Supplemental Material

Particle preparation and characterization

WC and WC-Co particles used in the biological tests represent pure tungsten carbide powder (6.9 m^2/g after milling, see Supplemental Material, Table 1 for levels of impurities) or a mixture of the tungsten carbide powder and a cobalt powder (6.6 m^2/g after milling).The tungsten carbide powder was produced via a chemical route resulting in a crystallite size of about 100 nm. The powder was then de-agglomerated in a ball-mill for 144 h either alone or in the presence of a 10% weight (wt) content of the cobalt powder, for the preparation of the WC-Co particle mixture. The ball-mill vessel was lined with tungsten carbide hard-metal and WC-Co hard metal balls were used. Contamination due to the milling procedure is caused by debris of WC and Co from the mill and the impurities contained in these debris. They mainly result in an increase of the Co content which corresponds to the weight loss of the balls. The weight loss of the balls amounted to less than 1 wt.-% of the powder milled, i. e., the cobalt content of the milled powder is increased by about 0.05 wt.-%. Scanning electron microscopy images of the resulting WC and WC-Co particles are provided in Supplemental Material, Figure 1. Images suggested a particle size distribution of approximately 40-300 nm. A similar size range was calculated, based on volume-weighted particle size distribution, from dynamic light scattering data (Supplemental Material, Figure 2).

Table 1. Analysis of metal impurities in the WC powder used to prepare the nanoparticles for this study (mass spectrometry, all values in 10^{-4} wt.-%)

Element	B	Na	Al	Si	K	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Sr	Y	Mo	Ba
10^{-4} wt.-%	2,1	3,5	3,7	9,1	5,3	14,0	10,0	0,6	6,2	0,4	14,0	1,0	2,2	2,6	1,7	1,7	9,9	6,0

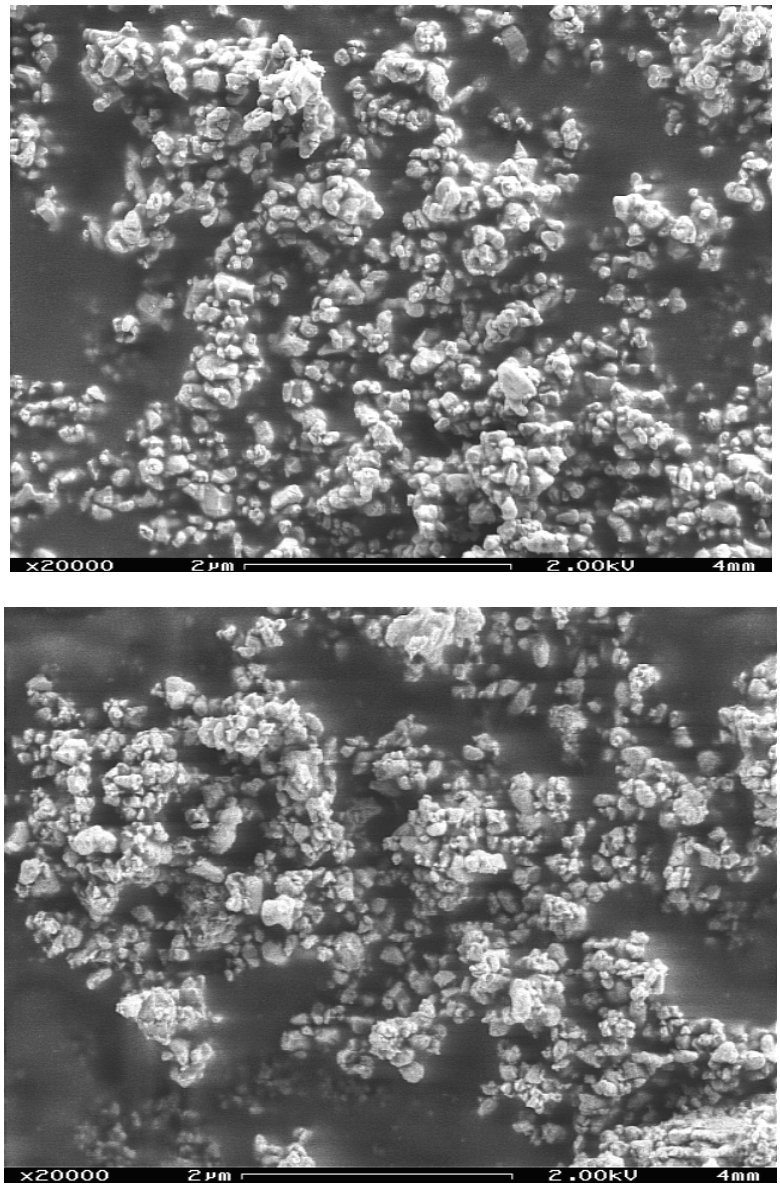

Figure 1. Scanning electron micrograph of WC nanoparticles (top image) and WC-Co nanoparticles with 10 wt.% cobalt (bottom image) after preparation with a ball-mill and drying of the particles on the SEM-slide.

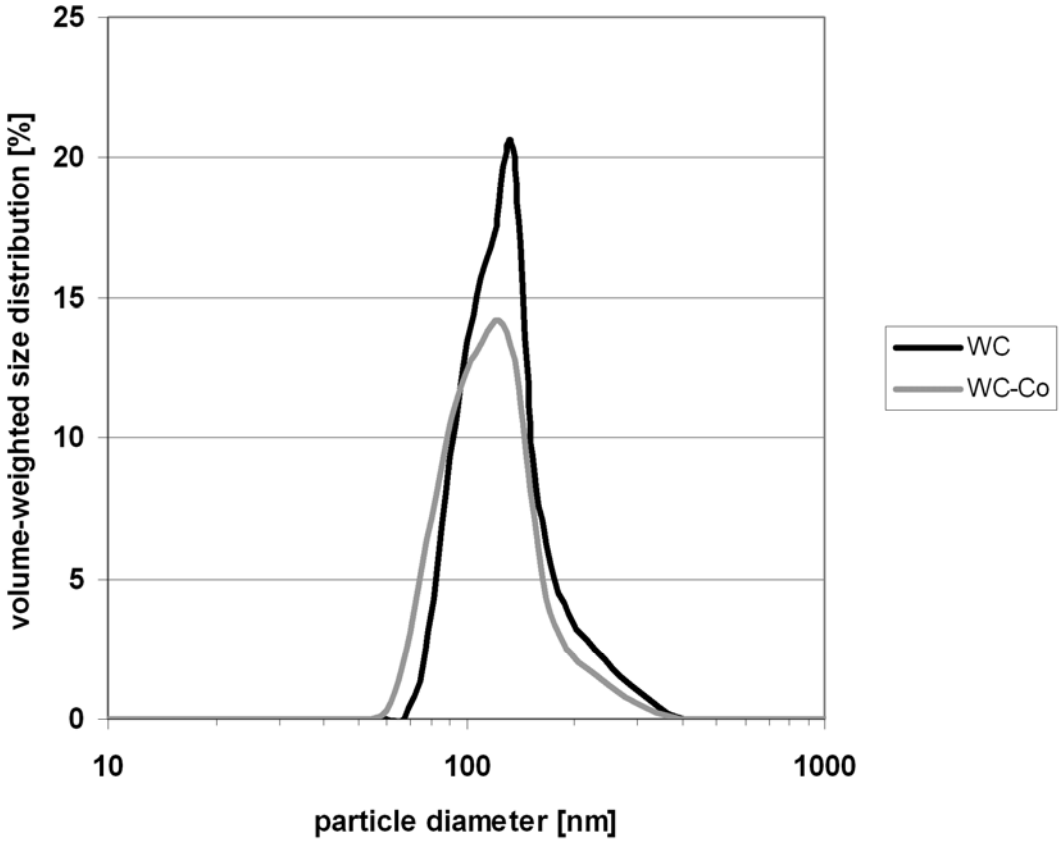

Figure 2. Volume-weighted particle size distribution calculated from dynamic light scattering (DLS) data. Based on volume-weighted particle size distribution, a particle size range of 50 to 300 nm was calculated.

Cell culture and assessment of cell viability

Cell culture

Cell lines - Human colon adenocarcinoma cells, CaCo-2 (HTB-37; American Type Culture Collection, Rockville, MD), were maintained in DMEM (Invitrogen) supplemented with 10% (v/v) FBS (Sigma, Chemicals, St. Louis, MO, USA) and 1% (v/v) penicillin/streptomycin (Sigma), in 75 cm^2 flasks (TPP, Trasadingen, Switzerland). Human keratinocyte cells, HaCaT (CLS - Cell Lines Service, Eppelheim, Germany) (Boukamp et al., 1988), and human lung carcinoma cells, A549 (CCL-185; American Type Culture Collection, Rockville, MD), were maintained in RPMI medium (Biochrom) supplemented with 5% (v/v) FBS and 1% (v/v) penicillin/streptomycin in 75 cm^2 flasks. The oligodendroglial cell line, OLN-93, originates from spontaneously transformed cells from rat brain. The cell line was kindly provided by the Department of Neonatology, Charité, Berlin, Germany. OLN-93 cells bear the morphological and antigenic properties of 5- to 10-day-old (postnatal time) cultured rat brain oligodendroglial cells (OLs). These resemble the intermediate stage between the pre-OL (O4_O1_MBP_) and the mature OL (O4_O1_MBP_). Cells were maintained in DMEM, containing 10% (v/v) FBS.

All cell lines were cultured in monolayers at 37°C in a humidified, 5% (v/v) CO_2 atmosphere with medium replenishment every 2 to 3 days (OLN-93) or subcultivation twice a week (all other cell lines). For sub-cultivation, cells were washed three times with Versene (Invitrogen) and detached by Trypsin (0.25% (v/v) in phosphate-buffered saline (PBS) (Biowest, Germany).

Primary neuronal and astroglial cell cultures - Neuronal cell cultures were prepared from cortices of 18-day-old Wistar rat foetuses according to Fedoroff & Richardson (1997). The tissue was pooled into ice-cold glucose (33 mM) HBSS (PAA Laboratories), cut into small pieces and incubated for 30 min at 37^{o}C with 0.25% (v/v) trypsin-EDTA solution (Sigma). Single cell suspension was obtained by gentle pipeting of the cortex fragments in the presence of 10% (v/v) FBS and 0.01% (v/v) DNase I (Sigma). Cells were then sieved through a 70 µM cell strainer (Falcon, Becton Dickinson, Franklin Lakes, New Jersey, USA), centrifuged at 800 rpm for 10 min and plated at a density of 5 x 10^5 cells/ml on poly-l-lysine (MW 70.000-150 000) coated 96 multiwell plates (BD Biocoat, Becton Dickinson). The culture medium consisted of B-27 supplemented Neurobasal Medium (Invitrogen), 0.5 mM L-glutamine and 1% (v/v) of antibiotic-antimycotic solution (Invitrogen). Incubation was carried out at 37^{o}C in a humidified 95% (v/v) air and 5% (v/v) CO_2 atmosphere. The culture medium was refreshed every three days by changing half of it, until the culture reached 6 days. Neuronal phenotype was confirmed by positive staining with mouse anti-neuron specific beta-III Tubulin monoclonal antibody (R&D Systems, Minneapolis, USA).

Astroglial cultures were prepared from cortices of 1-3 day old pups. The tissue was pooled into ice-cold glucose (33 mM) HBSS, washed twice with HBSS, incubated for 5-8 min at 37^{o}C with 0.25% (v/v) trypsin HBSS solution and 0.01% (v/v) DNase (Sigma) and washed twice with HBSS again. Single cell suspension was obtained by gentle pipeting of the cortex fragments. Cells were then centrifuged at 800 rpm for 10 min and plated in culture flasks (Greiner bio-one, Frickenhausen, Germany) with 50% (v/v) DMEM and

50% (v/v) Ham`s F-12 medium (PAA Laboratories) with 3.7g/L $NaHCO_3$, 25mM HEPES, 4.5g/L D-glucose, 4.4g/L NaCl, containing 10% (v/v) heat-inactivated FBS, 20 mM L-glutamine and 1% (v/v) of antibiotic-antimycotic solution. The cells were cultured at 37°C in a humidified, 5% (v/v) CO_2 atmosphere and the culture medium was changed every three days. After 12 days the cultures were shaken, which dislodged the microglial cells and oligodendrocytes. The remaining astrocytes were removed by trypsin treatment and plated at a density of 5×10^4 cells/ml in 96 multiwell plates (Greiner bio-one).

Assessment of Cell Viability

AlamarBlue/CFDA-AM – Cell viability in CaCo-2, HaCaT and A549 cells was determined using a mixture of two fluorescent indicator dyes - Alamar Blue (Bioscource, Nivelles, Belgium) and 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM; Molecular Probes, Carlsbad, CA, USA). Alamar Blue is a non-fluorescent dye which is reduced by cellular dehydrogenases to a fluorescent product, resorufin. The conversion to the fluorescent product indicates cellular metabolic activity. The CFDA-AM indicator dye is an esterase substrate that can be converted by non-specific esterases of living cells from a non-polar, non-fluorescent substance to a polar, fluorescent dye, carboxyfluorescein. The presence of carboxyfluorescein in cells indicates plasma membrane integrity. The Alamar Blue/CFDA-AM assay was performed as described previously (Schirmer et al., 1997) for fish cell lines with minor modifications for mammalian cells and the 24-well plate format. Briefly, cells were washed once with PBS prior to addition of the indicator dyes. Alamar Blue (5 % v/v) and CFDA-AM (4 µM) were combined in PBS and aliquots of 500 µl/well were added to the cells in 24 well

culture plates. After 1h of incubation, fluorescence was quantified with the GENios Plus fluorescence reader (Tecan, Grödig, Austria). The excitation/emission wavelengths were 530/595 nm for Alamar Blue and 493/541 nm for CFDA-AM. Background levels of the fluorescent indicator dyes in wells receiving the respective exposure media but no cells were also monitored and found to be stable and low (below 5% (v/v) for Alamar Blue and 1% (v/v) for CFDA-AM compared to the fluorescent values observed with cells). The OLN-93 cell line and the primary cells were also evaluated using Alamar Blue as fluorescent indicator dye in addition to the CCK-8 assay.

CCK-8 assay: The Cell Counting Kit-8 (CCK-8) utilizes Dojindo's highly water soluble tetrazolium salt (WST-8-[2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt]; Dojindo Laboratories, Kumamoto, Japan). WST-8 is reduced by dehydrogenases in cells to give an orange colored product (formazan) which is soluble in the tissue culture medium. Absorbance is measured at 450 nm wavelength using a microplate reader. Background levels of the indicator dyes in wells receiving the respective exposure media but no cells were also monitored and found to be stable and low.

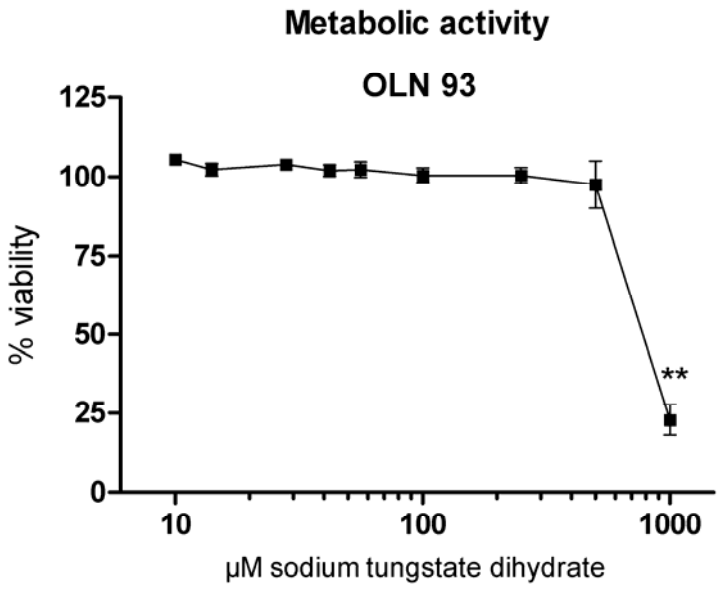

Figure 3. Example of impact of tungsten salt on cell viability. The oligodendroglial cell line OLN 93 was exposed to the tungsten salt (Na_2WO_4 x $2H_2O$) for three days and cell viability was measured using the Alamar Blue assay (indicating metabolic activity). Results are expressed as percentage of control values (Mean $\pm$ SD, n=3). Significant differences from control are indicated by the asterisks (** $p<0.01$; one-way ANOVA with Dunnett's post test).