis--a case report." Am Ind Hyg Assoc J **37**(11): 668-70.

Bentley, M. M., J. H. Williamson, et al. (1981). "The effects of molybate, tungstate and lxd on aldehyde oxidase and xanthine dehydrogenase in Drosophila melanogaster." Can J Genet Cytol **23**(4): 597-609.

The effects of dietary sodium molybdate and sodium tungstate on eye color and aldehyde oxidase and xanthine dehydrogenase activities have been determined in Drosophila melanogaster. Dietary sodium tungstate administration has been used as a screening procedure to identify two new lxd alleles. Tungstate administration results in increased frequencies of "brown-eyed" flies in lxd stocks and a coordinate decrease in AO and XDH activities in all genotypes tested. The two new lxd alleles affect AO and XDH in a qualitatively but not quantitatively similar fashion to the original lxd allele. AO and XDH activity and AO-CRM levels appear much more sensitive to mutational perturbations of this gene-enzyme than do XDH-CRM levels in the genotypes tested.

Bonde, J. P. (1990). "Semen quality and sex hormones among mild steel and stainless steel welders: a cross sectional study." Br J Ind Med **47**(8): 508-14.

Welding may be detrimental to the male reproductive system. To test this hypothesis, semen quality was examined in 35 stainless steel welders, 46 mild steel welders, and 54

non-welding metal workers and electricians. These figures represent a participation rate of 37.1% in welders and 36.7% in non-welding subjects. The mean exposure to welding fume particulates was 1.3 mg/m3 (SD 0.8) in stainless steel welders using tungsten inert gas, 3.2 mg/m3 (SD 1.0) in low exposed mild steel welders using manual metal arc or metal active gas (n = 31), and 4.7 mg/m3 (SD 2.1) in high exposed mild steel welders (n = 15). The semen quality of each participant was defined in terms of the mean values of the particular semen parameters in three semen samples delivered at monthly intervals in a period with occupational exposure in a steady state. The sperm concentration was not reduced in either mild steel or stainless steel welders. The sperm count per ejaculate, the proportion of normal sperm forms, the degree of sperm motility, and the linear penetration rate of the sperm were significantly decreased and the sperm concentration of follicle stimulating hormone (FSH) was non-significantly increased in mild steel welders. A dose response relation between exposure to welding fumes and these semen parameters (sperm count excepted) was found. Semen quality decreased and FSH concentrations increased with increasing exposure. Significant deteriorations in some semen parameters were also observed in stainless steel welders. An analysis of information from questionnaires obtained from the whole population including subjects who declined to participate indicated an underestimation of effects due to selection bias. Potential confounding was treated by restriction and statistical analysis. The results support the hypothesis that mild steel welding and to a lesser extent stainless steel welding with tungsten inert gas is associated with reduced semen quality at exposure in the range of the Danish process specific threshold limit values of welding.

Broeckaert, F., J. P. Buchet, et al. (1997). "Reduction of the ex vivo production of tumor necrosis factor alpha by alveolar phagocytes after administration of coal fly ash and copper smelter dust." J Toxicol Environ Health **51**(2): 189-202.

We investigated the effect of intratracheally instilled coal fly ash (FA) and copper smelter dust (CU) on the lung integrity and on the ex vivo release of tumor necrosis factor alpha (TNF-alpha) by alveolar phagocytes. Groups of female NMRI mice received a single intratracheal administration of different particles normalized for the arsenic content (20 micrograms/kg body weight, i.e., 600 ng arsenic/mouse) and the particle load (100 mg/kg body weight, i.e., 3 mg/mouse). Mice received tungsten carbide (WC) alone (100 mg/kg), FA alone (100 mg/kg, i.e., 20 micrograms arsenic/kg), CU mixed with WC (CU, 13.6 mg/kg, i.e., 20 micrograms arsenic/kg; WC, 86.4 mg/kg) and Ca3(AsO4)2 mixed with WC (20 micrograms arsenic/kg; WC, 100 mg/kg). Animals were sacrificed at 1, 6, or 30 d posttreatment and analyzed by bronchoalveolar lavage for total protein (TP) content, inflammatory cell number and type, and TNF-alpha production. Additional mice were studied to evaluate particle retention by measuring total arsenic retention in the lung at appropriate times. Instillation of WC induced a mild and transient (d 1) inflammatory reaction characterized by an increase of TP and an influx of polymorphonuclear leukocytes in the alveolar compartment. Compared to WC, Ca3(AsO4)2 produced a significant increase of TP content in BALF. CU particles caused a severe but transient inflammatory reaction, while a persisting alveolitis (30 d) was observed after treatment with FA. Compared to control saline, a marked inhibition of TNF-alpha release was observed in response to LPS in all groups at d 1. Cytokine production was upregulated in

WC- and Ca3(AsO4)1-treated animals after 6 and 30 d, respectively. However, a 90% inhibition of TNF-alpha production was still observed at d 30 after administration of CU and FA. Although arsenic was cleared from the lung tissue 6 d after Ca3(AsO4)2 administration, a significant fraction persisted (10-15% of the arsenic administered) in the lung of CU- and FA-treated mice at d 30. We hypothetize that suppression of TNF-alpha production is dependent upon the slow elimination of the particles and their metal content from the lung.

Bruckner, H. C. (1967). "Extrinsic asthma in a tungsten carbide worker." J Occup Med **9**(10): 518-9.

Capilna, S., L. Ababei, et al. (1963). "Effect of Molybdenum and Tungsten Ions on Intermediate Metabolism of Glutamine in Rat Liver and Brain." Nature **200**: 470.

Cardin, C. J. and J. Mason (1976). "Molybdate and tungstate transfer by rat ileum. Competitive inhibition by sulphate." Biochim Biophys Acta **455**(3): 937-46.

For both MoO42- and WO42- the maximum rate of uptake by the small intestine of the rat (studied in vitro using the everted sac technique) occurs in the lower ileum. Kinetic constants, derived by a least squares procedure, are compared with those previously obtained for SO42- transport. For both V and Ka, SO42- greater than MoO42- greater than WO42-, with only small differences between sacs IV and V. Mutual inhibition of MoO42- and WO42- transport and inhibition of both by SO42- are competitive processes. This is shown by the generally good agreement between Ka values and derived Ki values and by V values in the presence and absence of the inhibiting species. The three ions SO42-, MoO42- and WO42- are probably transferred across the intestine by a common carrier system. Implications for the sulphate-molybdenum interaction in molybdosis are discussed.

Chakraborty, D. and A. K. Das (1989). "Indirect determination of tungstate in rat tissues by atomic absorption spectrometry." Analyst **114**(1): 67-9.

An indirect method is described for the determination of tungsten as tungstate in tissue samples by atomic absorption spectrometry (AAS). Tungstate forms a stable ion-association complex [Fe(dipy)3]2+WO4(2-) (dipy = 2,2'-dipyridyl) in acidic solution, which can be extracted into chloroform with an efficiency of higher than 98%. The extract can be analysed for iron (and hence indirectly for WO4(2-] by flame AAS after stripping back into 60% perchloric acid. The calibration graph is linear up to 19 p.p.m. of WO4(2-) and the limit of detection is 0.17 p.p.m. Many foreign ions do not interfere and the method has been applied successfully to the determination of tungstate in rattus norvegicus tissue samples.

Chatterjee, G. C., R. K. Roy, et al. (1973). "Effect of chromium and tungsten on L-ascorbic acid metabolism in rats and chicks." J Nutr **103**(4): 509-14.

Coates, E. O., Jr. and J. H. Watson (1971). "Diffuse interstitial lung disease in tungsten carbide workers." Ann Intern Med **75**(5): 709-16.

Edel, J., E. Sabbioni, et al. (1990). "Trace metal lung disease: in vitro interaction of hard metals with human lung and plasma components." Sci Total Environ **95**: 107-17.

Hard metal pneumoconiosis is an occupational pulmonary disease caused by long-term exposure to dust produced in the hard metal industry. In vitro experiments have been carried out to study the solubility and metabolic behaviour in human lung tissue and plasma of hard metal alloy constituents such as cobalt, tungsten, tantalum, titanium and niobium. The experiments were carried out using 60Co, 187W, 182Ta, 44Ti and 95Nb radiotracers in combination with neutron activation, radio-release tests and gel filtration techniques. Leaching experiments from neutron-irradiated hard metal dust showed that cobalt was highly soluble, especially in the lung cytosol and plasma, in comparison with tantalum and tungsten. The gel filtration experiments showed three biochemical pools of cobalt in both lung and plasma components, in accordance with the hypothesis that cobalt represents the allergic factor in the development of hard metal disease. High affinity for proteins was observed for Nb, Ta and Ti, but not for W, in agreement with the dissimilar biological half-lives of these elements in the body. The different ability of the metals to interact with biochemical components and to be solubilized in biological media may explain the various degrees of retention in the lung, which would influence the metabolic pathways. This would explain the presence of Co, Ta and W in body fluids, as well as in the public hair and toenails of hard metal workers.

Fillat, C., J. E. Rodriguez-Gil, et al. (1992). "Molybdate and tungstate act like vanadate on glucose metabolism in isolated hepatocytes." Biochem J **282 (Pt 3)**: 659-63.

In rat hepatocytes, molybdate and tungstate inactivate glycogen synthase by a mechanism independent of Ca2+ and activate glycogen phosphorylase by a Ca(2+)-dependent mechanism. On the other hand, both molybdate and tungstate increase fructose 2,6-bisphosphate levels and counteract the decrease in this metabolite induced by glucagon. These effectors do not directly modify 6-phosphofructo-2-kinase activity, even though they partially counteract the inactivation of this enzyme induced by glucagon. These effects are related to an increase on the glycolytic flux, as indicated by the increase in L-lactate and CO2 production and the decrease in glucose 6-phosphate levels in the presence of glucose. All these effects are similar to those previously reported for vanadate, although molybdate and tungstate are less effective than vanadate. These results could indicate that molybdate, tungstate and vanadate act on glucose metabolism in isolated hepatocytes by a similar mechanism of action.

Galle, P., J. P. Berry, et al. (1992). "Role of alveolar macrophages in precipitation of mineral elements inhaled as soluble aerosols." Environ Health Perspect **97**: 145-7.

The lysosomes of several varieties of cells such as the tubular proximal cell of the kidney and the alveolar macrophage have the ability to concentrate and precipitate several elements inhaled in water-soluble form, usually as phosphate. The mechanism involved is

attributed to the high acid phosphatase activity of lysosomes and can be considered as an in vivo Gomori reaction. Among the elements studied, most of them are chemotoxic or radiotoxic (Cr; group IIIA: Al, Ga, In; rare earths: La, Ce, Tm; actinides: Th, U). In the lung macrophage, this mechanism of intralysosomal concentration and precipitation may prevent the diffusion of these toxic elements through the alveolar membrane.

Hartung, M. and H. Valentin (1983). "[Pulmonary fibrosis caused by hard-metal dusts]." Zentralbl Bakteriol Mikrobiol Hyg [B] **177**(3-4): 237-50.

Hardmetals are broadly used in many different branches. In the FRG lung fibrosis caused by hardmetal-dust are recognized as occupational diseases (no. 4107). New findings concerning pathogenesis of this disease should be considered. Not only the dust of the presintered material is hazardous but also the inhalation of the sintered material. There is a special risk for tool-grinders. Cobalt is suspected to be the causal agent. In tool-grinders an elevated external and internal Cobalt-burden has been objectified. We report about 14 persons who suffered from lung fibrosis and worked in the hard metal industry. X-ray and pathohistological examinations may give some hints but there is no specific histological correlate. So the notifying procedure may be difficult. For this reason any case of aetiologically unknown lung fibrosis the lung biopsy and quantitative chemical analysis using AAS should be performed. In one patient the Cobalt-level in lung-tissue was significantly elevated. Toleration of invasive diagnostic measurements can not be demanded. Tool-grinders need more medical surveillance in future. As a preventive strategy the cobalt-exposure is to be reduced to a minimum. The external cobalt-burden at work-place can be estimated by the determination of the renal Cobalt-excretion.

Higgins, E. S., D. A. Richert, et al. (1956). "Molybdenum deficiency and tungstate inhibition studies." J Nutr **59**(4): 539-59.

Huaux, F., G. Lasfargues, et al. (1995). "Lung toxicity of hard metal particles and production of interleukin-1, tumor necrosis factor-alpha, fibronectin, and cystatin-c by lung phagocytes." Toxicol Appl Pharmacol **132**(1): 53-62.

Hard metal alloys (WC-Co) are made of a mixture of cobalt (Co; 6%) and tungsten carbide (WC; 94%) particles. Chronic inhalation of hard metal dust can lead to the development of a fibrosing alveolitis, the pathogenesis of which is still undefined. The present investigation was undertaken to assess the effect of Co, WC, and WC-Co particles on the release by lung phagocytes of interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), fibronectin, and cystatin-c. The responses were compared with those induced by two other lung toxicants, i.e., crystalline silica (DQ12) and arsenic trioxide (As2O3). IL-1 and TNF-alpha activities produced in the presence and absence of LPS stimulation were measured with the aid of bioassays while fibronectin and cystatin-c were determined by latex immunoassays. In vitro, maximal noncytotoxic doses of As2O3, Co, WC, or WC-Co did not significantly affect the production of these mediators by rat alveolar macrophages. In contrast, DQ12 enhanced the production of TNF-alpha (with and without LPS stimulation) and IL-1 (after LPS stimulation) and decreased cystatin-c release (in the absence of LPS). Following a single intratracheal instillation of

the different test preparations in the rat, the response of the lung phagocytes obtained by bronchoalveolar lavage (BAL) 24 hr later was examined. We were unable to detect any consistent effect of Co (0.06 mg/100 g body wt), WC (1 mg/100 g body wt), or WC-Co treatment (1 mg/100 g body wt) on the production of the above mediators. In contrast, after LPS stimulation, As2O3 (0.5 mg/100 g body wt) and DQ12 (1 mg/100 g body wt) stimulated the production of TNF-alpha and IL-1. In the absence of LPS, As2O3 stimulated fibronectin and cystatin-c production and DQ12 stimulated cystatin-c release. Since the dose of WC-Co used in vivo (1 mg/100 g body wt) caused pronounced lung inflammation (increased LDH, protein, and albumin levels in BAL fluid), we conclude that the acute lung toxicity of WC-Co particles is not mediated through enhanced production of the examined mediators by lung phagocytes.

Hwang, P. L. and R. J. Ryan (1981). "Tungstate stimulates adenylate cyclase." Endocrinology **108**(2): 435-9.

Tungstate stimulates adenylate cyclase (E.C. 4.6.1.1) in ovarian homogenates of the rat; maximal stimulation (approximately 3-fold) is achieved at a concentration of 1 mM. This stimulation of cAMP production cannot be explained by the inhibition of phosphodiesterase activity or of ATP hydrolysis. Activation of adenylate cyclase by tungstate is rapid and reversible. The effects of tungstate and hCG on cyclase activity are additive, but tungstate does not augment fluoride-stimulated activity. At higher concentrations (5 and 10 mM), tungstate inhibits basal as well as hCG- and fluoride-stimulated cyclase activity in an irreversible manner. After solubilization by Lubrol-PX, the cyclase enzyme is inhibited by tungstate at concentrations ranging from 0.1-10 mM. Tungstate also activates adenylate cyclase in the brain, heart, lungs, kidneys, and liver of the rat. This suggests that tungstate activation of adenylate cyclase is a general phenomenon and may be mediated by a similar mechanism in different tissues. Tungstate provides an additional tool by which the molecular basis of adenylate cyclase activation can be probed. (Endocrinology 108: 435, 1981)

Iyengar, V. and J. Woittiez (1988). "Trace elements in human clinical specimens: evaluation of literature data to identify reference values." Clin Chem **34**(3): 474-81.

Reference values are proposed for the concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Pb, Se, and Zn in whole blood, blood serum, urine, milk, liver, and hair from adult human subjects. For F, I, and Ni, it was not possible to evaluate reference intervals for all the specimens mentioned above. For several elements, including Al, B, Br, Cs, Li, Rb, U, and V, the present status of the literature does not provide an adequate basis for formulating baseline concentrations; therefore, results from selected investigations are listed for information only. For elements such as Cu, Fe, and Zn, which are known to be homeostatically controlled, the concentrations in whole blood and blood serum follow a gaussian-like frequency distribution, and we could consider both median and mean values for evaluation. On the other hand, elements whose concentrations in tissues and body fluids are influenced by dietary availability (e.g., As and Se) or environmental factors (e.g., Cd, Hg, and Pb) show wide scatter. In these cases, the median appeared to be a

better indicator of the central tendency than the mean, when different populations are involved. These points are illustrated.

Jelmert, O., I. L. Hansteen, et al. (1995). "Cytogenetic studies of stainless steel welders using the tungsten inert gas and metal inert gas methods for welding." Mutat Res **342**(1-2): 77-85.

Cytogenetic damage was studied in lymphocytes from 23 welders using the Tungsten Inert Gas (TIG), and 21 welders using the Metal Inert Gas (MIG) and/or Metal Active Gas (MAG) methods on stainless steel (SS). A matched reference group I, and a larger reference group II of 94 subjects studied during the same time period, was established for comparison. Whole blood conventional cultures (CC), cultures in which DNA synthesis and repair were inhibited (IC), and the sister chromatid exchange (SCE) assay were applied in the study. For the CC a statistically significant decrease in chromosome breaks and cells with aberrations was found for both TIG/SS and MIG/MAG/SS welders when compared with reference group II. A non-significant decrease was found for the corresponding parameters for the two groups of welders when compared with their matched referents. A statistically significant negative association was found between measurements of total chromium (Cr) in inhaled air and SCE, and a weaker negative correlation with hexavalent Cr (Cr(VI)) in air. In conclusion, no cytogenetic damage was found in welders exposed to the TIG/SS and MIG/MAG/SS welding fumes with low content of Cr and Ni. On the contrary, a decline in the prevalence of chromosomal aberrations was indicated in the TIG/SS and MIG/MAG/SS welders, possibly related to the suggested enhancement of DNA repair capacity at slightly elevated exposures.

Jordan, C., R. D. Whitman, et al. (1990). "Memory deficits in workers suffering from hard metal disease." Toxicol Lett **54**(2-3): 241-3.

This study examined memory functioning on the Wechsler Memory Scale-Revised in a group of adult tungsten carbide workers with hard metal disease and a group of matched controls. The hard-metal-exposed group of workers showed memory deficits related to difficulties in attention and verbal memory, with an apparent sparing of visual-spatial memory. Implications of this finding for future research are discussed.

Kaye, S. V. (1968). "Distribution and retention of orally administered radiotungsten in the rat." Health Phys **15**(5): 399-417.

Kitamura, H., Y. Yoshimura, et al. (1980). "Effects of cemented tungsten carbide dust on rat lungs following intratracheal injection of saline suspension." Acta Pathol Jpn **30**(2): 241-53.

In order to examine the effect of cemented tungsten carbide dust on the animal lung, saline suspensions were intratracheally administered into the lungs of rats in a single dosage. About one-fifth of the animals died during the first three days. The acute response of the lungs was hemorrhagic edema with intense alveolar congestion. The animals killed at six months all presented pulmonary lesions of patchy fibrosis in the

vicinity of the deposited dusts, occasionally associated with focal traction emphysema and bronchobronchiolar ectasia. At twelve months, two-third of the animals had neither fibrosis nor dust deposition, although the remaining animals showed pulmonary lesions similar to those seen in the six-months responders. Fibrosis of the lungs seemed to consist of collapsed alveoli with condensation of the preexistent reticulin fibers, but without noticeable collagenization. It is supposed that both the early toxic and the late fibrogenic effects of the carbide dust are attributable to the cytotoxic action of cobalt present in the dust particles. It is possible that recovery of the pulmonary lesions results from removal of the dusts from the lesions.

Lasfargues, G., D. Lison, et al. (1992). "Comparative study of the acute lung toxicity of pure cobalt powder and cobalt-tungsten carbide mixture in rat." Toxicol Appl Pharmacol **112**(1): 41-50.

Alveolitis progressing to lung fibrosis has been reported in workers exposed to cobalt containing dust (e.g., tungsten carbide-cobalt mixture as produced by the hard metal industry) but rarely following exposure to pure cobalt dust (e.g., in cobalt-producing factories). We have previously demonstrated that tungsten carbide-cobalt mixture is more toxic toward rat alveolar macrophages in vitro than pure cobalt metal powder. The present study was undertaken to compare in female rats the acute pulmonary response (lung weight, lung histology, cellular and biochemical analyses of bronchoalveolar lavage fluid, and mortality) following the intratracheal instillation of pure cobalt (Co) particles (median particle size, d50:4 microns), pure tungsten carbide (WC) particles (d50:2 microns), tungsten carbide-cobalt (WC-Co) powder (d50:2 microns; cobalt 6.3%, tungsten 84%, carbon 5.4%) and crystalline silica (d50 less than 5 micron) used as pneumotoxic reference material. WC alone (15.67 mg/100 g body wt) behaves as an inert dust producing only a mild accumulation of macrophages in the alveolar duct walls. Co alone (1.0 mg/100 g) only causes a moderate inflammatory response. An identical amount of Co given as WC-Co mixture (16.67 mg/100 g; corresponding to 1.0 mg Co/100 g) produces a severe alveolitis and fatal pulmonary edema. Cellular and biochemical characteristics of bronchoalveolar lavage fluid collected 24 hr after the intratracheal instillation of WC (1.0 mg/100 g) or Co (0.06 mg/100 g) are not significantly different from those of control animals instilled with sterile saline. On the contrary, bronchoalveolar lavage fluid changes following administration of the WC-Co mixture (1.0 mg/100 g; corresponding to 0.06 mg Co/100 g) are very similar to those induced by crystalline silica (1.0 mg/100 g). The amount of cobalt excreted in urine is significantly higher when the animals are exposed to WC-Co powder as compared to an equivalent amount of pure cobalt particles, suggesting an increased bioavailability of cobalt metal when combined with tungsten carbide. This study demonstrates that the acute lung toxicity of tungsten carbide-cobalt mixture is much higher than that of each individual component and may explain why lung fibrosis is rarely if ever induced by exposure to pure cobalt dust.

Lison, D. (1996). "Human toxicity of cobalt-containing dust and experimental studies on the mechanism of interstitial lung disease (hard metal disease)." Crit Rev Toxicol **26**(6): 585-616.

In the industry, the potential for exposure to cobalt metal dust is particularly important during the production of cobalt powder and the processing and use of hard metals and other cobalt-containing alloys. The different adverse health effects reported in these workers are reviewed. One of the main target organs is the respiratory tract, and this article concentrates on the lung parenchymal reactions induced by cobalt-containing dust. Clinical and epidemiological data indicate that this manifestation is rarely, if ever, induced by pure cobalt metal dust alone, but requires the concomitant inhalation of other compounds such as tungsten carbide in the hard metal industry (hard metal disease). Experimental studies demonstrate that cobalt metal and metallic carbides interact to produce an elective lung toxicity. Recent work on the mechanism of this interaction, which is based on the production of activated oxygen species, is reviewed. A practical implication in industrial hygiene should be that permissible exposure levels to Co dust might have to be different when exposure is to pure Co particles or an association with carbides.

Lison, D., J. P. Buchet, et al. (1997). "Toxicity of tungsten." Lancet **349**(9044): 58-9.

Lison, D. and R. Lauwerys (1991). "Biological responses of isolated macrophages to cobalt metal and tungsten carbide-cobalt powders." Pharmacol Toxicol **69**(4): 282-5.

A previous study from this laboratory, using morphological and biochemical (LDH release) parameters, has shown that tungsten carbide-cobalt dust exhibits a greater cytotoxicity toward isolated macrophages than cobalt metal powder alone. The present study extends this comparison by examining additional biological parameters. Glucose uptake and superoxide anion production by isolated macrophages were significantly more depressed by the tungsten carbide-cobalt mixture (WC-Co) than by cobalt alone (Co) while pure tungsten carbide (WC) had no effect or even stimulated the cells. For glucose-6-phosphate dehydrogenase and cell-associated plasminogen activator (PA) activities, no difference between Co and WC-Co dusts was observed. These observations add further evidence to our previous findings regarding the different biological reactivity of cobalt metal alone or mixed with tungsten carbide.

Lison, D. and R. Lauwerys (1992). "Study of the mechanism responsible for the elective toxicity of tungsten carbide-cobalt powder toward macrophages." Toxicol Lett **60**(2): 203-10.

We have previously demonstrated that tungsten carbide-cobalt powder (WC-Co) is more toxic toward murine macrophages in vitro than pure cobalt metal particles and that the cellular uptake of cobalt is enhanced when the metal is present in the form of WC-Co mixture. The present study was undertaken to assess the possible mechanism(s) of this interaction. We found that solubilization of cobalt in the extracellular milieu was increased in the presence of WC. This phenomenon, however, is not the critical factor explaining the greater toxicity of the WC-Co mixture since increasing the amount of solubilized cobalt in the extracellular medium in the absence of WC did not result in increased toxicity. Moreover, the amount of cobalt solubilized from a toxic dose of WC-Co was insufficient to affect by itself macrophage viability. A toxic effect was only

observed when the WC-Co mixture came directly in contact with the cells. The elective toxicity of WC-Co can also not be explained by stimulation of phagocytosis of cobalt metal particles due to the simultaneous presence of other particles (WC) in the extracellular fluid since stimulation of phagocytosis by latex beads or zymosan particles did not amplify the toxicity of cobalt metal particles. These results indicate that the toxicity of the WC-Co mixture does not simply result from an enhanced bioavailability of its cobalt component. This suggests that hard metal dust behaves as a specific toxic entity.

Lison, D. and R. Lauwerys (1993). "Evaluation of the role of reactive oxygen species in the interactive toxicity of carbide-cobalt mixtures on macrophages in culture." Arch Toxicol **67**(5): 347-51.

The lung toxicity of a carbide-cobalt mixture is more important than that of each individual component; the mechanism of this interaction is not understood. The capacity of cobalt metal particles alone and mixed with different carbides to generate hydroxyl radicals was examined with the deoxyribose assay. In a chemical system, cobalt ions and cobalt metal particles (Co) were found to catalyse the degradation of deoxyribose in the presence of hydrogen peroxide. Carbides were able to directly oxidize deoxyribose, but their respective activities did not support such a mechanism to explain the carbide-cobalt interactive toxicity, since there was no direct relationship between deoxyribose degradation ability and cytotoxicity toward macrophages. Tungsten, niobium, titanium and chromium carbides (interactive carbides) were only weak oxidants and conversely molybdenum, vanadium and silicon carbides (non-interactive carbides) were the most potent ones. The ability of cobalt metal to produce hydroxyl radicals in the presence of hydrogen peroxide was not increased by tungsten carbide. The role of reactive radical formation in the toxicity of these particles was further assessed in a macrophage culture model. Catalase (4000 U/ml), superoxide dismutase (300 U/ml), sodium azide (1 mM), sodium benzoate, mannitol, taurine and methionine (all 20 mM) were all unable to protect against the cytotoxic effects of cobalt ions and cobalt metal alone or mixed with tungsten carbide. In conclusion, no evidence was found that production of reactive oxygen species contributes to the elective toxicity of carbide-cobalt mixtures.

Lunde, P. K. (1978). "[Essential drugs for basic health needs]." Lakartidningen **75**(5): 295-6.

Miller, C. W., M. W. Davis, et al. (1953). "Pneumoconiosis in the tungsten-carbide tool industry; report of three cases." A M A Arch Ind Hyg Occup Med **8**(5): 453-65.

Murakami, N., S. P. Healy, et al. (1982). "Interaction of rat liver glucocorticoid receptor with sodium tungstate." Biochem J **204**(3): 777-86.

Effects of sodium tungstate on various properties of rat liver glucocorticoid receptor were examined at pH7 and pH 8. At pH 7, [3H]triamcinolone acetonide binding in rat liver cytosol preparations was completely blocked in the presence of 10--20 mM-sodium tungstate at 4 degrees C, whereas at 37 degrees C a 30 min incubation of cytosol receptor

preparation with 1 mM-sodium tungstate reduced the loss of unoccupied receptor by 50%. At pH 8.0, tungstate presence during the 37 degrees C incubation maintained the steroid-binding capacity of unoccupied glucocorticoid receptor at control (4 degrees C) levels. In addition, heat-activation of cytosolic glucocorticoid-receptor complex was blocked by 1 mM- and 10 mM-sodium tungstate at pH 7 and pH 8 respectively. The DNA-cellulose binding by activated receptor was also inhibited completely and irreversibly by 5 mM-tungstate at pH 7, whereas at pH 8 no significant effect was observed with up to 20 mM-tungstate. The entire DNA-cellulose-bound glucocorticoid-receptor complex from control samples could be extracted by incubation with 1 mM- and 20 mM-tungstate at pH 7 and pH 8 respectively, and appeared to sediment as a 4.3--4.6 S molecule, both in 0.01 M- and 0.3 M-KCl-containing sucrose gradients. Tungstate effects are, therefore, pH-dependent and appear to involve an interaction with both the non-activated and the activated forms of the glucocorticoid receptor.

Murakami, N., T. M. Quattrociocchi, et al. (1982). "Effects of sodium tungstate on the nuclear uptake of glucocorticoid-receptor complex from rat liver." Arch Biochem Biophys **214**(1): 326-34.

Peao, M. N., A. P. Aguas, et al. (1993). "Inflammatory response of the lung to tungsten particles: an experimental study in mice submitted to intratracheal instillation of a calcium tungstate powder." Lung **171**(4): 187-201.

Tungsten has been implicated as a cause of a severe form of pneumoconiosis in humans, the so-called "hard metal" lung disease. We have investigated the effect of intratracheal instillation of a powder of calcium tungstate on the pulmonary tissue of CD-1 mice. The tungsten-induced alterations were studied using 3 microanatomical methods: cytologic study of exudates obtained by bronchoalveolar lavage (BAL); histologic examination of paraffin-embedded sections of the lung; and scanning electron microscopic (SEM) examination of lung samples using x-ray microanalysis to detect tungsten in situ. The animals were sacrificed 1, 3, 7, 14 and 21 days after a single intratracheal instillation of 250 micrograms calcium tungstate particles suspended in 100 microliters of saline. We found that the metal particles induced a marked inflammatory response in the bronchoalveolar space characterized by a biphasic attraction of leukocytes with cellular peaks observed at day 1 and 14. More than 50% of the BAL macrophages showed ingested tungsten. In the lung parenchyma, the inflammatory infiltrates were predominantly located at the periphery of the bronchiolar walls. From 7 days on after the tungsten deposition, large inflammatory exudates were seen invading focal areas of the alveolar domain of the lung. SEM views revealed that the tungsten particles could be inside alveolar macrophages, in cells making up the alveolar wall, or inside periacinar lymphatics. Our data document that tungsten particles cause a marked inflammatory response in the lung tissue and that the leukocyte exudates may invade alveolar areas of the lung.(ABSTRACT TRUNCATED AT 250 WORDS)

Peao, M. N., A. P. Aguas, et al. (1993). "Morphological evidence for migration of particle-laden macrophages through the interalveolar pores of Kohn in the murine lung." Acta Anat (Basel) **147**(4): 227-32.

We have investigated the topography of particle-laden macrophages in the pulmonary tissue of CD-1 mice after intratracheal instillation of a suspension of 250 micrograms of calcium tungstate. The mice were sacrificed 1, 3, 7 and 14 days after the particle deposition. Lung fragments were studied by scanning electron microscopy (SEM) coupled with X-ray microanalysis that allowed in situ elemental identification of tungsten in the lungs. Tungsten-positive macrophages were distinctly located in the lungs of mice sacrificed at 1-3 days when compared with samples from mice killed 7-14 days after the calcium tungstate instillation. At 1-3 days, the tungsten-carrying macrophages were accumulated near the terminal bronchioles whereas they were seen predominantly in the alveolar ducts and sacs in the 7- to 14-day groups of mice. This suggests that during pulmonary inflammation there is a redistribution of the particle-containing macrophages throughout the deep lung tissue. In high-magnification SEM views, we observed that the tungsten-positive macrophages presented numerous surface microvilli. Tungsten-laden phagocytes were detected in interalveolar fenestrae, at the so-called Kohn pores. This finding documents that the Kohn pores may be used by inflammatory cells as a pathway for the migration of phagocytes in between adjacent alveolar sacs.

Peao, M. N., A. P. Aguas, et al. (1992). "Cellular kinetics of inflammation in the pleural space of mice in response to the injection of exogenous particles." Exp Lung Res **18**(6): 863-76.

CD-1 mice were used to study the cellular kinetics of the inflammatory response of the pleural space to the injection of 250 micrograms of silica or of tungsten microparticles. The pleural exudates were collected by lavage of the serous cavity of mice that were sacrificed at 30 min and up to 7 days after the intrapleural instillation of the particles. The samples were studied by light and electron microscopy (transmission and scanning modes); the quantitative cellular kinetics of the inflammation was determined by leukocyte counting in exudates using cytocentrifuge preparations. The normal resident population of cells of CD-1 mice was made up of (2.47 +/- 0.37) x 10(6) cells. It consisted mostly of macrophage-like cells ((2.03 +/- 0.26) x 10(6) cells, 82% of total cells), some lymphocytes ((0.37 +/- 0.07) x 10(6) cells, 15% of total cells), a few mast cells and eosinophilic granulocytes (1-2% of total cells). The initial inflammatory reaction (30-60 min after injection) was characterized by a decrease in the number of cells harvested from the pleural space. This was followed by an intense recruitment of granulocytes and monocytes that resulted in a peak of intrapleural cells at 24 h ((16.8 +/- 4.0) x 10(6) cells induced by silica particles and (18.3 +/- 4.2) x 10(6) cells induced by tungsten particles). In tungsten-injected mice (but not in silica-treated animals) the enhancement in the number of intrapleural macrophages continued up to 72 h after particle injection. The highest percentage of macrophages with ingested tungsten (50% of total macrophages) was found early (6 h) and decreased thereafter; at day 7 it encompassed just 17% of the macrophages. Injection of any of the two particulates led to the disappearance of mast cells from the pleural space of mice. Silica particles attracted a high number of eosinophils to the pleural cavity of mice. Light and electron microscopy documented that pleural macrophages underwent striking morphological changes during the inflammatory response: the phagocytes showed marked increase in size and in number of surface processes, and their cytoplasm often contained large amounts of the

injected particles and also of cellular debris. This study establishes the mouse as a reliable animal model to study the dynamics of the pleural space and it offers a precise definition of the cellular kinetics of inflammation in this serous cavity. The data indicate that the kinetics of experimental pleural inflammation induced by particulates may depend on the nature of the injected particles.

Pereira Ade, S., N. R. Grande, et al. (1992). "Evidence of drainage of tungsten particles introduced in the pleural space through the visceral pleura into the lung parenchyma." Acta Anat (Basel) **145**(4): 416-9.

Studies of pleural clearance of calcium tungstate particles were made in the dog. By using scanning electron microscopy and elemental microanalysis, we show that mesothelial cells of the visceral leaflet of the pleura are also involved in the clearance of particles present in the pleural space. The histological study of lung parenchyma shows many macrophages loaded with tungsten particles, and we conclude that this way may be an important pathway for the transmission of pathologic processes from the pleural space to the lung.

Pham Huu, C. (1965). "The Comparative Toxicity of Sodium Chromate, Molybdate, Tungstate and Metavanadate. I. Experiments on Mice and Rats." Arch Int Pharmacodyn Ther **154**: 243-9.

Ratto, D., J. Balmes, et al. (1988). "Pregnancy in a woman with severe pulmonary fibrosis secondary to hard metal disease." Chest **93**(3): 663-5.

The effect of interstitial pulmonary fibrosis on pregnancy is unclear. We present the findings in a 31-year-old woman with severe pulmonary fibrosis (vital capacity, 37 percent of predicted) secondary to hard metal disease who went through a successful term pregnancy. The patient was a grinder of tungsten-carbide drill bits who developed pneumonitis and subsequent fibrosis. Her therapy required steroids and cyclophosphamide for stabilization of her pulmonary function prior to her pregnancy. At six months' gestation, right heart catheterization showed normal cardiac output and pulmonary arterial and wedge pressures. Stage 2 exercise study demonstrated a maximum oxygen consumption of 1.17 L/min (53 percent of predicted). The patient was able to exercise to a maximum workload of 300 kpm/min (32 percent of predicted). She became hypoxemic (arterial oxygen pressure, 54 mm Hg) at 150 kpm/min. Her pregnancy concluded with an uncomplicated normal vaginal delivery requiring only supplemental oxygen and spinal anesthesia. Review of the few similar cases suggests that a woman can have a successful pregnancy despite severe pulmonary dysfunction.

Rolfe, M. W., R. Paine, et al. (1992). "Hard metal pneumoconiosis and the association of tumor necrosis factor-alpha." Am Rev Respir Dis **146**(6): 1600-2.

Hard metal pneumoconiosis is a recently recognized occupational lung disease associated with the exposure to cobalt fumes in the workplace. Chronic exposure in susceptible individuals results in interstitial lung disease histopathologically manifested as interstitial

fibrosis with an associated mononuclear cell infiltrate and the presence of "cannibalistic" multinucleated giant cells in the alveolar airspaces. The majority of patients present with symptoms of chronic cough and dyspnea. Interestingly, in addition, patients uniformly report significant weight loss out of proportion to their degree of respiratory impairment. In this case report we demonstrate the association of tumor necrosis factor-alpha (TNF) and hard metal (cobalt) pneumoconiosis and suggest that TNF may have a potential role in the etiology of the constitutional symptoms and the pathogenesis of interstitial lung disease.

Schepers, G. W. (1955). "The biological action of tungsten carbide and cobalt; studies on experimental pulmonary histopathology." AMA Arch Ind Health **12**(2): 140-6.

Schroeder, H. A. and M. Mitchener (1975). "Life-term studies in rats: effects of aluminum, barium, beryllium, and tungsten." J Nutr **105**(4): 421-7.

Sivjakov, K. I. and H. A. Braun (1959). "The treatment of acute selenium, cadmium, and tungsten intoxication in rats with calcium disodium ethylenediaminetetraacetate." Toxicol Appl Pharmacol **1**: 602-8.

Sprince, N. L., R. I. Chamberlin, et al. (1984). "Respiratory disease in tungsten carbide production workers." Chest **86**(4): 549-57.

We carried out a medical and environmental survey to evaluate respiratory disease at two tungsten carbide (TC) production plants. The study population of 290 subjects (19.2 percent of the total work force) was chosen to focus on those with the greatest potential exposures to cobalt, a binding agent which is probably the cause of interstitial fibrosis and airways disease in TC workers. We found peak air concentrations of cobalt exceeding 500 micrograms/m3 during many major steps in TC production. Nine subjects at plant A and two at plant B had interstitial infiltrates. Two of these nine from plant A had restriction (total lung capacity less than 80 percent of predicted). A lung biopsy specimen in one showed interstitial fibrosis. Two nonsmokers at plant A and one nonsmoker at plant B had obstructive defects. These results suggest that interstitial and obstructive lung disease occur in TC workers in association with elevated peak air concentrations of cobalt.

Sprince, N. L., L. C. Oliver, et al. (1988). "Cobalt exposure and lung disease in tungsten carbide production. A cross-sectional study of current workers." Am Rev Respir Dis **138**(5): 1220-6.

A cross-sectional study of 1,039 tungsten carbide (TC) production workers was carried out. The purposes were (1) to evaluate the prevalence of interstitial lung disease (ILD) and work-related wheezing, (2) to assess correlations between cobalt exposure and pulmonary disease, (3) to compare lung disease in grinders of hard carbide versus nongrinders, and (4) to evaluate the effects of new and previous threshold limit values for cobalt of 50 and 100 micrograms/m3. We obtained medical and occupational histories, flow-volume loops, single breath carbon monoxide diffusing capacity (DLCO), and chest

radiographs. Time-weighted average cobalt levels were determined at every step in the production process. Work-related wheeze occurred in 113 participants (10.9%). Profusion greater than or equal to 1/0 occurred in 26 (2.6%) and interstitial lung disease (defined as profusion greater than or equal to 1M, FVC or DLCO less than or equal to 70%, and FEV1/FVC% greater than or equal to 75) in 7 (0.7%). The relative odds of work-related wheeze was 2.1 times for present cobalt exposures exceeding 50 micrograms/m3 compared with exposures less than or equal to 50 micrograms/m3. The relative odds of profusion greater than or equal to 1/0 was 5.1 times for average lifetime cobalt exposures exceeding 100 micrograms/m3 compared with exposures less than or equal to 100 micrograms/m3 in those with latency exceeding 10 yr. ILD was found in three workers with very low average lifetime exposures (less than 8 micrograms/m3) and shorter latencies. Grinders of hard carbide had lower mean DLCO than nongrinders, even though their cobalt exposures were lower.(ABSTRACT TRUNCATED AT 250 WORDS)

Stettler, L. E., D. H. Groth, et al. (1983). "Automated characterization of particles extracted from human lungs: three cases of tungsten carbide exposure." Scan Electron Microsc(Pt 1): 439-48.

An automated scanning electron microscope-energy dispersive x-ray analysis-image analysis system was used to characterize particles extracted from three human lung samples which had suspected occupationally-induced lung disease. The particles were isolated from the lung tissues by low temperature ashing and deposited on Nuclepore filters. Particles in randomly selected fields of view for each filter were automatically sized, analyzed for 32 elements, and classified according to their chemistry by the system. For each of the three lung specimens, large numbers of particles were found which indicated exposure to cemented tungsten carbide products. The particle analysis data was collected at a rate of 200 particles per hour which is considerably faster than the rate at which manual, in situ analyses can be performed.

Tajima, Y., Z. Nagasawa, et al. (1993). "A factor found in aged tungstate solution enhanced the antibacterial effect of beta-lactams on methicillin-resistant Staphylococcus aureus." Microbiol Immunol **37**(9): 695-703.

We have found a factor (Factor T) in aged mixtures of tungstate and phosphate which greatly enhances the antibacterial effects of beta-lactams on both inducible and constitutive methicillin-resistant Staphylococcus aureus, but not on methicillin-susceptible S. aureus. Factor T alone did not strongly inhibit bacterial growth. There was no synergism of Factor T with other classes of antibiotics, nor with other groups of bacteria, and it reduced the efficacy of amino-glycosides and tetracycline. Upon preparation of Factor T, acidifying and heating the mixture of tungstate and phosphate resulted in a high yield and reproducibility, and no enhancing effect was observed when other anions such as sulfate or molybdate were used instead. Factor T is heat- and acid-stable but labile to alkalization, and is probably a complex of phosphate and tungstate.

Terada, L. S., I. R. Willingham, et al. (1992). "Tungsten treatment prevents tumor necrosis factor-induced injury of brain endothelial cells." Inflammation **16**(1): 13-9.

Exposure to recombinant human tumor necrosis factor-alpha (TNF-alpha) or calcium ionophore (A23187) for 4 h increased (P less than 0.05) lactate dehydrogenase (LDH) release from cultured bovine brain endothelial cells (EC). In contrast, treatment with endotoxin or interleukin-1 did not increase (P greater than 0.05). LDH release from brain EC. Pretreatment with tungsten decreased (P less than 0.05) xanthine oxidase activity in brain EC and decreased (P less than 0.05) LDH release from brain EC following exposure to TNF. Our results suggest that TNF-alpha injures brain microvascular EC and that this effect may be mediated by xanthine oxidase.

Van Goethem, F., D. Lison, et al. (1997). "Comparative evaluation of the in vitro micronucleus test and the alkaline single cell gel electrophoresis assay for the detection of DNA damaging agents: genotoxic effects of cobalt powder, tungsten carbide and cobalt-tungsten carbide." Mutat Res **392**(1-2): 31-43.

Although it is well known that micronuclei may arise from either DNA breakage leading to acentric chromosome fragments or from chromosome/chromatid lagging in anaphase, the ratio between the amount of DNA breakage induced and the frequency of micronuclei expressed in the following interphase is unclear. With the development of the alkaline single cell gel electrophoresis assay, which measures single strand and/or double strand breaks in a cell by cell approach, it is new possible to address this question at the cellular level. We therefore compared the genotoxic potential of pure cobalt powder (Co) and a cobalt-containing alloy, cobalt-tungsten carbide (WC-Co), involved in specific lung disorders, in parallel with the alkaline single cell gel electrophoresis (SCGE) assay (comet assay) and the cytokinesis-blocked micronucleus (MN) test, both carried out in vitro on isolated human leukocytes. The comet assay indicated that the WC-Co mixture produced a higher level of DNA damage than Co alone; WC alone was not able to induce a dose-dependent DNA breakage effect as was seen for Co and WC-Co. Results from the MN test confirmed these observations. It was clear that the clastogenic property of Co-containing dust is significantly enhanced when the Co metal is mixed with WC and suggested that their physicochemical characteristics may act as one of the important parameters responsible for the increased incidence of lung cancers observed in the population of hard metal workers. In agreement with data obtained in the same laboratory on liposoluble chemicals (PCBs and chlorinated aliphatic hydrocarbons) and from the literature, the results indicate that both the comet assay and the micronucleus test were able to detect differences in the genotoxic potential of the compounds studied. Although the micronucleus test seemed to be less sensitive to assess a synergistic DNA damaging potential of the mixture involved, it detects chromosomal aberrations (chromosome/genome mutations) and not just repairable DNA breakage or alkali-labile sites. Combination of the comet assay and the in vitro MN test might therefore be recommended for genotoxins to understand the mechanisms underlying mutagenicity and to assess the lowest efficient dose.

Wase, A. W. (1956). "Absorption and distribution of radio-tungstate in bone and soft tissues." Arch Biochem Biophys **61**(2): 272-7.

Wei, H. J., X. M. Luo, et al. (1985). "Effects of molybdenum and tungsten on mammary carcinogenesis in SD rats." J Natl Cancer Inst **74**(2): 469-73.

Virgin female rats of the SD strain were fed ad libitum a nutritionally adequate semipurified diet and demineralized water (groups 1 and 2), or the same diet with 10 ppm molybdenum (group 3) or 150 ppm tungsten (group 4) added to the drinking water. The animals in groups 2-4 received a single iv injection of 5 mg N-nitroso-N-methylurea (NMU; CAS: 684-93-5)/100 g body weight at 50 days of age. One hundred and twenty-five days after the NMU treatment, group 2 exhibited a 50.0% incidence of mammary carcinoma. Group 4 exhibited a significant increase in carcinoma incidence (79.2%) and the value for group 3 (45.5%) was not significantly different from that of group 2. The carcinoma incidence of group 3 (50.0%) was significantly lower than that of group 2 (90.5%) or group 4 (95.7%) 198 days after NMU treatment.

Wesselius, L. J., I. M. Smirnov, et al. (1996). "Alveolar macrophages accumulate iron and ferritin after in vivo exposure to iron or tungsten dusts." J Lab Clin Med **127**(4): 401-9.

Extracellular iron present in alveolar structures may contribute to oxidative lung injury induced by toxic mineral dusts by enhancing dust-induced generation of hydroxyl radicals. Alveolar macrophages (AMs) can sequester iron within ferritin and limit generation of hydroxyl radicals. In the current study we sought to assess whether AMs accumulate iron and ferritin after in vivo exposure to a dust with high iron content, to iron oxide, or to an inflammatory dust, calcium tungstate. We performed lung lavage 1, 7, 14, 28, 42, and 56 days after intratracheal instillation of mineral dust in saline solution or instillation of saline solution alone and quantitated cell recovery and AM content of iron and ferritin. Instillation of iron oxide increased neutrophil recovery only on a day 1 when compared with results in controls, whereas calcium tungstate increased neutrophil recovery through day 14. AMs recovered after instillation of iron oxide contained increased amounts of iron and ferritin, beginning on day 1 and progressing through day 56 after treatment (7.57 +/- 0.38 microgram iron per 10(6) AMs vs 1.54 +/- 0.28 microgram iron per 10(6) AMs for controls, $p < 0.001$; and 5908 +/- 768 ng ferritin per 10(6) AMs vs 395 +/- 20 ng ferritin per 10(6) AMs, $p < 0.001$). AMs recovered after calcium tungstate instillation also contained increased amounts of iron and ferritin beginning 14 days after treatment, with greatest content 42 days after treatment (4.85 +/- 0.68 microgram iron per 10(6) AMs, $p < 0.001$, and 2274 +/- 736 ng ferritin per 10(6) AMs, $p < 0.001$). Tumor necrosis factor, which can enhance iron accumulation by macrophages, was spontaneously released by AMs recovered from tungsten-treated rats. These studies indicate that AMs accumulate iron and ferritin in response to both iron loading of the lungs with iron oxide exposure and lung inflammation induced by calcium tungstate exposure.

Wide, M. (1984). "Effect of short-term exposure to five industrial metals on the embryonic and fetal development of the mouse." Environ Res **33**(1): 47-53.

An increase is expected in the world's industrial use of several metals, Al, Co, Mo, V, and W, among others. Very little is known about their possible effects on early mammalian development. Groups of mice were injected with compounds of these metals either before implantation or at early organogenesis. None of the metal compounds showed any interference with implantation, but all of them significantly affected fetal development: Al caused an increased frequency of fetal internal hemorrhage, Mo inhibited fetal normal weight gain, W increased the frequency of resorptions, and Al, Co, Mo, and V all interfered with fetal skeletal ossification.

Wide, M., B. R. Danielsson, et al. (1986). "Distribution of tungstate in pregnant mice and effects on embryonic cells in vitro." Environ Res **40**(2): 487-98.

Whole-body autoradiography and impulse counting experiments were used to study the distribution and retention of radioactivity in the pregnant mouse after administration of [185W]tungstate. A rapid uptake was found in a number of tissues--skeleton, red pulp of the spleen, adrenal, liver, thyroid, pituitary, and ovary--and in the intestine and kidneys, through which it was rapidly excreted. 185W was also readily transported from mother to fetus, although more in late than in early gestation. The largest metal retention was found in the maternal skeleton, kidneys, and spleen and in the visceral yolk sac epithelium and the skeleton of the fetus. Furthermore, in vitro cytotoxicity experiments showed inhibition by tungstate of cartilage production in limb bud mesenchymal cultures at concentrations similar to those found in vivo.

Wide, M., B. R. Danielsson, et al. (1986). "Distribution of tungstate in pregnant mice and effects on embryonic cells in vitro." Environ Res **40**(2): 487-98.

Whole-body autoradiography and impulse counting experiments were used to study the distribution and retention of radioactivity in the pregnant mouse after administration of [185W]tungstate. A rapid uptake was found in a number of tissues--skeleton, red pulp of the spleen, adrenal, liver, thyroid, pituitary, and ovary--and in the intestine and kidneys, through which it was rapidly excreted. 185W was also readily transported from mother to fetus, although more in late than in early gestation. The largest metal retention was found in the maternal skeleton, kidneys, and spleen and in the visceral yolk sac epithelium and the skeleton of the fetus. Furthermore, in vitro cytotoxicity experiments showed inhibition by tungstate of cartilage production in limb bud mesenchymal cultures at concentrations similar to those found in vivo.