European Commission

Recommendation from the Scientific Committee on Occupational Exposure Limits for Copper and its inorganic compounds

SCOEL/SUM/171
March 2013

Employment, Social Affairs and Inclusion

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8-hour TWA:	0.01 mg/m^3 (respirable fraction)
STEL (15-min):	None
BLV:	None
Notation:	None

This evaluation is based on ACGIH 2001, ATSDR 2004, HCN 1999, Greim 2004, Greim 2006, ECB 2000, Schneider and Kalberlah 1999, WHO 1998, WHO 2002 and the references cited in these reviews and a literature update (time period 2004-2012).

1. Substance identification, physico-chemical properties

Substance	CAS No.	Molecular formula	Molecular weight	Solubility in water
Copper	7440-50-8	Cu	63.55	Insoluble
Copper(II) acetate*	142-71-2	$Cu(CH_3–COO)_2$	181.64	Soluble
Copper(II) carbonate*	1184-64-1	$CuCO_3$	123.56	Insoluble
Copper(I) chloride	7758-89-6	CuCl	99.00	Barely soluble
Copper(II) chloride	1344-67-8 7447-39-4	$CuCl_2$	134.45	Soluble
Copper(II) hydroxide	20427-59-2	$Cu(OH)_2$	97.56	Insoluble
Copper(II) nitrate	3251-23-8	$Cu(NO_3)_2$	187.56	Soluble
Copper(II) oxide	1317-38-0	CuO	79.55	Insoluble
Copper(II) oxysulfate	12158-97-3	$Cu_3O_2SO_4$	318.71	Barely soluble
Copper(II) sulphate	7758-98-7	$CuSO_4$	159.60	Soluble
Copper(II) sulphate pentahydrate	7758-99-8	$CuSO_4 \times 5\ H_2O$	249.68	Soluble

* Copper(II) acetate and copper(II) carbonate are included in the evaluation, even though the compounds are not inorganic.

The copper compounds used in the cited studies were mostly copper(II) oxide, acetate, chloride, sulphate or sulphate pentahydrate.

EU classification:

Copper(I) chloride and copper(I) oxide

Acute Tox. 4	H302	Harmful if swallowed
Aquatic Acute 1	H400	Very toxic to aquatic life
Aquatic Chronic 1	H410	Very toxic to aquatic life with long lasting effects

Copper sulphate

Acute Tox. 4	H302	Harmful if swallowed
Eye Irrit. 2	H319	Causes serious eye irritation
Skin Irrit. 2	H315	Causes skin irritation
Aquatic Acute 1	H400	Very toxic to aquatic life
Aquatic Chronic 1	H410	Very toxic to aquatic life with long lasting effects

1.1 Physico-chemical properties

Metallic copper is a solid with a characteristic reddish colour. The preferred oxidation state of copper is +2 (cupric salts). Cuprous salts (oxidation state +1) are chemically less stable than cupric salts and can easily be oxidised.

The melting point of metallic copper is 1 083 °C, the boiling point is about 2 600 °C, and it is non-volatile at 20 °C and insoluble in water. The density of metallic copper is 8.9 g/cm^3. Many cupric compounds such as copper sulphate, nitrate, chloride and acetate are readily soluble in water (> 100 g/l) and have a characteristic blue-green colour. Copper carbonate, oxide and hydroxide are almost insoluble in water (ATSDR 2004, WHO 1998).

1.1.1 Mechanism of Action

As a transition element, copper is able to accept or donate one electron and thereby initiate redox reactions resulting in the formation of oxygen radicals. Copper ions are thus important catalytic co-factors for enzymatic redox reactions. Examples of copper binding enzymes are copper-zinc superoxide dismutase, cytochrome c oxidase, dopamine β hydroxylase and ceruloplasmin (ferroxidase). Copper-zinc superoxide dismutase plays an essential role in the cellular defence against reactive oxygen species, such as the superoxide radical, which are normally formed during the cellular metabolism (WHO 1998).

Copper is an integral part of many proteins and more than 20 enzymes with important functions in cellular respiration, cellular energy metabolism, connective tissue biosynthesis, and iron metabolism. It also plays an important role in the regulation of gene transcription (WHO 1998).

Nevertheless, besides its essential functions, copper ions may exert toxic properties on conditions of disturbed homeostasis due to overload or unphysiological exposure routes such as inhalation. Thus, ionic copper binds with a high affinity to histidine or sulphur in cysteine and methionine. This can lead to inactivation of proteins and enzymes (Rae *et al* 1999). Reactive copper(II) can oxidise thiol groups located in the membranes to form disulphides, thereby being able to disturb structural or functional properties of membranes (Kumar *et al* 1978). The copper(I) thus formed can be oxidized again to form copper(II) via endogenous oxygen or via hydrogen peroxide from the respiratory chain. In this redox cycle, reactive oxygen radicals can be produced through Fenton-like reactions (Goldstein and Czapski 1986). Therefore, it is assumed that reactive copper can lead to oxidative cell damage, such as lipid

peroxidation, thiol oxidation, and DNA damage (Glass and Stark 1997, Li and Trush 1993).

With respect to potential mechanisms involved in respiratory toxicity, Gu and Lin (2010) showed that copper (as $CuCl_2$) stimulated pulmonary sensory neurons via a direct activation of TRPA1 in pulmonary C-fibre sensory nerves in mice.

Several studies support a high toxicity of copper oxide (CuO) nanoparticles in comparison to other metal oxide nanoparticles in pulmonary epithelial cells *in vitro* and also when compared to CuO microsize particles or water soluble copper salts (Karlsson *et al* 2008, 2009; Fahmy and Cormier 2009, Lanone *et al* 2009, Ahamed *et al* 2010). These observations appear not to be due to differences in the extracellular solubility of the metal compounds, but rather due to higher intracellular levels of copper ions. Effects appear to involve sustained oxidative stress possibly due to redox cycling (Fahmy and Cormier 2009). One key mechanism may be the ability of CuO to damage the mitochondria (Karlsson *et al* 2009). Furthermore, Hsp70, p53 and DNA damage repair proteins Rad51 and MSH2 expression and/or protein levels were up-regulated, demonstrating that CuO nanoparticles possess a genotoxic potential in A549 cells which may be mediated through oxidative stress (Ahamed *et al* 2010).

2. Occurrence/use and occupational exposure

Copper and its compounds occur naturally in rock, soil, water and (in low amounts) in air, as well as in plants and animals. Anthropogenic sources of copper are predominantly ore mining, smelting and refinery. There is a widespread use of copper and its alloys in the production of various electrical equipments, cookware, water pipes, coins, or processing in the fabrication e.g. of dyes or wood preservatives. Additional sources of exposure are the use of copper-containing fertilisers, animal food additives, bactericides, fungicides, insecticides and antifouling agents (ATSDR 2004, WHO 1998).

Analytical methods of copper at the workplace have been published, i.e. by NIOSH (1987) (with detection limits of 1 µg and 0.05 µg copper per sample, respectively, for IPCS-AES or AAS), and by the DFG (2012) with a detection limit of 27.1 µg copper/m^3 at an air sample volume of 0.42 m^3 (ICP-OES).

3. Health significance

3.1 Toxicokinetics

3.1.1 Human data

Copper is an essential element, which is incorporated in various proteins. It is a constituent of more than 20 enzymes.

No quantitative data exist for the rate of absorption by the inhalation route (the absorption rate of fine particles is generally in the order of 50 %). An oral daily uptake of 1–3 mg/day is considered necessary to avoid copper deficiency (ATSDR 2004, WHO 1998, WHO 2002). Age-specific "normative requirements" are given by WHO as 1.35 mg/day for an adult male and 1.15 mg/day for an adult female. Estimates of typical copper intakes (mainly through food, less by inhalation) for the EU population are in the range 0.8–1.8 mg/day. Typical copper intakes of men are higher than those of women while the intake among the general adult population is higher than that of the elderly. Intakes of both men and women are generally close to the WHO normative requirements but may be somewhat lower in specific locations where background

levels of copper are unusually low (Sadhra *et al* 2007). The uptake of copper by the gastrointestinal tract is regulated homeostatically by specific mechanisms, which reduce absorption at higher exposure levels by enhancing faecal elimination. The oral absorption rate is usually in the range of 20–60 %. *In vitro* studies with copper compounds (chloride or sulphate) as well as *in vivo* dermal application of copper salts or dermal exposure to metallic copper fumes suggest that copper is poorly absorbed through the skin. After absorption, copper is transported by the blood (bound to ceruloplasmin and albumin) mainly to the liver and, to a lesser extent, to the kidney. The predominant elimination pathway is the bile. Small amounts are excreted via urine. Specific population groups with genetic defects or abnormalities in the metabolism of copper (e.g. individuals with Menkes disease or Wilson disease) may be sensitive to levels of copper exposure that are non-toxic to persons without these defects (ATSDR 2004, WHO 1998, WHO 2002).

3.1.2 Animal data

No quantitative data exist for the absorption rate by the inhalation route. The half-life of copper sulphate in rat lungs after tracheal instillation was 7.5 hours. The degree of absorption of orally administered copper in animals is dependent on the dose and the copper status. The mechanisms of regulation of absorption, distribution and elimination are similar to those in humans. Dermal absorption of copper is enhanced in the presence of compounds like salicylic acid (ATSDR 2004, WHO 1998).

3.1.3 Biological monitoring

There is obviously a great variation of copper background levels in the European population. Several studies report mean background serum or plasma values of European healthy adults in the range of 70–137 µg/dl (Karadag *et al* 2004, Terrés-Martos *et al* 1997, Hamilton *et al* 1994, Rükgauer *et al* 1997, Walther *et al* 2000, Cornelis *et al* 1994, Kouremenou-Dona *et al* 2006, Sánchez *et al* 2010). The individual variations in these studies were generally high. The individual serum values covered a range of 30–200 µg/dl, and the standard deviations were up to 50 % of the mean values.

After occupational inhalation exposure to 0.64–1.05 mg Cu/m^3, copper plasma concentrations in exposed workers were not significantly different from controls (108 ± 4 µg/dl vs. 99 ± 3 µg/dl) (Finelli *et al* 1981). In a recent study with workers exposed to 0.001–0.082 mg Cu/m^3 copper serum values were 104.5 ± 15.1 µg/dl vs. 99.7 ± 12.1 µg/dl in the control group ($p = 0.05$) (Kossowska 2010). Therefore, there are no clear or dose dependent elevated internal copper levels in blood at exposure concentrations up to about 1 mg Cu/m^3. After oral exposure, there was no significant correlation between copper serum values and ingested copper doses in a range of < 0.01 and 6 mg Cu/l drinking water over a period of 2 months (Araya *et al* 2003b). The higher concentration in drinking water was correlated to the onset of gastrointestinal symptoms and, thus, the occurrence of (local) effects was unrelated to copper concentrations in serum.

Copper exposure for longer periods (several months) may be assessed by determination of hair or nail copper levels (ATSDR 2004). In control persons, mean values were 8.9 ± 0.9 µg/g in hair (Finelli *et al* 1981), in another study, 89.1 µg/g in hair and 18.1 µg/g in nails (Georgopoulos *et al* 2001). In the exposed group of the study by Finelli *et al* (1981), the content of copper in hair was 706 ± 167 µg/g, which was significantly higher than the control values. Another study reported an increased level of copper in hair (up to 109 µg/g) in some individuals working in braiding division in cables factory. Mean values of copper reported for different countries were given as

4.6–83 µg/g in human hair (Khuder *et al* 2008). Kempson reviewed typical concentrations of copper in human hair from population studies of 7.6 ± 9.12 – 44.1 ± 3.5 µg/g (Kempson *et al* 2007). An investigation of biomarkers for copper in 280 healthy adults revealed no statistical correlation between the levels of copper in hair and blood or plasma (Rodrigues *et al* 2008). Another recent evaluation of copper in scalp, blood and urine samples of steel mill workers (exposure data not given) presented significantly elevated levels in all three biological samples when compared to normal unexposed referents. Values were 1.85, 2.97 and 3.84 mg Cu/l blood, respectively, in non-exposed referents (N), production (P) and quality control workers (Q); 0.19 (N), 0.37 (P) and 0.53 (Q) mg/urine; 12.2 (N), 15.3 (P) and 17.9 (Q) µg/g in scalp hair (Afridi *et al* 2009).

No data for nail concentrations of occupationally copper exposed persons were available. Due to the limited and (for background hair levels) inconsistent data base, there is need for further investigation.

3.2 Acute toxicity

3.2.1 Human data

The inhalation of copper fumes (copper oxide) or fine copper dusts was associated with "metal fume fever" with a burning sensation, redness of the throat, coughing, sneezing, shortness of breath, nausea, rigor and fever. These effects occurred usually within a few hours after exposure and lasted for 24–48 hours (ATSDR 2004, Greim 2004). Quantitative data on exposure concentrations were scarce. A recent analysis of seven published studies with reports of copper-induced metal fume fever could not find clear evidence that copper was indeed the causative agent (because of lack of valid exposure assessment, atypical symptoms and lack of consistency among the types of work associated with the effects) (Borak *et al* 2000). A cross-sectional study by Jayawardana (2004) on brass workers also mentioned the occurrence of acute symptoms of metal fume fever, but the workers were also exposed to zinc (exposure concentrations not stated). Zinc oxide is a well-known inducer of metal fume fever. According to some unpublished data from occupation in the copper-welding and refining industry, concentrations up to 0.4 mg Cu/m^3 resulted in no ill effects (ACGIH 2001). Because no further details are given, these data are not suitable to derive a no observed adverse effect concentration (NOAEC).

There is a single case report of a 2-year-old female patient who unintentionally inhaled copper metal dust, developed respiratory failure a few hours later, and developed acute respiratory distress syndrome after three days. She also developed haemolytic anaemia, liver failure, oliguric renal failure and evidence of acute tubular injury. A sample of bronchoalveolar lavage showed macrophages that stained positive for copper (Donoso *et al* 2007).

There are several case reports of single oral exposures to copper compounds (accidents, suicide attempts, uptake of contaminated beverages). The observed symptoms included metallic taste, epigastric burning, nausea, abdominal pain, vomiting and, in more severe cases, lethargy, haemolytic anaemia, damage of liver and kidney as well as sometimes coma and death (ATSDR 2004, WHO 1998). Several controlled studies with human exposure to a single oral dose of copper sulphate in drinking water after an overnight fast revealed a lowest observed adverse effect level (LOAEL) for first gastrointestinal effects (nausea) of 0.011–0.017 mg Cu/kg bw and a no observed adverse effect level (NOAEL) of 0.0057–0.011 mg Cu/kg bw (Araya *et al* 2001 and 2003a, Olivares *et al* 2001).

3.2.2 Animal data

The inhalation LC_{50} for copper (II) hydroxide was > 1 303 mg/m^3 (no further details) (WHO 1998).

Drummond *et al* (1986) studied the effects of single inhalation exposure of Syrian golden hamsters and CD1 mice to a copper sulphate aerosol. Both species were exposed once for 3 hours to concentrations of 1.2 and 3.3 mg Cu/m^3 (MMAD 0.75 µm) and examined for reductions of cilia beating and histological alterations of the tracheal tissue (decrease of normal epithelium with smooth surface and beating cilia). Four animals per group were tested in this experiment. In hamsters, both endpoints were significantly altered after single inhalation exposures to 3.3 mg Cu/m^3 (3 hours) compared to the control animals. The corresponding NOAEC was 1.2 mg Cu/m^3. No effects were seen in parallel studies on mice at both concentrations, but the mouse model is probably not suited for the assessment of these endpoints: "…a significant finding in these studies was the poor quality of the respiratory epithelium in control CD1 mice. Because of the large areas of cellular necrosis with accompanying loss of cilia and desquamation, the tracheas of the CD1 mice did not appear appropriate for the assessment of air pollutant effects and future studies should be performed in hamsters." (Drummond *et al* 1986).

Drummond *et al* (1986) also exposed CD1 mice (23–100 per sex and group) to concentrations of 0, 0.56, 1.2 and 3.3 mg Cu/m^3 (3 hours, MMAD 0.54 µm) and analysed the impairment of the pulmonary defence mechanisms by concurrent inhalation exposure of the animals to streptococcus bacteria (10 colony forming units per mouse). This treatment produced a significant and dose-dependent increase in mortality within 14 days (mean values for males and females: increase in mortality of 62, 70 and 100 %, respectively). For this effect there was no NOAEC. The bactericidal activity of lung macrophages was tested after a single exposure to 1.2 and 3.3 mg Cu/m^3 for 3 hours in 23–44 mice per sex and group and was found to be significantly reduced (mean of males and female: 59 % of control value) at the higher concentration (Drummond *et al* 1986). The mouse is the most sensitive rodent species in this assay and humans could be expected to respond in a similar manner to the presence of infectious agents. The impairment of the host defence appears to be caused by the damage of the alveolar macrophage system (Ehrlich 1980).

In a study by Skornik and Brain (1983), Syrian golden hamsters (6–12 per group) were exposed to copper sulphate aerosol in concentrations of 0.13– 2.7 mg Cu/m^3 for 4 hours. These authors detected a dose-dependent decrease of the endocytotic capacity of intratracheally instilled colloidal gold by lung macrophages after inhalation exposure to concentrations of 1.2 mg Cu/m^3 and above. The effects lasted up to 24 hours after exposure but were reversible after 48 hours. The NOAEC in this study was 0.13 mg Cu/m^3. Amongst four metal sulphates, copper sulphate was the most potent compound.

Chen *et al* (1991) exposed 10 guinea pigs of the Hartley strain once for 1 hour to ultrafine copper oxide aerosols (diameter < 0.1 µm) at a concentration of 1.3 mg Cu/m^3. The animals showed reductions in the tidal volume and the minute volume during and post exposure as well as a decreased lung compliance 1 hour post exposure. No other concentrations were tested in this study.

Oral LD_{50} values for various copper salts are in the range of 15–857 mg/kg bw, depending on the species and the compound. Water soluble salts are generally more toxic than those with lower solubility. Symptoms in these studies included salivation, vomiting, diarrhoea, gastric haemorrhage, hypotension, haemolytic crisis, convulsions and paralysis. LD_{50} values for the dermal route are > 1 000 mg/kg bw (copper,

rabbits), > 1 124 mg/kg bw (copper oxisulphate, rats) and > 2 058 mg/kg bw (copper hydroxide, rabbits) (WHO 1998).

3.3 Irritation and corrosivity

3.3.1 Human data

Occupational exposure to 111–464 mg/m^3 metallic copper dust caused symptoms of irritations of the respiratory tract (Suciu *et al* 1981). Irritation of the respiratory tract and the eyes were noted in other studies with occupational exposure to copper dust or oxide, but exposure concentrations were not determined (Askergren and Mellgren 1975, Jayawardana 2004). Finelli *et al* (1981) stated the occurrence of conjunctivitis in workers exposed to copper dust concentrations of 0.64–1.05 mg/m^3 (co-exposure with iron, lead and cadmium). In the study by Gleason (1968), no irritation of the lower respiratory tract was reported (exposure to 0.12–0.36 mg Cu/m^3 as copper dust).

Dermal contact with copper salts may cause irritation to the skin, itching and erythema. Contact of copper salts with the eye may lead to conjunctivitis, ulceration, turbidity of the cornea and adhesion of the eyelids to the eye (no further details) (WHO 2002).

3.3.2 Animal data

Skin

Dermal contact with copper salts may cause irritation to the skin, itching and erythema (no further details) (WHO 2002).

Dermal application of metallic copper caused follicular reactions in guinea pigs (Greim, 2004). Necroses were observed after dermal exposure of mice to copper chloride in DMSO at concentrations ≥ 2.5 % (Basketter *et al* 1999).

Eyes

Contact of copper salts with the eye may lead to conjunctivitis, ulceration, turbidity of the cornea and adhesion of the eyelids to the eye (no further details) (WHO 2002).

3.4 Sensitisation

3.4.1 Human data

Copper and copper sulphate may evoke allergic contact dermatitis. Testing of patients with contact eczema or of workers occupationally exposed to copper dust or fumes provoked dermal reactions following testing with copper sulphate in concentrations up to 5 % copper sulphate. However, the number of reported cases with a clear copper-induced sensitisation is very low and was observed only at high concentrations of 5 % of copper salts (Walton *et al* 1983a,b). The observed dermal reactions were mostly either unspecific or cross reactions to a nickel allergy. In some cases, they may have been provoked by nickel contaminations of the copper (Greim 2004).

A single case of occupational respiratory sensitisation is reported. A worker in the galvanic industry showed a 30 % decline of the forced expiratory volume after provocation with 1 mg copper sulphate/m^3 (Cirla 1985).

3.4.2 Animal data

Two maximisation tests in guinea pigs with the pentahydrate of copper sulphate in petrolatum yielded conflicting results (Boman *et al* 1979, Karlberg *et al* 1983). As these studies were done by the same working group at similar conditions, the reason for this discrepancy is unknown. One Local Lymph Node Assay (LLNA) in mice with 10 % copper sulphate pentahydrate in ethanol failed to show a positive reaction (Ikarashi *et al* 1992). Another LLNA with copper chloride (1–5 % in DMSO) exhibited a strong lymphocytic proliferation, but this was attributed to the local necrotic action of the compound (Basketter *et al* 1999).

3.5 Repeated dose toxicity

3.5.1 Human data

Gleason (1968) reported symptoms similar to metal fume fever (Section 3.2) in an unknown number of workers after occupational exposure to copper (fine metal dust, most probably not copper oxide) during polishing of copper plates with aluminium oxide abrasive. The effects (general feeling of discomfort, slight sensations of chills and warmth, stuffiness of the head) were first reported some weeks after the start of exposure. Measured exposure was 0.12 mg Cu/m^3 but, according to the author, the workers may sometimes have been exposed possibly to two- to threefold higher concentrations. The effects did not disappear until an exhaust system was installed, which reduced exposure to 0.008 mg Cu/m^3. However, the reported symptoms differed from the typical metal fume fever in that there was no acute onset and no acclimatisation. The exposure was to copper dust (metallic copper) with co-exposure to aluminium oxide dust. Metal fume fever is mainly attributed to exposure to metal fumes (oxides). For these reasons, Borak *et al* (2000) questioned that the effects observed in the Gleason (1968) study were indeed copper-induced metal fume fever and assumed other (unknown) agents to be responsible for the complaints.

Suciu *et al* (1981) examined about 100 workers chronically exposed to 111–464 mg Cu/m^3 as copper dust. At the higher concentration levels, the authors reported an increased incidence in respiratory effects, gastrointestinal complaints, neurotoxic symptoms, cardiovascular and peripheral vascular disorders, hepatomegaly and impotence. No control group was included in this study. Finelli *et al* (1981) observed mild anaemia, hepatomegaly and bronchitis in workers who were exposed to copper dust concentrations of 0.64–1.05 mg/m^3. These workers were also exposed to iron, lead and cadmium. A more recent cross-sectional study by Jayawardana (2004) of brass workers reported anorexia, distaste, aches and pain after chronic occupational exposure (exposure concentration not stated, co-exposure with zinc).

Repeated oral exposure to copper by contaminated drinking water led to gastrointestinal effects similar to those observed after acute exposure (abdominal pain, nausea, vomiting, diarrhoea) (ATSDR 2004, Greim 2004). The most reliable case study in terms of exposure characterisation is that by Spitalny *et al* (1984), which documents effect concentrations of 3.1–7.8 mg/l drinking water and a NOAEL of 1.58 mg/l. Controlled, well conducted studies with subacute to subchronic exposure of 60–340 volunteers to copper (added to drinking water) revealed a LOAEL of 3–4 mg/l (0.073–0.092 mg Cu/kg × day) and a NOAEL of 1–2 mg/l (0.027–0.042 mg Cu/kg × day) for first gastrointestinal complaints (Araya *et al* 2003b, Pizarro *et al* 1999).

3.5.2 Animal data

Inhalation

Drummond *et al* (1986) exposed CD1 mice and Syrian golden hamsters on 3 hours/day to 0.12 mg Cu/m^3 as copper sulphate for 5 days and to 0.13 mg Cu/m^3 (MMAD 0.54 µm) for 10 days. These authors examined disturbances of pulmonal defence mechanisms (decreased bactericidal activity in alveolar macrophages, increase in mortality following the concurrent inhalation of *Streptococcus* bacteria) in mice as well as histological alterations in the respiratory tract (alterations of cilia beats in trachea, reduction of the percentage of normally appearing tracheal tissue with smooth surface and beating cilia) in both species. The exposure concentrations in this study were chosen to obtain the same concentration x time product as in the acute studies of these authors (Section 3.2.2). Inhalation exposure of 22 male and 24 female mice to 0.12 mg Cu/m^3 as copper sulphate (5 days, 3 hours/day, MMAD 0.54 µm) induced a small but significantly decreased bactericidal activity of alveolar macrophages only in females (94 % of the control value). Exposure of 22 male and 18 female mice to 0.13 mg Cu/m^3 for 10 days (3 hours/day) significantly decreased bactericidal activity of alveolar macrophages to 95 % (males) and 85 % (females) of control values. There was no increase in mortality after 5 days of exposure to 0.12 mg Cu/m^3 (n = 47–48 per sex) and inhalation of *Streptococcus* bacteria (10 colony forming units per mouse). However, a significantly increased mortality of mice (mean of males and females: increase of 28 % compared to controls, n = 48 per sex) was reported following exposure to 0.13 mg Cu/m^3 for 10 days and inhalation of *Streptococcus* bacteria, showing a clear time-dependence of the immunosuppressive effect.

There were no effects on cilia beatings or other tissue alterations in hamsters as a result of copper exposure for 5 or 10 days. Tissue alterations in the mouse experiments could not be evaluated due to a poor quality of the respiratory epithelium in control CD1 mice (see Section 3.2.2) (Drummond *et al* 1986).

No effects on respiratory function were observed in groups of 8 rabbits after 4–6 weeks of intermittent exposure (5 days/week, 6 hours/day) to 0.6 mg Cu/m^3 as copper chloride (only one concentration tested). However, there was an increased density of type-II alveolar cells and of membrane damage in the lung macrophages (Johansson *et al* 1983, 1984, Lundborg and Camner 1984).

In a study by Ginoyan (1976), two groups of rats were exposed to either variable exposure concentrations within a range of 0.01–0.1 or to 1 mg/m^3 copper oxide aerosol for 90–100 days (0.008–0.08 and 0.8 mg Cu/m^3). At the lower exposure level, there was an increase in serum protein levels. At the higher concentration, increased blood haemoglobin levels and higher erythrocyte counts were observed in addition. These data are insufficiently reported and could therefore not be used for risk assessment.

In a 4-week study (OECD guideline 412, whole body, 6 hours/day, 5 days/week) Sprague-Dawley-rats were exposed to 0.17, 0.35, 0.7 or 1.7 mg Cu/m^3 as Cu_2O (MMAD = 1.725 µm ± 1.73 µm GSD) with a recovery period of 13 weeks. Satellite groups were exposed to the high and low dose to evaluate whether a plateau was observed at week 1, 2 or 3 (ICA 2010).

Following 4 weeks of exposure, the test substance related effects included higher blood neutrophil counts in all exposed groups, with a significant increase at ≥ 0.35 mg Cu/m^3. This effect is probably related to the inflammation of the lung. At ≥ 0.17 mg/m^3, increases in lactate dehydrogenase (LDH) and total protein in the bronchoalveolar lavage fluid (BALF) were observed at the end of week 4 and also

following week 1, 2, and 3 of exposure at 2.0 mg/m^3 with a plateau. At 0.35 mg/m^3, there was a slight increase in total cell count in the BALF, significant at 0.7 mg/m^3. The majority of cells present were alveolar macrophages, a small number of lymphocytes, neutrophils, and/or epithelial cells. The increase in total cell count was associated with a higher proportion of neutrophils in all test substance-exposed rats (strong increase of 45 % at 0.17 mg/m^3 versus 0–1.1 % in control animals). These effects were also seen in the satellite groups (1.7 mg/m^3) at weeks 2 and 3 with a plateau at days 12–19. Macroscopically, enlarged bronchial and/or mediastinal lymph nodes were observed at 0.7 and 1.7 mg/m^3. At 0.17 mg/m^3, absolute and relative lung weights were increased, which was statistically significant at the next higher dose. At the end of the recovery period this effect was not completely reversible (ICA 2010).

After 4 weeks of exposure, there were histopatological findings in lung, lymph nodes (bronchial and mediastinal) and nose (level II, III, IV and V). In the lung, a dose-dependent histiocytosis (foamy macrophages; minimal at 0.17 mg/m^3 and moderate at 1.7 mg/m^3) was observed and a dose-dependent acute inflammation occurred at 0.35 mg/m^3 and higher. Lymphoid hyperplasia of bronchial lymph node was observed in the majority of rats at ≥ 0.35 mg/m^3 and in 1 female at 0.17 mg/m^3. Lymphoid hyperplasia was also present in mediastinal lymph nodes at ≥ 0.35 mg/m^3, but with a lower incidence. Minimal to slight subacute inflammmation in nasal levels II and III were present in 3 male rats at 1.7 mg/m^3 and in 1 rat at 0.17 mg/m^3 (ICA 2010). As nasal levels were investigated only in 5 animals per dose group, a final evaluation of this effect is not possible.

Histopatological findings were reversible within the recovery period. The satellite group at 1.7 mg/m^3 showed minimal to slight alveolar histiocytosis, acute inflammation and lymphoid hyperplasia in all rats without a clear time-dependence. According to the study authors, except lung weight and incidence of lymphoid hyperplasia, effects at 1.7 mg/m^3 appeared to have a peak prior to completion of the 4-week exposure time. Except lung weights, which were greatly reduced, and still slightly detectable following the recovery period, all test substance related effects were reversible within 13 weeks recovery (ICA 2010). The LOAEL of this study is 0.17 mg Cu/m^3 (as Cu_2O). A calculation of the human equivalent concentration (HEC) based on the 4-week rat study and using the Multiple-Path Particle Deposition (MPPD) model resulted in a human NO(A)EC_{HEC} of 0.006 mg Cu/m^3.

Oral

In studies with rats and mice, copper sulphate was given orally either by drinking water or feed for 14 days (NTP 1993, Hébert *et al* 1993). The exposure caused gastrointestinal irritation (only in the studies with dietary exposure), nephrotoxicity, hepatotoxicity, haematological alterations including anaemia and, at higher doses, body weight reduction and mortality. The LOAEL for the 14-day drinking water rat study was 10 mg Cu/kg bw and day, based on nephrotoxicity in males (no NOAEL). In the 14-day feeding study with rats, the most sensitive effect was the occurrence of forestomach lesions (LOAEL 45 mg Cu/kg bw and day, NOAEL 26 mg Cu/kg bw and day). In parallel studies, mice appeared to be less susceptible than rats. In a 13-week feed rat study of the NTP, the LOAEL was 34 mg Cu/kg bw and day and the effects observed at this dose were kidney and liver toxicity, forestomach lesions as well as alterations in haematological and clinical chemistry parameters. The NOAEL was 17 mg Cu/kg bw and day. In parallel studies, mice appeared to be less susceptible than rats (NTP 1993, Hébert *et al* 1993). The occurrence of immunosuppression was reported in mice following subacute oral exposure to copper sulphate at doses of 19 mg Cu/kg bw and day (NOAEL 9.5 mg Cu/kg bw day) (Pocino *et al* 1991).

The combined repeated dose and reproductive/developmental toxicity study of copper monochloride was investigated in rats given the test substance once daily by gavage at 0, 1.3, 5, 20 or 80 mg copper monochloride/kg bw and day (0.83, 3.2, 12.8 or 51.3 mg Cu/kg bw and day). Male rats were dosed for a total of 30 days beginning 14 days before mating. Female rats were dosed from 2 weeks before mating to day 3 of lactation. There was a dose-dependent reduction in the food consumption and increase in the incidence of clinical signs. At 51.3 mg Cu/kg bw and day, deaths were observed in 3 out of 12 females, haematological parameters were affected, and there was an increased incidence of squamous cell hyperplasia of the stomach in both genders as well as increased haematopoiesis of the femur in males. At 12.8 mg Cu/kg bw and day, there was an increase in squamous cell hyperplasia of the stomach in both genders. At 3.2 mg Cu/kg bw and day, an increase in the incidence of squamous cell hyperplasia of the stomach was observed in females. Based on these findings, the NOAEL were concluded to be 3.2 mg Cu/kg bw and day in male rats and 0.83 mg Cu/kg bw and day in female rats (Chung *et al* 2009).

Dermal

No animal studies with dermal exposure were available.

3.6 Genotoxicity

3.6.1 In vitro

Copper compounds were not mutagenic in most studies in bacteria and yeasts. Copper sulphate and chloride produced no mutations in *Salmonella* strains TA98, TA100, TA102, TA1535 and TA1537 with or without metabolic activation, even at cytotoxic concentrations or at the limit of solubility. A lack of response was also reported up to cytotoxic concentrations without metabolic activation in the SOS Chromotest (*Escherichia coli* PQ37), in *E. coli* WP2, in rec assays with *Bacillus subtilis* (H17 and M45), in a test for streptomycin independence in *E. coli* Sd4-73 and in tests for penicillin or streptomycin resistance in *Micrococcus aureus* FDA209 (ATSDR 2004, Greim 2004, WHO 1998).

Copper nitrate induced dose-dependent gene mutations, sister chromatid exchange and DNA strand breaks in V79 hamster cells (0.01–0.5 mmol/l, without metabolic activation) (Sideris *et al* 1988). DNA single strand breaks in rat hepatocytes were reported after exposure to copper sulphate, but only at a cytotoxic concentration of 1 mmol/l (Sina *et al* 1983). Copper sulphate induced a roughly dose-dependent increase in unscheduled DNA synthesis and an accumulation of copper in the nucleus of rat hepatocytes in the range of 7.9–78.5 µmol/l (Denizeau and Marion 1989). In Chinese hamster ovary (CHO) cells, and to a lesser extent also in human fibroblasts, DNA-protein crosslinks were induced following exposure to copper sulphate at 1–2 mmol/l, but not at 0.5 mmol/l (Olin *et al* 1996). In HeLa cells, copper sulphate interfered with the repair of oxidative DNA damage and inhibited poly(ADP-ribosyl)ation at concentrations starting from 100 µmol/l, while in the same study the induction of DNA strand breaks and oxidative DNA base modifications was restricted to cytotoxic concentrations of 300 µmol/l and higher (Schwerdtle *et al* 2007). Cu(II) chloride induced minimal DNA double-strand breaks (single cell electrophoresis assay at neutral pH) in human CD4+ T cells at 0.5 mM, but no viable cells were found in the subsequent higher concentrations (Caicedo *et al* 2008). DNA strand breaks were also observed with Cu(II) chloride in peripheral mouse blood lymphocytes at 100 µM (Urbina-Cano *et al* 2006) without data on cytotoxicity.

Karlsson *et al* (2008, 2009) showed that CuO nanoparticles were highly potent regarding cytotoxicity, mitochondrial damage, the induction of reactive oxygen

species, DNA strand breaks and oxidative DNA base modifications (Comet assay) when human lung epithelial cell line A549 was exposed to the particles.

Another study showed also a strong induction of genotoxic response towards CuO nanoparticles in human pulmonary epithelial cells (A549) by activating the p53 pathway and up-regulation of the DNA damage repair proteins Rad51 and MSH2 (Ahamed *et al* 2010).

3.6.2 In vivo – Human data

No human data on genotoxic effects were available.

3.6.3 In vivo – Animal data

Single intraperitoneal injection of copper sulphate pentahydrate to Albino mice induced a significant and dose-related increase in chromosomal aberrations (chromatid type) at doses of 1.1–6.6 mg Cu/kg bw. There was also an increase of chromosomal breaks at the highest dose (Agarwal *et al* 1990).

A study by Bhunya and Pati (1987) reported an increase in chromosomal aberrations (chromatide gaps) in Swiss mice, which were intraperitoneally injected in single doses of 1.3–5 mg Cu/kg bw as copper sulphate, either given as a single dose or in five daily doses. Further studies were carried out with single doses of 5.1 mg Cu/kg bw by the oral or subcutaneous route. All exposures resulted in significant increases in chromosomal aberrations. In the mice dosed once intraperitoneally, the effect was dose-dependent. In parallel studies with the same strain of mice, these authors also reported a significant and dose-dependent increase in the incidence of micronuclei after two intraperitoneal injections (24 hours apart) of doses of 1.3–5 mg Cu/kg bw and day as copper sulphate. The authors used no positive controls and there were signs of cytotoxic effects at all doses.

A significant and dose-dependently increased rate of micronuclei was also reported in a study by Rusov *et al* (1997). These authors exposed BALB/c mice twice intraperitoneally at 14-hour intervals to copper acetate at doses of 0.3–13.0 mg Cu/kg bw. Male and female CF1 mice were gavaged for six consecutive days with $CuSO_4$ (8.25 mg Cu/kg bw and day). This dose regimen induced micronuclei in bone marrow cells and was genotoxic when evaluated in the neutral and the alkaline version of the comet assay in whole blood (Prá *et al* 2008). Data on cytotoxic effects on bone marrow were not given. Saleha *et al* (2004) also detected DNA single-strand breaks by the comet assay in leukocytes from male Swiss albino mice administered orally up to 4.9 mg Cu/kg bw as copper sulphate. The trypan blue exclusion technique showed a cell viability ranging from 90–95 %. DNA single-strand breaks, detected by the comet assay (Franke *et al* 2006), were also induced in blood cells from male and female Swiss Webster mice after oral administration of copper sulphate (8.50 Cu mg/kg bw). In contrast to these findings, Tinwell and Ashby (1990) did not observe an increase in micronuclei following a single intraperitoneal injection of copper sulphate pentahydrate at doses of 1.7–5.1 mg Cu/kg bw to CBA mice.

3.7 Carcinogenicity

3.7.1 Human data

Epidemiological studies reported increased incidences for the overall cancer mortality as well as mortality due to lung and stomach cancer in workers exposed to copper, especially in copper smelting processes (copper oxide). Due to the lack of exposure characterisation and the possible influence of confounding factors (smoking, co-

exposure to arsenic and elevated individual copper serum levels in consequence of several diseases including cancer) these studies are not adequate to derive a causal relationship between inhalation exposure to copper compounds and cancer (ATSDR 2004, Greim 2004). Suciu *et al* (1981) reported the occurrence of 7 pituitary adenomas in workers, who were exposed to 111–464 mg Cu/m^3 as copper dust. Due to the insufficient diagnosis and description of these tumours, it is not possible to draw a firm conclusion regarding the carcinogenic potency of copper dust (Greim 2004). There are no qualified studies on the carcinogenic action of copper in humans via the oral route (ATSDR 2004, WHO 1998).

3.7.2 Animal data

There are no adequate studies on the carcinogenicity of copper compounds in laboratory animals with oral or inhalation exposure (ATSDR 2004, WHO 1998).

3.8 Reproductive toxicity

3.8.1 Human data

Suciu *et al* (1981) reported sexual impotence after chronic occupational exposure to 111–464 mg Cu/m^3 as copper dust, especially in persons with obesity and hypertension. Intrauterine copper pessaries impair the implantation of embryos and are therefore used as contraceptives (Greim 2004). There are no qualified studies on fetotoxic effects in humans of copper by the oral route (Greim 2004, Schneider und Kalberlah 1999, WHO 1998).

3.8.2 Animal data

Fertility

Subchronic inhalation exposure of rats to ≥ 2.5 mg Cu/m^3 as copper chloride caused a decrease in sperm motility, testes weight, blood sexual hormone concentrations and an increase in sperm anomalies (Gabuchyan 1987). Toxic effects on the testes were also observed after 90–100 days of inhalation exposure to copper oxide aerosol in two groups of rats (0.008-0.08 and 0.8 mg Cu/m^3; Ginoyan 1976). The authors reported testicular atrophy, inhibition of spermatogenesis and altered functional state of spermatozoa, but did not differentiate between the two exposure groups. These two Russian studies are not suitable for risk assessment (insufficient data presentation). Some older studies with insufficient data presentation reported effects on reproductive organs in rats at oral doses of about 30 mg Cu/kg bw and day but the results are inconsistent (WHO 1998). In 13-week NTP studies, no effects on reproductive organs, sperm quality or oestrous cycle were detected at doses up to 140 mg Cu/kg bw and day as copper sulphate in mice or rats (NTP 1993, Hébert *et al* 1993).

No effects on reproduction were seen in a 2-generation study with rats at doses up to 23.6–43.8 mg Cu/kg bw and day as copper sulphate pentahydrate (Greim 2006) and in studies by Lecyck (1980) with rats at doses up to 213 mg Cu/kg bw day and Aulerich *et al* (1982) with minks at doses up to 24 mg Cu/kg bw and day (both as copper sulphate).

Developmental toxicity

No studies were available for the inhalation route. Copper sulphate induced reduced postnatal weight gain and organ weights in the offspring of mice at oral doses of 1.3–1.6 mg Cu/kg bw and day, but only when the exposure lasted through lactation (Kasama and Tanaka *et al* 1988). After oral exposure of rats or mice, embryolethality and foetotoxicity were seen in rats exposed to copper acetate or sulphate at doses of

greater than about 60 mg Cu/kg bw and day with additional teratogenic effects at higher doses (Haddad *et al* 1991, Lecyck 1980). In a 2-generation study with rats, a NOAEL for developmental toxic effects of 26.7 mg Cu/kg bw and day given as copper sulphate pentahydrate was determined. In a prenatal developmental toxicity study with rabbits, the NOAEL for maternal toxicity was evaluated as less than 6 mg Cu/kg bw and day, the NOAEL for developmental toxicity as 9 mg Cu/kg bw and day given as copper hydroxide (Greim 2006). In a combined repeated dose and reproductive/ developmental toxicity study with copper monochloride in rats (see also Section 3.5.2), at 51.3 mg Cu/kg bw and day, there was an increase in the number of icteric and runt pups at birth (NOAEL 12.9 mg Cu/kg bw and day) (Chung *et al* 2009). Aulerich *et al* (1982) reported an increased foetal mortality in minks after subchronic dietary exposure to 12 mg Cu/kg bw and day as sulphate (NOAEL 6 mg Cu/kg bw and day).

4. Recommendations

The critical effect of inhalation exposure to copper is the local action on the respiratory tract, which includes an immunosuppression that is attributable to the disturbance of alveolar macrophage function.

No qualified human data for deriving an OEL for copper were available. One study reported symptoms similar to metal fume fever in workers at concentrations in the range of 0.12–0.36 mg Cu/m^3 for copper dust (Gleason *et al* 1968), but these results were questioned by Borak *et al* (2000). However, it cannot be excluded that the effects observed in the study by Gleason (1968) were produced by the high degree of absorption of fine metallic copper particles. The effects did not disappear until an exhaust system was installed, which reduced exposure to 0.008 mg Cu/m^3. In other exposure situations (brass foundry), copper aerosols with greater particle sizes are generated, which may be respirable only to a minor degree (Borak *et al* 2000).

Single inhalation exposure (4 hours) of hamsters to copper sulphate produced a dose-dependent decrease of the endocytotic capacity of lung macrophages at 1.2 mg Cu/m^3 and above, with the NOAEC being 0.13 mg Cu/m^3 (Skornik and Brain 1983). After single inhalation exposure to copper sulphate (3 hours), mice suffered from a dose-dependent increase in mortality following the inhalation of *Streptococcus* bacteria, starting at 0.56 mg Cu/m^3 (LOAEC) (Drummond *et al* 1986). After exposure of mice to a copper(II) sulphate aerosol for 5–10 days (3 hours/day), alterations in immune function of the respiratory tract (decreased bactericidal activity of alveolar macrophages, increased mortality following the inhalation of *Streptococcus* bacteria) were observed at 0.12–0.13 mg Cu/m^3 and showed a clear time dependence (Drummond *et al* 1986). A 4-week inhalation study with rats according to OECD guideline 412 revealed a LOAEL of 0.17 mg/m^3, because of inflammatory effects in the lung. The most sensitive parameter was an increase in neutrophils in the bronchoalveolar lavage fluid (BALF) (ICA 2010). A calculation of the human equivalent concentration (HEC) based on the 4-week rat study and using the Multiple-Path Particle Deposition (MPPD) model resulted in a human NO(A)EC$_{HEC}$ of 0.006 mg Cu/m^3.

Although one of the studies was performed according to current standard protocols, none of them is suitable to derive an overall NOAEC. In workers, available data revealed a NOAEC and a LOAEC of 0.008 and 0.12–0.36 mg Cu/m^3 (fine metal dust, most likely not copper oxide), respectively (Gleason *et al* 1968). Also, exposure in mice towards 0.12 mg Cu/m^3 (copper sulphate, MMAD 0.54 µm) for 5 days and only 3 hours/day still resulted in alterations of immune functions (Drummond *et al* 1986). Furthermore, exposure of rats to 0.17 mg Cu/m^3 (Cu_2O, MMAD 1.7 µm) as the lowest applied concentration for 4 weeks, 5 days/week, 6 hours/day resulted in clear signs of

lung inflammation (ICA 2010). Even though some of the effects in the latter study were reversible 4 weeks after exposure, this is not relevant for chronic workplace exposure. Additionally the high toxicity of copper nanoparticles (Karlsson *et al* 2008, 2009; Fahmy and Cormier 2009, Lanone *et al* 2009, Ahamed *et al* 2010) in pulmonary epithelial cells gives rise to specific concern.

Based on these data, an OEL of 0.01 mg/m^3 for the respirable fraction is proposed. This value is based primarily on the LOAEL of 0.12 mg Cu/m^3 in humans (fine metal dust, most likely not copper oxide) and experimental animals (copper sulphate, MMAD 0.54 µm) and on the NOAEL of 0.008 mg Cu/m^3 in workers. Since no sufficient data are available to recommend OELs for defined copper species and metallic copper, this OEL applies to copper and its inorganic compounds. This approach is supported by the fact that poorly water soluble and water soluble copper compounds appear to be equally toxic in the few experimental inhalation studies available. It has to be noted that the OEL recommended for the respirable fraction does not apply to nanoparticles, since no quantitative data suitable for risk assessment were available. Nevertheless, ultrafine copper particles are included at least to some extent, since such particles were present at the workplace in the Gleason study.

With regard to a potential OEL for the inhalable fraction, a subacute inflammation in the nose was observed in one male rat at 0.17 mg/m^3 (ICA 2010). However, a final evaluation of this effect to derive a recommendation for an OEL for the inhalable fraction is not possible. One other approach consists in the consideration of the upper tolerable intake level for copper presented by the Scientific Committee on Food (SCF, today EFSA). SCF derived a tolerable upper intake of 5 mg/day for adults. Daily intakes of copper from food in EU countries ranged from mean values of 1.1 mg/day (the Netherlands) to 2.2 mg/day (Germany) with the highest 97.5 % upper confidence limit of 4.2 mg/day (Austria) (SCF 2003, see Annex 1). Assuming an oral absorption rate of 30–40 %, which is typical for diets in developed societies (SFC 2003), and an assumed 100 % absorption by inhalation, the daily difference of 0.8 mg/day would correspond to an inhalable air concentration of copper of 0.03–0.04 mg/m^3 (5 days exposure/week with a breathing volume of 10 m^3/8-hour day). To avoid systemic toxicity, the inhalable exposure to copper should be below this value.

A NOAEL of 0.36 mg/m^3 has been estimated for acute sensory irritation in humans. It is not known, whether metal fume fever-like symptoms observed in employees exposed to copper dust at 0.12–0.36 mg/m^3 is primarily dependent on concentration or on total dose (concentration × time product). Given all the uncertainties, a scientifically based STEL cannot be recommended.

At the recommended OEL of 0.01 mg/m^3, no developmental effects are expected to occur. The lowest effect dose is that for postnatal developmental delay in the offspring of mice exposed to 1.3–1.6 mg Cu/kg bw and day as copper sulphate (Kasama and Tanaka *et al* 1988). This dose corresponds to an air concentration of about 20 mg Cu/m^3.

A clastogenic action of copper compounds cannot be excluded, but the data are inconsistent. The carcinogenic potential of copper cannot be evaluated on the basis of existing studies.

There are only few reports of sensitisation to copper with an immunological aetiology. Most of the documented cases were regarded as either unspecific or cross reactions to nickel allergy (ATSDR 2004, Greim 2004). With regard to the extensive use of copper and its compounds and the small number of case reports, there is little concern about the sensitising properties of copper.

A "skin" notation is not recommended. The dermal uptake of copper compounds is considered to be low.

Biological Limit Values cannot be derived. First, there is a large range of variation of mean and individual background serum or plasma copper concentrations in the European population. Second, inhalation exposure to copper concentrations up to 1 mg Cu/m^3 did not result in significantly or dose-dependent elevated copper plasma concentrations compared to controls (Finelli *et al* 1981, Kossowska 2010). After oral exposure up to 6 mg/l drinking water there was no significant elevation of serum copper levels. This concentration already produced local effects. Therefore, copper levels in blood are no suitable biomarker, presumably due to tight copper homeostasis.

No measurement difficulties are foreseen at the recommended OEL.

The present Recommendation was adopted by SCOEL on xx Date Month Year.

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ANNEX 1.

Table 1. Daily intakes of copper from food in EU countries (mg/day). Table adapted from SCF (2003).

Country	Type of survey	n	Method	Supplements [a]	Mean	97.5 %
Austria [b]	Individual	2 488	24-h recall	Not defined	2.0	4.2
Germany [c]	Individual (m)	854	7-day dietary	-	2.2	4.0
	Individual (f)	1 134	record	-	1.8	3.3
United Kingdom [d]	Individual (m)	1 087	7-day weighed	-	1.6 (1.5)	3.5
	Individual (f)	1 110	inventory	-	1.2 (1.1)	2.8
	Individual(m)	1 087		+	1.6 (1.5)	3.5
	Individual (f)	1 110		+	1.2 (1.1)	2.8
Italy [e]	Household	2 734	7-day record	+	1.4	2.8
The Netherlands [f]	Individual (m, f)	5 958	2-day record	-	1.1	1.2
Ireland [g]	Individual (m)	662	7-day estimated	+	1.5	3.1
	Individual (f)	717	food record	+	1.2	2.7

[a] + data included supplements; - data excluded supplements
[b] Elmadfa *et al* 1998.
[c] Heseker et al 1994 (VERA study) – median values.
[d] Gregory et al 1990 – values are the mean with the median in parenthesis.
[e] Turrini 1996.
[f] Hulshof and Kruizinga 1999.
[g] IUNA 2001.