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MUT/06/2

COMMITTEE ON MUTAGENICITY OF CHEMCIALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

BIOLOGICAL EFFECTS OF WEAR DEBRIS GENERATED FROM METAL ON METAL BEARING SURFACES: EVIDENCE FOR GENOTOXICITY.

Introduction

1. The MHRA- Biosciences and Implants unit have forwarded a request for advice from the COM on the evidence for genotoxicity arising from biomonitoring studies of individuals who have had metal-on-metal athroplasty. The background information to the request can be found in a paper presented to the Committee on Safety of Devices (CSD). In brief In 2004, about 50,000 hip replacement procedures were recorded in the National Joint Registry. Metal-on metal hip procedures can involve total hip replacement or hip resurfacing.

2. Particulate debris can be generated from articulating surfaces, metal-on-metal couples and from any modular or fixation interface as a result of corrosion, abrasion and differential micromovement. There are reports available regarding patients with particulate metal debris in the local periprosthetic tissue and in distant organs such as spleen, liver and lymph glands. Nickel, cobalt and other metal ions are released through these articulations and are subsequently found at an increased level in patient's blood, urine, hair and regional lymph glands. Larger metallic particles are associated with a foreign body giant cell reaction and smaller particles accumulate in cells and may cause histopathological damage locally in the periprosthetic tissue and systemically.

3. The generation of wear debris and the reported biological effects are dependent on various factors such as

- types of metal used in the alloy of the prosthesis
- nature of the break-down products
- size and number of the particles generated
- the amount of metal debris in particulate form
- the amount dissolved in tissue fluids- ionic form
- prior exposure to metal components
- how long the implant are *in situ*
- age and activity level of patients etc.

Advice requested from COM.

4. There are three papers cited in the CSD paper which refer to evidence for genotoxicity in patients who have undergone MoM arthroplasty (appended as Annex 1). The MoM replacements refer to the more recent 2nd generation MoM hip replacements, of which there are two basic Co/Cr alloy types (wrought/cast with high and low carbon levels, Case et al J of Arthroplasty, 26, (2), 174-188, 2005). The COM is asked to provide advice on the

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interpretation of these studies. The relevant studies are reviewed below. In assessing the studies members may wish to be aware that a typical alloy used for cobalt chromium prostheses would contain 63% cobalt, 26%-30% chromium, 5%-7% molybdenum, 1% nickel, 1%manganese, 1% silicon and small amounts of iron and carbon. A typical stainless steel alloy contains 17% chromium, 12% nickel, 2.5% molybdenum, 2% manganese, 1% silicone and small amounts of sulphur and carbon. It is noteworthy that SS prostheses do not contain cobalt. The structure of prostheses presumably vary between manufacturers even for products of similar chemical composition. Data summarised below and other published studies show elevated blood/serum levels of chromium three years after surgery. All papers summarised below are appended in full (Annex 2).

Overview of studies cited in CSD paper (Appended as Annex 2)

Studies in patients

Doherty AT et al, The Journal of Bone and Joint Surgery (Br), 83-B, 1075-1081, 2001.

5. Chromosome translocations and aneuploidy in peripheral blood lymphocytes were compared between a group of revision arthroplasty patients (n=31, mean age=71±13.4y, average implantation time 11.5 years, range= 3-21 y) and controls undergoing total hip athroplasty (n=-30, mean age =63.9±12.7 y). All patients had osteoarthritis except two at primary arthroplasty. All took non steroidal anti inflammatory medicines (NSAIDs). 11 patients had cobalt-chromium (Co-Cr) prostheses, 13 had titanium-aluminium-vanadium- (TiAlV), six had stainless steel, and one a hybrid titanium- Co/Cr prosthesis. [In a subsequent paper (see para 12 below) it was reported that all these patients had metal-on-polyethylene prostheses]. Data on diagnostic radiography (in past 8 years), drugs taken, smoking and type of prosthesis were obtained. Blood was taken (forearm) and 10 ml frozen at -80°C for Cr, Co and Ni analysis using high resolution inductively-coupled mass spectrometry (ICPMS, levels below detection limit 0.2ng/ml for all three metals were recorded at the detection limit). Another 10 ml was used for DNA isolation (stored at -70°C), and a further 10 ml sample was collected in a lithium-heparin tube for metaphase analysis. Cultures (37°C) were set up within 24 h. metaphase preparation (replicate cultures) were initiated with PHA and colchicines added at 72 h. Simultaneous fluorescence *insitu* hybridisation (FISH) analysis (chromosomes 1,2,3) was undertaken using commercial chromosome paints (as described in Ellard *et al* Mutagenesis, 11, 537-46, 1995) and 300 metaphases per patient were recorded on duplicate slides. For detection of non-disjunction, cytocholasin B was added to cultures at 44 h and cells harvested at 72h. FISH for chromosomes 1 and 2 was undertaken using commercial centromere specific paints (method Doherty *et al*, Mutation Research, 372, 221-31, 1996).

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6. Rate ratios for chromosome aberrations were calculated using log-linear mean rates. Three categories of prosthesis were examined (Ti alloy, CoCr, and Stainless steel (SS)). Unadjusted and adjusted (for gender, smoking, radiography, age) analyses were performed. Adjusted data were estimated relative to a baseline of a 65 y female, non-smoker with 10 radiographs in previous 8 years. Levels of Co/Cr were compared between different prostheses.

7. The mean incidence of aneuploidy and translocations in peripheral blood lymphocytes in revision arthroplasty patients was increased by three fold and two-fold respectively. The initial analyses suggested there was an increase in aneuploidy in patients with TiAlV prostheses (but no significant increase in translocations) and an increase in translocations in patients with Co-Cr prostheses (but without an increase in aneuploidy). There was no evidence for an increases in either endpoint in patients with stainless steel prostheses.

8. A log-linear analysis revealed minor (non-significant) effects for age (aneuploidy decrease, translocations increase), radiography and smoking (both endpoints increase). Adjusted analyses reported a statistically significant five-fold increase in aneuploidy in patients with Ti (without any increase in translocations). In contrast adjusted analyses reported a 2.5 fold increase in aneuploidy and a 3.5 fold increase in translocations in patients with Co-Cr prostheses. No increase in either end point was reported for stainless steel.

9. The authors conclude that the findings could not be accounted for by confounding, that the specificity of finding (Ti alloy compared to Co-Cr) was unexpected, and that the results could not be proven to be associated with effects of metals.

10. The authors did report that 5/10 individuals with high translocations had elevated blood levels of Co-Cr, and that 6/8 individuals with elevated Co-Cr levels also had elevated translocations. In contrast only one individual with a high aneuploidy index had an elevated blood level of Co-Cr.

11. What are members' views of this study? Are the findings biologically significant?

Ladon D et al, The Journal of Arthroplasty, 19, 8 suppl 3, 78-83, 2004

12. 95 patients with metal on metal total hip arthroplasty (Metasul®; head and articulation (Co-Cr high carbon), acetabular cup (large cup shaped cavity on the lateral surface of the oscoxae in which the head of the femur articulates); polyethylene; stem Protasul S30 (stainless steel)) were recruited. Patients with existing prostheses, previous radiotherapy, or chemotherapy were excluded. Blood

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samples (10 ml) were obtained at prior to operation (95) and at 6 months (80), 1 year (89), and 2 years (54) post operation. Another 5 ml sample was taken at each time point for trace metal analysis. Cultures were set up within 24 h of collection. Whole blood cultures (37°C) were initiated with PHA, colchicine was added at 72h. Methods were reported to be those used in the previous study (see para 5, details of cytocholasin B addition not given in this report). FISH analyses were undertaken using aged cells (ethanol 94°C for 20 seconds, Henegaritu et al, Cytometry, 43, 101, 2001). Simultaneous evaluation for aneuploidy/translocations using chromosome 1,2,3 were undertaken. There were two scorers for cytogenetics, and one for anueploidy. Metals were analysed by ICPMS.

13. A log linear Poisson regression model was used to compare pre and post operative aberration data. In subsequent analyses patients with a tendency to have higher metal levels and high cytogenetics/aneuploidy scores than patients with lower metal levels was assessed.

14. Post operative blood levels of Co-Cr were elevated at 2 years. The highest level of Cr was at 2 years and the highest level of Co was at 1 year. A much smaller but statistically significant increase in Molybdenum was reported at the time points used. There was a statistically significant increase in translocations and aneuploidy at all time points after operation. This was evident if the data from both scorers were combined and if the data from the single scorer of both translocations and aneuploidy (both chromosome gain and loss) were analysed separately. The increase in aneuploidy was much greater than that of chromosome translocation and both were progressive over time. Patients with higher molybdenum concentrations had higher translocation indices. There were no statistically significant correlations between chromosome translocations and chromium concentrations. There were no correlations between metal concentrations and aneuploidy.

15. The authors reported that Co-Cr levels increased by approximately 6-7 fold and the incidence of translocations by 1.5 fold and aneuploidy by 2-4 fold. The authors considered that the data did not reflect an effect of age, smoking or previous X-ray exposure. It was suggested that the data were consistent with a biological effect of metals. It was reported that there were some in-vitro studies to support this conclusion (see paras 17-24 below). The apparent discrepancy between soluble metal levels in blood and cytogenetic/aneuploidy in peripheral blood lymphocytes was discussed. Overall it was considered that the measurements reported might not reflect the exposure of bone-marrow cells to particulate metals. The authors considered there was a need for epidemiological studies in which direct comparisons can be made between patients with metal-on-metal and metal-on-polystyrene

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prostheses as well as between patients with implants of different metal alloy composition and at long postoperative survivals.

16. What are members views of this study? Does the data support a biological effect of prosthesis-derived metals?

In-vitro studies of synovial fluid taken at revision arthroplasty

Davies AP et al, The Journal of Bone and Joint Surgery (Br), 87-B, 1439-1444, 2005.

17. This study examined the proposal that there would be metal-specific DNA damage following incubation of synovial fluid from patients undergoing revision arthroplasty. It was considered appropriate to use the Comet assay to measure DNA strand breaks, cross links and alkali labile sites. 24 patients were included in the study at revision surgery. The joint was aspirated before opening using a plastic cannula. Samples were collected by the same procedure. Patients had been treated for osteoarthritis. Patients with inflammatory arthroplasty were excluded. (Age range of patients 45-80 y). None of the patients had impingement of the prosthesis. All prostheses had been introduced with cement. Tabulated information regarding groups studied is given below;

Group	Time since initial surgery	Reason for surgery
Group 1; six types of Co-Cr MoM hip replacements at revision. All cemented at femoral side, uncemented at acetabular side	Three at 2 y, two at 5y, one at 6 year after implantation	Asceptic loosening of acetabular side.
Group 2; six Co-Cr-on polyethylene knee replacements at revision. Five total knee replacements and one unicompartmental prosthesis.	6y,6y,7y,8y,8y,9y	Asceptic loosening of one or more components.
Group 3; SS –on-polyethylene total hip replacements.	2y,10y,12y,21y,22y,26y	At 2y instability. For others asceptic loosening.
Group 4; six control synovial fluid samples..	N/A	Patients with osteoarthritis at time of primary joint replacement

18. Primary human fibroblasts (2 x10^5) were placed in wells in complete medium and 250 µml of test fluid added (HBSS used for control). Cultures were incubated at 37°C for 48h and then incubated with trypsin. Cells were isolated washed (4°C) and resuspended in low melting agar, pipetted onto slides and the slides refrigerated for 30

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mins. A further layer of agarose gel was added and the slides immersed in lysis fluid in darkness overnight at 4°C. The slides were washed in electrophoresis buffer and electrophoresis was carried at 40 V and 300 mA for 30 minutes. Slides were washed and stained with ethidium bromide, stored at 4°C and scored within 72 h. Olive tail moment was determined (tail length x percent fluorescence in the tail, Olive PL, Radiation Research, 122, 86-94, 1990). Trace metal levels in synovial fluid were determined by Electrothermal atomic absorption spectroscopy (detection limit 0.5 ppb.). A model corrosion of P21 Co-Cr alloy was undertaken by incubating pellets in buffered salt solution at 37°C. Artificial synovial-type fluids were made using Cr (VI), Cr (III) and Co(II) in buffered salt solution. Each sample analysed provided comet data on 150 cells. The ratio of arithmetic mean of comet tail moment for each sample was compared between tests and controls. The authors report adjusting analyses for multiple comparisons. Data are presented graphically as median, interquartile range and full range of results for each test.

19. All six samples from Co-Cr MoM hip revisions induced a statistically significant increase in DNA damage. Four/six samples from Co-Cr-on-polyethylene knee joints induced statistically significant DNA damage. None of the samples from SS-on-polyethylene prostheses induced statistically significant DNA damage. All samples from osteoarthritic joints caused a low level but statistically significant increase in DNA damage. The level of Cr in synovial fluid from MoM hips at revision was between 0.95-6.88 µM and Co varied from 0.92-2.64 µM. In the group with Co/Cr-on-polyethylene implants concentrations of chromium varied between 0.07-2.06 µM and those of Co between 0.01-0.62 µM. In the SS implant group, Cr levels were reported to vary between 0.07-2.76 µM whilst Co were below the detection limit in four cases and 0.05 µM in the two other patients. Low but measurable concentrations of Cr were documented in the osteoarthritic group whereas the level of Co was below the limit of detection in all individuals in the osteoarthritic group. .

20. The solution of corroded Co-Cr alloy contained 0.27 µM Cr and 0.84 µM of Co. It produced statistically significant levels of DNA damage. Spiked fluid containing Co alone (at 0.84 µM) produced a lower level of DNA damage. Similarly experiments with Cr(VI) or Cr (III) (at 0.27 µM) induced a lower level of DNA damage than Co/Cr alloy alone. A comet test using a test solution containing both 0.27 µM Cr and 0.84 µM Co demonstrated higher levels of DNA damage comparable to that seen with Cr-Co alloy.

21. The authors consider that the lower levels of DNA damage seen in the studies using artificial synovial fluid reflect the simple nature of the model used (lacking inflammatory factors or proteins).The authors argue the data are consistent with an interaction between

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Co and Cr and this would explain why no DNA damage is seen in studies using SS implants. This argument is supported by reference to a study of the interaction between Co and particulate metals (which is summarised below).

Supporting in-vitro study

De Boeck M et al, Mutagenesis, 18, 177-186, 2003

22. The authors investigated the DNA damage response and CBMN response in isolated peripheral blood lymphocytes using particulate Co (SA 0.448 m^2/g, median size 4μm) and Cr_3C_2 (SA 0.983 m^2/g, median size 1.2μm) and combinations of these two particulates. (The reference is appended as Annex 3). Briefly blood was obtained from two donors (one male, one female aged <28y). 10 μl of particulate suspension was added to isolated lymphocyte cultures for a period of 15 minutes, 24 h after PHA treatment. Cells were immediately isolated after treatment for the Comet assay (De Boeck, Carcinogeneisis, 19, 2021-2029, 1998). 50 comets/duplicate culture were randomly assessed using tail length and tail DNA (total) as criteria. Cell were further incubated for MN assay and cytocholasin B added at 44 h (6.0μg/ml). Cells were harvested at 72h. 1000 CB binucleated lymphocytes were evaluated.

23. The authors noted considerable inter-donor variance in the results of both Comet and MN assays. There was no effect of Cr_3C_2 in the Comet assay. No statistically significant effect was documented with Co in the Comet assay (but a wide inter-trial and inter-donor variance was reported). A slight increase in DNA migration was documented in trials using co-exposure to Cr_3C_2 and Co (ca 1.33 fold, not statistically significant). In the CB MN assay, no significant dose-dependent increase in MN frequency was reported for Cr_3C_2. A significant dose-related effect was reported for Co. It was reported that the mixture of Cr_3C_2 and Co resulted in a non significant increase in MN frequency of 1.61 fold compared to Co. Evidence for an interaction was also documented with other carbides (tungsten and niobium). More clearly defined evidence for an interaction was reported when the doses of carbides were documented in terms of surface area. The interpretation of these data are complicated by the variance between donors and provide limited support for an interactive effect of Co and Cr.

24. What are members views on the evidence that the genotoxic effects reported in individuals who have had MoM implants may result from release of both Cr and Co and that an interaction between these two metals may be important?

COM discussion

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25. The Committee has been asked to provide advice in respect of the evidence for genotoxicity associated with metal on metal hip replacements (MoM). In this respect some of the data presented for metal-on-polyethlene hip replacements (e.g TiAlV) are less relevant. The data has to be assessed in the context of the composition of MoM hip replacement devices, the evidence for leakage of potentially DNA damaging metals, and the form and kinetics of release of metals (particulate v solution of ions). The cross sectional study of Doherty et al (2001) provided evidence for translocations and aneuploidy in peripheral blood lymphocytes of patients undergoing revision arthroplasty. There was limited evidence to associate high Cr-Co blood with translocations. The authors cited a previous preliminary study where there was evidence for a higher incidence in chromosome aberrations in bone marrow samples adjacent to the prosthesis (i.e. the femur) compared to iliac crest marrow from the same patients (Case CP *et al, Clin Orthop Relat Res*, 329 (suppl), S269-279, 1996, it is noted that it is unclear whether these patients had MoM or metal-on-polyethylene hip replacements*). A copy of this paper is appended in Annex 4. (A further preliminary report (Jenkin L et al, *J of Bone and Joint Surgery*, 81B (suppl III), 319, 1999) documented a higher incidence of 14:18 translocations in peripheral blood lymphocytes in patients undergoing revision hip arthroplasty. The authors do not appear to have published a full account of this study).

Ring -CoCr *Charnley-SS ,Howse - titanium , Harris-Galantem - CoCr , McKee-Farrar - CoCr , Exeter –SS, Stanmore -CoCr , Ring -CoCr

26. The prospective study undertaken by Landon et al (2004) provides evidence of chromosome translocations and aneuploidy in lymphocytes from whole blood cultures from patients undergoing primary hip replacement using MoM devices. The authors report on blood levels of Cr-Co and chromosome damage over a period of up to 2 years post surgery. There is clear evidence of increased DNA damage in patients following surgery but the authors couldn't correlate this to whole blood Cr-Co levels. An argument is presented that a clear correlation might not be expected as the key exposure index would be levels of particulate metals in the bone marrow.

27. The in-vitro studies of synovial fluid from patients with revision hip operations who had Cr-Co MoM prostheses provides confirmatory evidence of DNA damage using the Comet assay in fibroblasts. It is noteworthy that no such DNA damage could be identified from patients with SS implants, although these would also presumably release chromium. (This is consistent with the data from patients with SS-on-polyethylene implants where there is no evidence of DNA damage). Davies and colleagues present an argument that a key interaction between Cr and Co is required to produce the DNA damaging effect in-vitro. A number of studies using artificial synovial fluid and spiked samples is presented to support the argument and

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reference is made to work of De Boeck *et al* (2003) on mixtures of Co and heavy metal carbides (including Cr_3C_2).

28. The available information suggests that metal-on-metal hip replacement results in elevated blood levels of Co and Cr ions.(Milosev et al *J Orthop Res*, 2005, 23, 526-535, 2005) Post-mortem histological evaluations have shown that widespread metal debris in individuals with SS and Co-Cr implants which could be detected even in when there was no apparent wear of the replacement hip. Metal debris was detected in both local and distant lymph nodes, bone marrow, liver and spleen. (Case CP et al *J of Bone and Joint Surgery*, 76-B, 701-712, 1994.) In a further post-mortem histological evaluation study metallic wear particles were more prevalent in patients who a failed hip arthroplasty compared with patients with a primary hip or knee replacement. (Urban RM et al, *J of Bone and Joint Surgery*, 82-A, 457-477, 2000).

29. Case and colleagues have published a recent review of the biological effects of hip replacement. (The J of Arthroplasty, 26, (2), 174-188, 2005). They report that although the rate of wear of MoM bearings is low, considerable amounts of metal generation may occur. Most studies report ion level of metals in blood or serum. (No data on speciation is given.) There is a wide inter-individual variation in levels of metals. In some instances blood levels of Co-Cr have exceeded occupational biological limits (e.g. those set in Germany and Italy). The average rate of wear for a Metasul 2nd generation hip replacement is approximately 5µm/year.

30. Thus there is evidence for the dispersion of both soluble and particulate metallic material from hip replacements. Members will be aware that a number of the metals considered in the submitted studies have been evaluated by IARC for potential carcinogenicity. Data on mutagenicity, if available, has been considered during the preparation of IARC monographs and is appended for members information. (www.iarc.fr) A brief tabulation of the IARC evaluations is given below for ease of reference.

Compound/Metal	**Evaluation**
Co metal	Sufficient evidence for carcinogenicity in animals, *in-vitro* mutagen (see De Boeck) Group 2B* (Evidence from additional paper De Boeck M et al, Mutation Research, 533, 135-152, 2003, reports metallic Co to be *in-vivo* mutagen and suggests the IARC classification is revised.)
Co salts	Sufficient evidence for carcinogenicity in animals, *in-vitro* mutagen. Group 2B*(Evidence from additional paper De Boeck M et al, Mutation Research, 533, 135-152, 2003, reports metal ions of Co to be *in-vivo* mutagen and suggests the IARC classification is revised.)
Cr metal	Inadequate data for carcinogenicity in animals, no relevant study of mutagenicity, Group 3
Cr (VI)	Sufficient evidence in humans and animals, in-vivo mutagen, group 1
Cr (III)	Inadequate data for carcinogenicity in animals, in-

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	vitro mutagen, group 3
Ni metal	Inadequate data for carcinogenicity in humans, limited data for carcinogenicity in animals, not *in-vitro* mutagen, evidence of cell transformation *in-vitro* reported. Group 2B
Ni salts	Sufficient evidence for carcinogenicity in humans and animals, in-vivo mutagen (*in-vitro* positive, *in-vivo* DNA damage). Group 1

*PAPER APPENDED AS ANNEX 5.

31. Members are asked to consider the available information and the following questions. A short statement will be prepared for MHRA.

i) Is there convincing evidence that MoM hip replacements can result in increased genotoxicity in patients?

ii) Can any conclusions be made with regard to the chemical(s) responsible, in part, of fully for the observed responses?

iii) Is there convincing evidence that an interaction between Cr and Co may be important in the observed mutagenic responses?

Secretariat January 2006

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APPENDED INFORMATION FROM IARC EVALUATIONS ON MUTAGENICITY OF CERTAIN METALS

Cobalt in Hard-metals and Cobalt Sulfate, Gallium Arsenide, Indium Phosphide and Vanadium Pentoxide (Volume 86, 7–14 October 2003) (In preparation)

A working group of 17 experts from 10 countries met in Lyon to evaluate the evidence of carcinogenicity of metallic cobalt particles with or without tungsten carbide, to which workers in the hard-metal industry are exposed, and of cobalt sulfate and other soluble cobalt (II) salts. It also evaluated three other particulate compounds: gallium arsenide and indium phosphide, which are used extensively in the microelectronics industry; and vanadium pentoxide, a contaminant at facilities refining and processing vanadium-rich ores and in various workplaces that use oil-fired boilers and furnaces.

Cobalt and cobalt compounds were previously evaluated in Volume 52 of the IARC *Monographs* (1991) as *possibly carcinogenic to humans* (Group 2B), with *inadequate evidence* of carcinogenicity in humans, and, in particular, *sufficient evidence* for the carcinogenicity of cobalt metal powder in experimental animal on the basis of an increased incidence of sarcomas at injection sites, though no inhalation studies of the metal were reported. Since that evaluation, an animal carcinogenesis bioassay of inhaled cobalt sulfate heptahydrate reported an increase in bronchiolo-alveolar neoplasms in exposed male and female mice and rats. In addition, several epidemiological studies addressed cancer risks among workers exposed to dusts containing cobalt with or without tungsten carbide in hard metal production facilities. Those conducted in France provided evidence of an increased lung cancer risk related to exposure to hard-metal dust containing cobalt and tungsten carbide, taking into account potential confounding by smoking and other occupational carcinogens. Hence, cobalt metal with tungsten carbide was evaluated as *probably carcinogenic to humans* (Group 2A) on the basis of *limited evidence* in humans for increased risk of lung cancer and *sufficient evidence* in experimental animals for the carcinogenicity of cobalt sulfate and of cobalt metal powder. The evidence of carcinogenicity for exposure to cobalt in the absence of tungsten carbide was considered *inadequate*, so that with *sufficient evidence* in experimental animals for the carcinogenicity of cobalt sulfate and of cobalt metal powder, the overall evaluation of cobalt metal without tungsten carbide was *possibly carcinogenic to humans* (Group 2B). Cobalt sulfate and other soluble cobalt (II) salts were evaluated as *possibly carcinogenic to humans* (Group 2B).

For mutagenicity data see De Boeck et al Mutagenesis, 18, 1777-186, 2003, appended as Annex 2.

--Extract from IARC monograph on Chromium compounds, re mutagenicity

5.4 Other relevant data

Inhaled chromium[VI] from welding and chrome-plating aerosols is readily absorbed from the respiratory tract. The degree of absorption depends on the extent of reduction of the hexavalent form to chromium[III], which is absorbed to a much lesser extent. The same factors apply to absorption from the gastrointestinal tract, although absorption by this route is generally much less than that from the respiratory tract.

Chromium[VI] compounds may cause adverse effects to the skin, the respiratory tract and, to a lesser degree, the kidneys in humans, while chromium[III] is less toxic.

Elevated levels of sister chromatid exchange were observed in workers exposed to chromium[VI] compounds in electroplating factories in four out of six studies. Chromosomal aberrations were found in all three studies of exposed workers; an increase frequency of aneuploidy was reported in one study. The two available studies on chromium[III] were inadequate to evaluate its cytogenetic effect in humans.

Chromates enter cells more readily than chromium[III] compounds and are reduced ultimately to chromium[III]. The reduction process and the subsequent intracellular activity of reduced chromium species are important for the mechanism of toxicity and carcinogenicity of chromium[VI]. Particulate chromium[III] compounds can also enter cells by phagocytosis.

Chromium[VI] compounds cross the placental barrier in greater amounts than chromium[III] compounds. Chromium trioxide increased fetal death rate, caused growth retardation and increased the frequency of

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skeletal deformities and of cleft palate in rodents. Developmental effects have also been reported in mice exposed to chromic chloride.

Chromium[VI] compounds of various solubilities in water were consistently active in numerous studies covering a wide range of tests for genetic and related effects. In particular, potassium dichromate, sodium dichromate, ammonium dichromate, potassium chromate, sodium chromate, ammonium chromate, chromium trioxide, calcium chromate, strontium chromate and zinc yellow induced a variety of effects (including DNA damage, gene mutation, sister chromatid exchange, chromosomal aberrations, cell transformation and dominant lethal mutation) in a number of targets, including animal cells *in vivo* and animal and human cells *in vitro*. Potassium chromate induced aneuploidy in insects, while chromium trioxide did not; various compounds induced gene mutation in insects. Potassium dichromate produced recombination, gene mutation and aneuploidy in fungi. All of these chromium[VI] compounds induced DNA damage and gene mutation in bacteria. Similar patterns were observed with zinc chromate, barium chromate, lead chromate and the derived pigments chromium orange, chromium yellow and molybdenum orange, which, however, often required preliminary dissolution in alkali or acids. A liquid chromium[VI] compound (chromyl chloride) and its vapours induced gene mutation in bacteria.

Although chromium[III] compounds were generally even more reactive than chromium[VI] compounds with purified DNA and isolated nuclei, 12 compounds of various solubilities (chromic chloride, chromic acetate, chromic nitrate, chromic sulfate, chromic potassium sulfate, chromium alum, neochromium, chromic hydroxide, chromic phosphate, chromic oxide, chromite ore and cupric chromite) gave positive results in only a minority of studies using cellular test systems, often under particular treatment conditions or at very high concentrations, which were generally orders of magnitude higher than those needed to obtain the same effects with chromium[VI] compounds. Some of the positive results could be ascribed to contamination with traces of chromium[VI] compounds. In particular, no DNA damage was observed in cells of animals treated *in vivo* with chromic chloride, and no micronuclei were seen in cells of animals given chromic nitrate. The chromium[III] compounds tested generally did not produce DNA damage, gene mutation, sister chromatid exchange or cell transformation in cultured animal and human cells. Chromosomal aberrations were often observed with high concentrations of chromium[III] compounds. Weak effects on gene mutation and mitotic gene conversion were observed in fungi. Negative results were obtained in the large majority of tests for DNA damage and gene mutation in bacteria. Certain complexes of chromium[III] with organic ligands, which favour the penetration of chromium[III] into cells, were reported to induce DNA damage and gene mutation in bacteria and in cultured mammalian cells.

A chromium[II] compound (chromous chloride) gave negative results in in-vitro tests with animal cells (DNA damage, chromosomal aberrations and aneuploidy). A water-insoluble chromium[0] compound (chromium carbonyl) did not induce DNA damage in bacteria.

No relevant study on the genetic and related effects of metallic chromium was available to the Working Group

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Extract from IARC monograph on Nickel compounds, mutagenicity

5.4 Other relevant data

Nickel and nickel compounds are absorbed from the respiratory tract, and to a smaller extent from the gastrointestinal tract, depending on dissolution and cellular uptake. Absorbed nickel is excreted predominantly in the urine. Nickel tends to persist in the lungs of humans and of experimental animals, and increased concentrations are seen notably in workers after inhalation of nickel. The nasal mucosa may retain nickel for many years.

Nickel carbonyl is the most acutely toxic nickel compound and causes severe damage to the respiratory system in experimental animals and in humans. Nickel causes contact dermatitis in humans. In experimental animals, adverse effects have also been documented in the respiratory system and in the kidney.

In four studies, the frequency of sister chromatid exchange did not appear to be increased in peripheral blood lymphocytes of nickel workers exposed during various processes. Enhanced frequencies of chromosomal gaps and/or anomalies were observed in single studies in peripheral blood lymphocytes of employees engaged in: (i) crushing, roasting and smelting (exposure mainly to nickel oxide and nickel subsulfide); (ii) electrolysis (exposure mainly to nickel chloride and nickel sulfate); and (iii) electroplating

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(exposure to nickel and chromium compounds). Enhanced frequencies were also seen in lymphocytes from retired workers who had previously been exposed in crushing, roasting and smelting and/or electrolysis.

Some nickel compounds have adverse effects on reproduction and prenatal development in rodents. Decreased fertility, reduction in the number of pups per litter and birth weight per pup, and a pattern of anomalies, including eye malformations, cystic lungs, hydronephrosis, cleft palate and skeletal deformities, have been demonstrated.

In one study, metallic nickel did not induce chromosomal aberrations in cultured human cells, but it transformed animal cells *in vitro*. Nickel oxides induced anchorage-independent growth in human cells *in vitro* and transformed cultured rodent cells; they did not induce chromosomal aberrations in cultured human cells in one study.

Crystalline nickel subsulfide induced anchorage-independent growth and increased the frequency of sister chromatid exchange but did not cause gene mutation in human cells *in vitro*. Crystalline nickel sulfide and subsulfide induced cell transformation, gene mutation and DNA damage in cultured mammalian cells; the sulfide also induced chromosomal aberrations and sister chromatid exchange. Amorphous nickel sulfide did not transform or produce DNA damage in cultured mammalian cells. In one study, crystalline nickel sulfide and crystalline nickel subsulfide produced DNA damage in *Paramoecium*.

Nickel chloride and nickel nitrate were inactive in assays *in vivo* for induction of dominant lethal mutation and micronuclei, and nickel sulfate did not induce chromosomal aberrations in bone-marrow cells; however, nickel chloride induced chromosomal aberrations in Chinese hamster and mouse bone-marrow cells.

Soluble nickel compounds were generally active in the assays of human and animal cells *in vitro* in which they were tested.

Nickel sulfate and nickel acetate induced anchorage-independent growth in human cells *in vitro*. Nickel sulfate increased the frequency of chromosomal aberrations in human cells, and nickel sulfate and nickel chloride increased the frequency of sister chromatid exchange. Nickel sulfate did not induce single-strand DNA breaks in humans cells. Nickel sulfate and nickel chloride transformed cultured mammalian cells. Chromosomal aberrations were induced in mammalian cells by nickel chloride, nickel sulfate and nickel acetate, and sister chromatid exchange was induced by nickel chloride and nickel sulfate. Nickel chloride and nickel sulfate also induced gene mutation, and nickel chloride caused DNA damage in mammalian cells. In one study, nickel sulfate inhibited intercellular communication in cultured mammalian cells.

Nickel sulfate induced aneuploidy and gene mutation in a single study in *Drosophila*; nickel chloride and nickel nitrate did not. Nickel chloride induced gene mutation and recombination in yeast.

In single studies, nickel acetate produced DNA damage in bacteria, while nickel nitrate did not; the results obtained with nickel chloride were inconclusive. In bacteria, neither nickel acetate, sulfate, chloride nor nitrate induced gene mutation.

Nickel carbonate induced DNA damage in rat kidney *in vivo*. Crystalline nickel subselenide transformed cultured mammalian cells, and nickel potassium cyanide increased the frequency of chromosomal aberrations. Nickelocene did not induce bacterial gene mutation. DNA damage was induced in calf thymus nucleohistone by nickel[III]-tetraglycine complexes.