Danish Ministry of the Environment
Environmental Protection Agency

Cobalt(II), inorganic and soluble salts

Evaluation of health hazards and proposal of a health based quality criterion for drinking water

Environmental Project No. 1520, 2013

Content

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to Cobalt(II), inorganic and soluble salts, and a proposal of health based quality criteria for drinking water. This resulted in 2010 in the present report, which was prepared by Elsa Nielsen, Krestine Greve and Ole Ladefoged, Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Nature Agency,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
Danish Regions,
The Danish Environmental Protection Agency.

The Danish Environmental Protection Agency
Copenhagen, December 2013.

1 General description

Cobalt is a naturally occurring metal. It exists in the oxidation states 0, +2 and +3 and belongs to the group of transition elements of the periodic table together with iron and nickel. ^{59}Co is the only stable isotope of cobalt. Twenty-six radioactive isotopes of cobalt are known of which two are of commercial importance (^{57}Co and ^{60}Co). Cobalt usually exists under naturally conditions associated with other elements. In this evaluation only soluble inorganic and non-radioactive cobalt salts are considered in relation to an estimation of a health based quality criterion in drinking water and soil.

This document is based on evaluations prepared by ATSDR (2004) and IARC (2006).

In this evaluation, the term "cobalt" is used in a generic sense and refers to the cobalt content of the various cobalt salts mentioned in this document. For the purpose of comparison, concentrations and dose levels of the various cobalt salts are expressed in terms of cobalt equivalents (Co) whenever possible.

1.1 Identity and physico-chemical properties

The identity of selected inorganic soluble cobalt salts is presented in Table 1.1 and the physico-chemical properties are presented in Table 1.2.

1.2 Production and use

The most important cobalt minerals used in the production of pure cobalt metal are linnaeite (Co_3S_4), carrolite ($CuCo_2S_4$), safflorite ($CoAs_2$), skutterudite ($CoAs_3$), erythrite ($Co_3(AsO_4)_2$ $8H_2O$) and glaucodot (CoAsS) (Hodge 1993, IARC 1991, Merian 1985, Smith and Carson 1981 – quoted from ATSDR 2004).

Cobalt is primarily produced as a by-product of the mining and processing of copper and nickel ores and to a lesser extent of silver, zinc, iron, lead and gold ores. The three main processes for leaching cobalt from ores and ores concentrates (e.g. oxide or sulphide ore concentrate) are acid sulphate leaching, acid chloride leaching and ammoniacal solution leaching (IARC 1991).

Cobalt is used in magnetic alloys and in alloys that require hardness, wear resistance and corrosion resistance. Cobalt super alloys are used in gas turbines aircraft engines exposed to elevated temperatures and high mechanical stress. Cobalt compounds are used as pigments in glass, ceramics, paints, as catalysts in the petroleum industry, as paint driers and as trace element additives in agriculture and medicine (ATSDR 2004).

Cobalt sulphate is the usual source of water-soluble cobalt since it is the most economical salt and shows less tendency to deliquesce or dehydrate than the chloride or nitrate salts. Cobalt sulphate is used in storage batteries, in cobalt electroplating baths, as a drier for lithographic inks and varnishes, in ceramics, enamels and glazes to prevent discolouring and in cobalt pigments for decorating porcelain (O'Neil 2001 – quoted from IARC 2006).

Table 1.1 Identity of selected inorganic soluble cobalt salts (Weast 1985, ChemIDplus Advanced, ATSDR 2004)
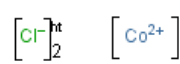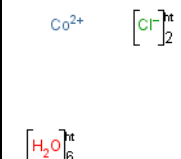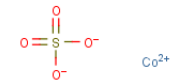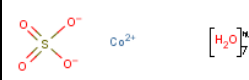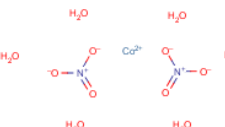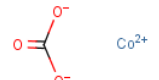
	Cobalt(II) Chloride	Cobalt(II) Chloride hexahydrate	Cobalt(II) sulphate	Cobalt(II) sulphate heptahydrate	Cobalt(II) nitrate hexahydrate	Cobalt(II) carbonate
Molecular formula	$CoCl_2$	$CoCl_2\ 6H_2O$	$CoSO_4$	$CoSO_4\ 7H_2O$	$Co(NO_3)_2\ 6H_2O$	$CoCO_3$
Structural formula						
Molecular weight	129.84	237.93	154.99	281.099	291.03	118.94
CAS-no	7646-79-9	7791-13-1	10124-43-3	10026-24-1	10026-22-9	513-79-10
Synonyms	Cobaltous dichloride	Cobaltous dichloride hexahydrate	Cobaltous sulphate	Cobaltous sulphate heptahydrate, bieberite	Cobaltous nitrate hexahydrate	Cobaltous carbonate, spherocobaltite

Table 1.2 Physico-chemical properties of selected inorganic soluble cobalt salts (Weast 1985, ChemIDplus Advanced, ATSDR 2004)

	Cobalt(II) Chloride	Cobalt(II) Chloride hexahydrate	Cobalt(II) sulphate	Cobalt(II) sulphate heptahydrate	Cobalt(II) nitrate hexahydrate	Cobalt(II) carbonate
Description	Blue solid	Red solid	Dark blue solid	Red-pink solid	Red solid	Red solid
Melting point °C	724	86	Decomposes at 735	96.8	55-56, -3H$_2$O at 55	Decomposes
Boiling point °C	1049	-6H$_2$O at 110	-	-7H$_2$O at 420		-
Density g/cm^3	3.356	1.924	3.71	1.948	1.87	4.13
Solubility in water g/100 g H$_2$O	45 at 7°C	76.7 at 0°C	3.83 at 25°C	60.4 at 3°C	133.8 at 0°C	0.18 at 15°C

Cobalt sulphate is used in electrowinning magnetic recording tapes and as a food and animal feed additive (IUCLID 2000).

Of the total cobalt production, the use in the different sectors are as follows: Alloys 34%, ceramics 12%, hard metal 11%, magnets 10%, catalysis 8%, cutting tools 6%, batteries 6%, magnetic tapes 4% and others 9% (EU 2001).

In Denmark, cobalt(II) nitrate hexahydrate is used in surface treatment materials (2006, 2007: 0.5 tonnes per year) (MST 2009).

1.3 Environmental occurrence and environmental fate

With an average concentration of 20-25 mg/kg, cobalt is the 33rd most abundant element in the earth's crust (Abbasi et al. 1989, Merian 1985, Smith and Carlson 1981 – quoted from ATSDR 2004).

Cobalt is widely distributed throughout the earth with the largest concentrations found in mafic (igneous rocks rich in magnesium and iron and low in silica) and ultramafic rocks (average 270 mg/kg). Sedimentary rocks contain varying amounts of cobalt (average 4 mg/kg in sandstone, 6 mg/kg in carbonate rocks and 40 mg/kg in clays and shales) (Donaldson et al. 1986, O'Neil 2001, Donaldson 2003 – quoted from IARC 2006).

The natural content of cobalt in fossil fuels is 5 mg/kg in coal and 0.2 mg/kg in oil (Bertine and Goldberg 1971 – quoted from IARC 2006).

1.3.1 Air

Cobalt is naturally released into the atmosphere by windblown soil, seawater spray, volcanic eruptions and forest fires (ATSDR 2004).

Primary anthropogenic sources include fossil fuel and waste combustion, vehicular and aircraft exhausts, processing of cobalt and cobalt containing alloys, copper and nickel smelting and refining and the manufacture and use of cobalt chemicals and fertilizers derived from phosphate rocks (Barceloux 1999, Lantzy and Mackenzie 1979, Nriagu 1989, Smith and Carson 1981 – quoted from ATSDR).

The mean cobalt levels in air at unpolluted sites are generally <1-2 ng/m^3. In several open-ocean environments, geometric mean concentrations ranged from 0.0004 to 0.08 ng/m^3. The highest measured average cobalt concentration of 48 ng/m^3 was recorded at the site of a nickel refinery in Wales (Chester et al. 1991, Hamilton 1994, Smith and Carson 1981 – quoted from ATSDR 2004).

In Denmark, the concentration of cobalt in the atmosphere was below the detection limit of 0.1-0.2 ng/m^3 (MST 1986a). More recent data have not been located.

1.3.2 Water

Cobalt is naturally occurring in seawater and in some surface water and groundwater at low levels. Anthropogenic sources of cobalt release into water include disposal of cobalt-containing wastewater and atmospheric deposition from activities such as the burning of fossil fuels and smelting and refining of metals (Smith and Carson 1981 – quoted from ATSDR 2004).

Soluble cobalt in the waterways will sorb to particles and may settle into the sediment or be sorbed directly by sediment. It may precipitate out as carbonates and hydroxides or with mineral oxides. It may also sorb to or complex with humic acid substances in the water. These processes are sensitive to environmental factors such as pH and the proportion of dissolved cobalt will be higher at low pH. Cobalt can also be transported in dissolved form or as suspended sediment by rivers to lakes and the sea or by ocean currents. The proportion of cobalt transported in each form is highly variable (Smith and Carson 1981 – quoted from ATSDR 2004).

In the US, the concentration of cobalt measured in surface and groundwater was <1 µg/l in pristine and 1-10 µg/l in populated areas. Measured levels in most drinking water samples were <1-2 µg/l although levels up to 107 µg/l were recorded (Hamilton 1994, Smith and Carson 1981, Greathouse and Craun 1978, Meranger et al. 1981, NAS 1977 – quoted from ATSDR 2004).

In Denmark, the concentration of cobalt was measured in a few samples in the area of Copenhagen. With the exception of one sample with a concentration of 20 µg Co/l, all were below the detection limit (detection limit not stated in the report) (MST 1986b).

1.3.3 Soil

Cobalt occurs naturally in soil. Anthropogenic sources of cobalt release into soil include activities such as the mining and processing of cobalt-bearing ores, the application of cobalt-containing sludge or phosphate fertilizers, the disposal of cobalt-containing wastes and atmospheric deposition from activities such as the burning of fossil fuels and smelting and refining of metals. Particles with aerodynamic diameters >2 µm may deposit within 10 km from the point of emission, while finer particles may travel longer distances. Cobalt originating from combustion sources exists primarily as the oxide, arsenides or sulphides (Smith and Carson 1981, Schroeder et al. 1987 – quoted from ATSDR 2004).

In the US, the average concentration of cobalt measured in soils was 7.2 mg Co/kg. Most soils contain 1-40 mg Co/kg. Soils containing <0.5-3 mg Co/kg are considered cobalt-deficient because plants growing on them have insufficient cobalt (<0.08-0.1 mg Co/kg) to meet the dietary requirements of cattle and sheep (Smith and Carson 1981 – quoted from ATSDR 2004).

In Denmark, the concentrations of cobalt measured at 44 farmlands were in the range 0.4-5 mg/kg (dry weight) with an average concentration of 2.1 mg Co/kg (MST 1986a).

1.3.4 Foodstuffs

Cobalt may be taken up from soil by plants. Elevated levels of cobalt have been found in the roots of sugar beets and potato tubers in soils with high cobalt concentrations (e.g., fly ash-amended soil). However, the translocation of cobalt from roots to above-ground parts of plants is not significant in most soils, as indicated by the lack of cobalt in seeds of barley, oats and wheat grown in high-cobalt soil. Surface deposition of cobalt on leaves of plants from airborne particles may also occur. A mean cobalt concentration of 0.48 µg/g was reported for terrestrial plants (Mermut et al. 1996, Smith and Carson 1981, Outridge and Noller 1991 – quoted from ATSDR 2004).

In Canada, the level of cobalt in most foods was low. Items with the highest concentrations were waffles (76 µg/kg), corn cereal (74 µg/kg) and potato chips (70 µg/kg) (Dabeka and McKenzie 1995 – quoted from ATSDR 2004).

In Sweden, the levels of cobalt were determined in 50 different food items, mainly meat, fish, fruit, vegetables, pulses, and cereals on the market during the years 1983-1990. Beef liver and seeds were fairly high in cobalt. The cobalt levels in µg/kg fresh weight were highest in alfalfa seeds (860 µg/kg), linseed (560 µg/kg), milk chocolate (340 µg/kg), dark chocolate (240 µg/kg), white poppy seeds (300 µg/kg), blue poppy seeds (150 µg/kg), soya beans (84 µg/kg), green lentils (54 µg/kg) and beef liver (43 µg/kg). Fish, fruit, roots and leafy vegetables were under 1 µg Co/kg fresh weight. (Jorhem and Sundström 1993 – quoted from ATSDR 2004).

In 20 brands of alcoholic and non-alcoholic beer widely consumed in Spain the cobalt concentration ranged from 0.16-0.56 µg/l with a median of 0.39 µg/l (Cameán et al. 1998 – quoted from ATSDR 2004).

In Denmark, the median concentrations of cobalt in onions and peas were 1.51 µg/ kg (range 0.119-5.1 µg/kg) and 4.6 µg/kg (range 0.57-17 µg/kg), respectively (Bibak et al. 1998a, 1998b – quoted from ATSDR 2004).

1.3.5 Bioaccumulation

Available data indicate that cobalt is not taken up appreciably by plants and does not bio magnify up the food chain (Baudin and Fritsch 1987, Baudin et al. 1990, Boikat et al. 1985, Francis et al. 1985, Kloke et al. 1984, Lux et al. 1995, Mascanzoni 1989, Mejstrik and Svacha 1988, Mermut et al. 1996, Palko and Yli-Hala 1988, Smith and Carson 1981, Tolle et al. 1983, Watabe et al. 1984 – quoted from ATSDR 2004).

1.4 Human exposure

Most of the cobalt ingested is inorganic (Jenkins 1980 – quoted from ATSDR 2004).

In Canada, the estimated average daily cobalt intake from diet was 11 µg/day ranging from 4-15 µg/day between the various age/sex groups. The contributions of various food groups to cobalt intake were: bakery goods and cereals 29.8%, vegetables 21.9%, beverages 9.8%, milk and milk products 9.4%, meat and poultry 9.1%, soups 6.4%, fruit and fruit juices 5.0%, sugar and candies 2.8%, fish 2.7%, fats and oils 2.2% and miscellaneous 1.1% (Barceloux 1999, Dabeka and McKenzie 1995 – quoted from ATSDR 2004).

In the 1960s, some breweries added cobalt sulphate to beer to stabilize the foam (resulting in exposures of 0.04-0.14 mg Co/kg). Cobalt is no longer added to beer (ATSDR 2004).

In France, the average daily intake of cobalt was estimated to be 7.5 µg/day for adults (from 15 years old) and 7.3 µg/day for children (3-14 years old). The 2.5[th] and 97.5[th] percentile daily exposure was 3.5 and 14 µg/day for adults and 3.0 and 15 µg/day for children, respectively (INRA 2004).

Using the highest reported concentration of cobalt in the US drinking water of 107 µg/l (Greathouse and Craun 1978 – quoted from ATSDR 2004), and the consumption rate of 0.08 l/kg bw/day (for children 1-10 years old), the intake from drinking water would be 8.56 µg Co/kg bw/day. For an adult (body weight of 70 kg), the daily exposure of cobalt from drinking water would be 599 µg.

Using the highest reported concentration of cobalt in Danish ground water of 20 µg/l (MST 1986b), and the consumption rate of 0.08 l/kg bw/day (for children 1-10 years old), the intake from drinking water would be 1.6 µg Co/kg bw/day (assuming no dilution of groundwater). For an adult (body weight of 70 kg), the daily exposure of cobalt from drinking water would be 112 µg.

Using the highest measured average cobalt concentration of 48 ng/m^3 recorded at the site of a nickel refinery (Smith and Carson 1981 – quoted from ATSDR 2004) and assuming the inhalation rate as 0.5 m³/kg bw/day (for children 1-5 years old), the exposure to cobalt from air would be 24 ng Co/kg bw/day. For an adult (body weight of 70 kg), the daily exposure of cobalt from air would be 1.68 µg.

2 Toxicokinetics

2.1 Absorption, distribution and elimination

As a component of vitamin B_{12} (cyanocobalamin) cobalt is an essential element and is found in most body tissues. Absorbed cobalt is transported throughout the body in the blood and has been identified in liver, muscle, lung, lymph nodes, heart, skin, bone, hair, stomach, brain, pancreatic juice, kidneys, plasma and urinary bladder of non-exposed subjects. The highest cobalt concentration was found in the liver, followed by the kidney (Collecchi et al. 1986, Forbes et al. 1954, Hewitt 1988, Ishihara et al. 1987, Muramatsu and Parr 1988, Teraoka 1981, Yamagata et al. 1962, Yukawa et al. 1980, Ayala-Fierro et al. 1999, Greenberg et al. 1943, Gregus and Klaassen 1986, Patrick et al. 1989 – quoted from ATSDR).

The cobalt tissue levels reflect exposure from all routes. The total body burden of cobalt was estimated to be 1.1-1.5 mg. About 0.11 mg cobalt was found in the liver (ICRP 1979, Yamagata et al. 1962 – quoted from ATSDR 2004).

2.1.1 Inhalation

Following inhalation exposure, cobalt compounds deposit in the lungs based on their aerosol characteristics. The absorption of deposited cobalt compounds seems to be related to their biological solubility. Physiologically insoluble cobalt particles are generally cleared by phagocytosis and/or mucociliary transport, and thus, have a low systemic absorption. To some extent, cobalt particles may be dissolved within alveolar macrophages. More soluble forms of cobalt may enter the bloodstream through the alveolar or bronchial walls (Kreyling et al. 1990 – quoted from ATSDR 2004).

In metal workers, increased cobalt levels were found in the lymph nodes, liver, spleen, and kidneys. (Hillerdal and Hartung 1983, Teraoka 1981 – quoted from ATSDR 2004).

2.1.2 Oral intake

In humans, the gastrointestinal absorption of cobalt varied considerably (18-97% of the given dose) based on the type and dose of cobalt compound given and the nutritional status of the subjects (Harp and Scoular 1952, Smith et al. 1972, Sorbie et al. 1971, Valberg et al. 1969 – quoted from ATSDR 2004).

In humans, iron deficiency led to increased absorption of cobalt from the gastrointestinal tract (31-71% in iron deficient individuals, 18-44% in controls) and simultaneous administration of cobalt and iron reduced the amount of cobalt absorbed. It has been suggested that cobalt and iron share a common absorptive pathway in the intestines, though the cobalt absorption takes place without ferritin (Sorbie et al. 1971, Valberg et al. 1969, Reuber et al. 1994, Schade et al. 1970, Thomson et al. 1971 – quoted form ATSDR 2004).

A 3-compartment model of the kinetics of ingested cobalt in humans that is applicable to infants, children, adolescents and adults was developed. Absorption

of ingested cobalt is assumed to be 60% in infants up to 3 months of age, 30% from 3 months to 15 years of age, and 10% after age 15 years. Absorbed cobalt is assumed to distribute as follows: 50% is excreted (urine and faeces combined in a 6:1 ratio), 5% is transferred to the liver, and 45% is transferred to other tissues. Elimination from tissue compartments is described by three first order rate constants representing slow, medium, and fast elimination pools with half-times of 6, 60, and 800 days, respectively. The elimination half-times are assumed to be independent of age (ICRP 1979, 1993 – quoted from ATSDR 2004).

In several studies in rats, soluble cobalt chloride was absorbed in the range of 13-34%. Administration of cobalt chloride (with ^{58}Co tracer) in cows' milk to the rats permitted a significantly greater (about 40%) absorption through the gastrointestinal tract (Ayala-Fierro et al. 1999, Barnaby et al. 1968, Hollins and McCullough 1971, Kirchgessner et al. 1994, Schade et al. 1970, Taylor 1962, Bailey et al. 1989, Collier et al. 1989, Patrick et al. 1989 – quoted from ATSDR 2004).

Absorption of soluble cobalt compounds was greater in rats (13-34%) than in dairy cows (1-2%) and guinea pigs (4-5%) following oral exposure (Bailey et al. 1989, Ayala-Fierro et al. 1999, Barnaby et al. 1968, Hollins and McCullough 1971, Kirchgessner et al. 1994, Naylor and Harrison 1995, Schade et al. 1970, Taylor 1962, van Bruwaene et al. 1984 – quoted form ATSDR 2004).

In rats and guinea pigs, the cobalt absorption was 3- to 15-fold greater in younger animals (1-60 days of age) than in adult (200 days of age) animals (Naylor and Harrison 1995 – quoted from ATSDR 2004).

In rats, long-term oral exposure to cobalt chloride resulted in significantly increased levels of cobalt in the liver, kidney, muscle, brain and testes of treated rats (Barnaby et al. 1968, Bourg et al. 1985, Thomas et al. 1976 – quoted from ATSDR 2004).

In rats, reported faecal elimination levels of soluble cobalt(II) chloride ranged from 70 to 83% of the administered dose, with urinary excretion accounting for the majority of the remainder of the dose (Ayala-Fierro et al. 1999, Barnaby et al. 1968, Hollins and McCullough 1971 – quoted form ATSDR 2004).

In Beagle dogs, exposed to a single oral dose of soluble cobalt nitrate, 70% was eliminated in the faeces and 25% in the urine (Kreyling et al. 1986 – quoted form ATSDR 2004).

2.1.3 Dermal contact

In guinea pigs, the absorption of cobalt chloride (in 1.4 N HCl) through intact skin 3 hours after exposure was <1%, while absorption through abraded skin was almost 80% (Inaba and Suzuki-Yasumoto 1979 – quoted from ATSDR 2004).

2.1.4 Transport through placenta and breast milk

In pregnant rats, oral exposure to cobalt sulphate showed a dose-dependent increase in cobalt levels in foetal blood and amniotic fluid (Szakmary et al. 2001 – quoted from ATSDR 2004).

In lactating dairy cows, about 97% of an oral dose of cobalt chloride was recovered in the faeces by day 70 post-exposure, while the urine and milk contained 0.26 and

0.012% of the dose, respectively (van Bruwaene et al. 1984 – quoted from ATSDR 2004).

2.2 Mode of action

Exposure to soluble cobalt increases indices of oxidative stress, including decreased levels of reduced glutathione, increased levels of oxidized glutathione, activation of the hexose monophosphate shunt and free-radical-induced DNA damage (Hoet et al. 2002, Kasprzak et al. 1994, Lewis et al. 1991, Zhang et al. 1998a – quoted from ATSDR 2004).

Soluble cobalt has been shown to alter calcium influx into cells, functioning as a blocker of inorganic calcium channels. This mechanism has been linked to a reduction of steroidogenesis in isolated mouse Leydig cells. Cobalt may also affect neuromuscular transmission though antagonism with calcium (Henquin et al. 1983, Moger 1983, Yamatani et al. 1998, Moger 1983, Weakly 1973 – quoted from ATSDR 2004).

Cobalt is thought to inhibit haem synthesis *in vivo* by acting upon at least two different sites in the biosynthetic pathway, which might result in the formation of cobalt protoporphyrin rather than haem. Effects on haem synthesis may potentially affect a wide variety of haem-containing proteins, including monooxygenase enzymes (i.e., cytochromes P450) and catalase (de Matteis and Gibbs 1977, Sinclair et al. 1979, Legrum et al. 1979, Yasukochi et al. 1974 – quoted from ATSDR 2004). Conversely, cobalt acts through a mechanism believed to involve a haem-containing protein, to increase erythropoietin, which stimulates the production of red blood cells (Di Giulio et al. 1991, Goldberg et al. 1988, Smith and Fisher 1973 – quoted from ATSDR 2004).

A gene expression mechanism is involved in several tissue and cellular responses induced by soluble cobalt compounds (generally cobalt chloride) mimicking the pathophysiological response to hypoxia, a response which involves various genes including those coding for erythropoiesis and for growth factors for angiogenesis. Up regulation of erythropoietin gene expression was observed in rats after a single intraperitoneal injection of cobalt chloride (60 mg/kg bw) (Gleadle et al. 1995, Steinbrech et al. 2000, Beyersmann 2002, Göpfert et al. 1995 – quoted from IARC 2006).

The results of genotoxicity assays have indicated a genotoxic potential of cobalt(II) compounds. In mammalian cells *in vitro*, two mechanisms seem to operate: a direct effect of cobalt(II) ions causing DNA damage through a Fenton-like mechanism, and an indirect effect of cobalt(II) ions through inhibition of repair of DNA damage caused by endogenous events or induced by other agents. As the repair of DNA damage is an essential homeostatic mechanism, this inhibition may account for a mutagenic or carcinogenic effect of cobalt(II) ions. Competition with essential magnesium ions and binding to zinc finger domains in repair proteins have been identified as potential modes of the indirect genotoxic activity (IARC 2006).

2.3 Vitamin B_{12}

Cobalt is a component of vitamin B_{12} (cyanocobalamin), the generic name for a specific group of cobalt-containing corrinoids (compounds with a corrin ring system, a tetrapyrrole ring system resembling the porphyrin ring system of haemoglobin) with biological activity in humans. Vitamin B_{12} functions primarily as a coenzyme in intermediary metabolism and plays a specific role in amino acid

metabolism. One key symptom in vitamin B_{12} deficiency is macrocytic megaloblastic anaemia, which is indistinguishable from that seen in folate deficiency, because of the interrelated function of both vitamins. Another key symptom are neurological disorders (paraesthesia, leg weakness, memory loss). (EFSA 2006)

According to EFSA (EFSA 2006), the average dietary requirement for vitamin B_{12} is 1.0 µg/day, with a population reference intake (PRI) for adults of 1.4 µg/day. According to WHO/FAO (WHO 2004), the estimated average requirement for vitamin B_{12} is 2.0 µg/day for adults with a recommended dietary allowance of 2.4 µg/day.

The average dietary requirement for vitamin B_{12} of 1.0 µg/day corresponds to about 0.04 µg Co/day. It should be noted, however, that the human requirement for cobalt is not the ionic form of the metal, but for a preformed metallovitamin that cannot be synthesised from dietary metal.

3 Human toxicity

3.1 Single dose toxicity

3.1.1 Inhalation

No data have been located.

3.1.2 Oral intake

A 19-month-old male child who swallowed an unknown amount of a cobalt chloride solution died approximately 6.5 hours after ingestion, despite repeated induced vomiting, gastric lavage and supportive therapy (Jacobziner and Raybin 1961 – quoted form ATSDR 2004).

In a case report of a 6-year-old boy, who ingested approximately 1.7 mg of cobalt chloride, neutropenia (condition where there are fewer neutrophils than normal in the blood) by 7 hours post-exposure was reported (Mucklow et al. 1990 – quoted from ATSDR 2004).

3.1.3 Dermal contact

No data have been located.

3.2 Irritation and sensitisation

According to IUCLID (IUCLID 2000) industry labels cobalt sulphate "Irritating to the eyes"; no specific studies were reported into IUCLID.

Cobalt-induced dermatitis is well documented in the literature, and the studies indicate that cobalt is a skin sensitizer (Alomar et al. 1985, Bedello et al. 1984, Dooms-Goossens et al. 1980, Fischer and Rystedt 1983, Goossens et al. 2001, Kanerva et al. 1988, 1998, Kiec-Swierczyńska and Krecisz 2002, Marcussen 1963, Minamoto et al. 2002, Pryce and King 1990, Swennen et al. 1993, Romaguera et al. 1982, Valer et al. 1967 - quoted from ATSDR 2004).

It appears that the allergic properties of cobalt result mainly from exposure to the metal itself, rather than a salt, as daily repeated exposure to aqueous cobalt salts did not result in hand eczema in patients known to have cobalt allergy (Nielsen et al. 2000 - quoted from ATSDR 2004).

Allergic dermatitis was reported in cobalt-sensitized people following oral challenge with cobalt. Several patients with eczema of the hands were challenged orally with 1 mg cobalt (as cobalt sulphate) given in tablet form once per week for 3 weeks (0.014 mg Co/kg/day). A flaring of the eczema was considered to be a positive allergic response to cobalt (Veien et al. 1987 – quoted from ATSDR 2004).

3.3 Repeated dose toxicity

3.3.1 Inhalation

No human data were located that evaluated the toxicity of inhalation exposure specifically to soluble cobalt salts.

Following occupational exposure of humans to cobalt metal or cobalt-containing hard metal the primary target of exposure is the respiratory tract. These effects include decreased pulmonary function, asthma, interstitial lung disease, wheezing and dyspnoea. These effects were reported at occupational exposure levels ranging from 0.015-0.13 mg Co/m^3 (ATSDR 2004).

Other effects of occupational exposure of cobalt in humans include cardiomyopathy, congestion of liver, kidney and conjunctiva and allergic dermatitis, manifesting as eczema and erythema. For further information see ATSDR (2004).

3.3.2 Oral intake

In beer drinkers who ingested an average of 0.04 mg Co/kg/day (n = 50, Morin et al. 1971) to 0.14 mg Co/kg/day (n = 28, Alexander 1972) for a period of years (approximately 8-30 pints of beer each day), cardiomyopathy and deaths were observed. The first signs of the cardiomyopathy were gastrointestinal effects and included nausea, vomiting, and diarrhoea followed by sinus tachycardia, left ventricular failure, cardiogenic shock, diminished myocardial compliance, absence of a myocardial response to exercise or catecholamine, enlarged heart, pericardial effusion, and extensive intracellular changes (changes in the myofibers, mitochondria, glycogen, and lipids). The beer-cobalt cardiomyopathy appeared to be similar to alcoholic cardiomyopathy and beriberi (disease of the nervous system caused by lack of vitamin B$_1$), but the onset of beer-cobalt cardiomyopathy was very abrupt. The cardiomyopathy may also have been due to the fact that the beer-drinkers had protein-poor diets and may have had prior cardiac damage from alcohol abuse.
Death within several days of admission accounted for 18% of the deaths. Approximately 40-50% of the patients admitted to the hospital died within several years of diagnosis. In a follow-up study of four different sites, 0-43% of the survivors, depending on the site, showed a residual cardiac disability and 23-41% had abnormal electrocardiograms (Alexander 1972).
Liver injury was also evident in the patients, which was characterized by central hepatic necrosis accompanied by increased levels of serum bilirubin and serum enzymes. However, the hepatic injury may have resulted from ischemia, secondary to the cardiac effects of cobalt and/or from excessive alcohol consumption (Alexander 1972, Morin et al. 1971 – quoted from ATSDR).

Seventy-eight pregnant women were treated with 0.5-0.6 mg Co/kg/day (as cobalt chloride, alone or combined with iron) for 90 days, to prevent the decrease in haematocrit and haemoglobin levels commonly found during pregnancy. The treatment did not prevent the reduction in haematocrit and haemoglobin levels, however, a small percentage of those treated complained of gastric intolerance. Liver function tests were found to be normal (Holly 1955 – quoted from ATSDR 2004).

Six apparently normal men, ages 20-47, were exposed to a daily dose of cobalt chloride (as a 2% solution diluted in either water or milk) for up to 22 days. Five of

the six received 150 mg CoCl$_2$/day (68 mg Co/day) for the entire exposure period, while the sixth was started on 120 mg CoCl$_2$/day (54 mg Co/day) and later increased to 150 mg CoCl$_2$/day. Blood samples were obtained daily from free-flowing punctures of fingertips at least 2 hours after eating and at least 15 hours after the last dosage of cobalt. Blood was analyzed for red blood cell counts, haemoglobin percentage, leukocyte counts, reticulocyte percentages and thrombocyte counts. Exposure to cobalt resulted in the development of polycythemia in all six subjects, with increases in red blood cell numbers ranging from 0.5 to 1.19 million (~16-20% increase above pre-treatment levels). Polycythemic erythrocyte counts returned to normal 9-15 days after cessation of cobalt administration. Haemoglobin levels were also increased by cobalt treatment, though to a lesser extent than the erythrocyte values, with increases of 6-11% over pre-treatment values. In five of the six subjects, reticulocyte levels were elevated, reaching at least twice the pre-experiment values. Thrombocyte and total leukocyte counts did not deviate significantly from pre-treatment values (Davis and Fields 1958 – quoted from ATSDR 2004).

From the LOAEL of 1 mg/kg/day identified in this study by ATSDR, an intermediate-duration oral MRL of 1×10^{-2} mg/kg/day was derived.

In anephric patients (with resulting anaemia), treated with 0.16-1.0 mg Co/kg/day daily (as cobalt chloride) for 3-32 weeks, increased levels of erythrocytes were observed. This increase resulted in a decreased need for blood transfusions (Duckham and Lee 1976b, Taylor et al. 1977 – quoted from ATSDR 2004).

In 14 Québécois patients who died of myocardosis related to beer drinking, three thyroids were normal but 11 thyroids showed irregular follicle morphology and decreased follicular size (Roy et al. 1968 – quoted from ATSDR 2004).

Three of five patients who received cobalt therapy for sickle-cell anaemia or renal amyloidosis developed goitre. One case was severe, while four of five showed microscopic alterations of the thyroid gland. Two of the patients died from non-cobalt-related causes, while the other three recovered once the cobalt treatment ceased (Kriss et al. 1955 – quoted from ATSDR 2004).

In four cases of sickle-cell anaemia, the treatment with cobalt resulted in an enlargement of the thyroid gland, which was reversible upon cessation of cobalt therapy (Gross et al. 1955 – quoted from ATSDR 2004).

In several other cases where cobalt was used therapeutically for anaemia similar effects on the thyroid (including enlargement, hyperplasia and an increased firmness), have been reported (Chamberlain 1961, Little and Sunico 1958, Soderholm et al. 1968, Washburn and Kaplan 1964 – quoted from ATSDR 2004).

In a man, who was treated with 1.3 mg Co/kg daily (as cobalt chloride) for four series of treatments with a total duration of 6 weeks for pancytopenia and hypercellular bone marrow, severe visual disturbances (optic atrophy, impaired choroidal perfusion) were observed (Licht et al. 1972 – quoted from ATSDR 2004).

3.3.3 Dermal contact

See section *3.2.*

3.4 Toxicity to reproduction

3.4.1 Inhalation

No data have been located.

3.4.2 Oral intake

Pregnant women were treated with up to 0.6 mg Co/kg/day (as cobalt chloride) for 90 days to raise haematocrit and haemoglobin levels that are often depressed during pregnancy. No developmental effects on the foetuses were observed, however, examination of the foetuses was limited to the reporting of obvious birth defects, and exposure only occurred in the final trimester (Holly 1955 – quoted from ATSDR 2004).

3.4.3 Dermal contact

No data have been located.

3.5 Mutagenic and genotoxic effects

No data have been located.

3.6 Carcinogenic effects

3.6.1 Inhalation

Several studies have noted increased mortality rates resulting from lung cancer following occupational exposure to cobalt, either as a mixture of cobalt compounds or as hard metal. However, in the majority of these and other reported occupational studies, co exposure to other substances was common, and was unable to be corrected for in the analysis (ATSDR 2004).

Further details on cancer risk from inhalation exposure to cobalt can be found in ATSDR (2004) and IARC (2006).

3.6.2 Oral intake

In a survey assessing the correlation between cancer mortality and trace metals in water supplies throughout the United States (10 basins, 1-19 µg Co/l), no correlation was found between cancer mortality and the level of cobalt in the water (Berg and Burbank 1972 – quoted from ATSDR 2004).

3.6.3 Dermal contact

No data have been located.

4 Animal toxicity

4.1 Single dose toxicity

Significantly increased erythrocyte (polycythemia), haematocrit and haemoglobin levels were found in animals exposed to a single oral dose of 161 mg Co/kg bw (as cobalt chloride) (Domingo and Llobet 1984 – quoted from ATSDR 2004).

Hyperemia of the liver and cytoplasmic changes in hepatocytes (clumpy cytoplasm located along the cell membrane) were observed in rats exposed to a single oral dose of 68.2 mg Co/kg (as cobalt fluoride) or a single dose of 157.3 mg Co/kg (as cobalt oxide). Renal injury (histological alteration of the proximal tubules) was observed in rats exposed to a single oral dose of 42 mg Co/kg (as cobalt fluoride). Hypothermia occurred in rats exposed to a single oral dose of 157 mg Co/kg (as cobalt oxide) or a single dose of 110 mg Co/kg (as cobalt fluoride) (Speijers et al. 1982 – quoted from ATSDR 2004).

Moderate reduction in spontaneous activity, muscle tone, touch response, and respiration were observed in Wistar rats exposed to a single oral dose of 4.25 mg Co/kg (as cobalt chloride), while a mild reduction in the same parameters was observed after 19.4 mg Co/kg (as cobalt sulphate) (Singh and Junnarkar 1991 – quoted from ATSDR 2004).

Other physiological signs noted in LD_{50} studies include decreased activity, ataxia, diarrhea and salivation (FDRL 1984a, 1984b – quoted from ATSDR 2004).

Oral LD_{50}-values in animals are listed in Table 4.1.

Table 4.1. LD_{50}-values of cobalt compounds (from ATSDR 2004).

Compound/	Species	LD_{50} Mg Co/kg bw	Reference
Cobalt chloride			
	Rat (Sprague-Dawley)	161.1	Domingo and Llobet (1984)
	Rat (Wistar)	42.4	Singh and Junnarkar (1991)
	Rat (Wistar)	190	Speijers et al. (1982)
	Mouse (Swiss webster)	89.3	Singh and Junnarkar (1991)
Cobalt sulphate			
	Rat (Wistar)	194	Singh and Junnarkar (1991)
	Rat (Wistar)	161	Speijers et al. (1982)
	Mouse (Swiss webster)	123	Singh and Junnarkar (1991)
Cobalt carbonate			
	Rat (Wistar)	149	FDRL (1984b)

4.2 Irritation and sensitisation

According to IUCLID (IUCLID 2000) industry labels cobalt sulphate "Irritating to the eyes"; no specific studies were reported in IUCLID.

Single or multiple dermal exposures of BALB/c mice to cobalt chloride in dimethylsulfoxide or in ethanol resulted in an increased cellular proliferation in the local lymph node assay in a concentration-dependant manner (Ikarashi et al. 1992a – quoted from ATSDR 2004).

4.3 Repeated dose toxicity

The toxicity of cobalt compounds following repeated exposure have been extensively studied in a number of animal species using inhalation (rat, mouse, hamster, rabbit, guinea pig, dog, pig) and oral (rat, mouse, rabbit, guinea pig, dog) routes, in studies with durations ranging from 3 days to 2 years. Inhalation repeated dose toxicity studies are only briefly described in section 4.3.1. Further details of cobalt toxicity following inhalation can be found in ATSDR (2004).

4.3.1 Inhalation

In a 13-week inhalation study, F344 rats and B6C3F1 mice (10 animals/sex per group) were exposed to cobalt sulphate heptahydrate at concentrations of 0, 0.3, 1, 3, 10 or 30 mg/m^3 (calculated on the basis of the anhydrous salt, corresponding to 0, 0.11, 0.38, 1.1, 3.8 or 11 mg Co/m^3) 6 hours/day, 5 days/week for 13 weeks (NTP 1991).
The cobalt content in the urine of rats increased with increasing atmospheric cobalt exposure and the amount of cobalt excreted in urine over 16 hours of rats exposed to 0.3 mg/m^3 was approximately 10 times that excreted by controls.
The absolute and/or relative lung weights were significantly increased in rats from 0.3 mg/m^3 and in mice from 10 mg/m^3. Relative kidney weights were significantly increased in male rats at all exposure concentrations. Absolute and relative testis weights and the epididymal weight were significantly decreased in male mice at 30 mg/m^3.
Polythaemia (indicated by significant increases in erythrocytes, mean haemoglobin concentrations, and in the haematocrit value) was observed in rats from 3 mg/m^3; no consistent or dose-related haematological effects were observed in mice.
In mice, the number of abnormal sperm was significantly increased at 30 mg/m^3, and sperm motility was significantly reduced in mice from 3 mg/m^3. No significant effects on sperm motility, sperm counts, or the incidence of abnormal sperm were observed in male rats.
Histopathological lesions in the rat were limited to the respiratory tract and in mice generally limited to the respiratory tract (primarily severe necrotising injury with the larynx appearing to be the most sensitive tissue. In male mice, histopathological lesions were also observed in the testis (atrophy at 30 mg/m^3). Overall, a NOAEC could not be determined from these studies. The LOAEC was 0.3 mg/m^3 (0.11 mg Co/m^3).

4.3.2 Oral administration

Oral repeated dose toxicity studies on cobalt compounds are summarised in Table 4.3.1 and supplementary information on the studies are given in the text.
The NOAELs and LOAELs presented in this section are those stated in ATSDR (2004).

Table 4.3.1. Animal repeated dose toxicity studies on cobalt, oral administration

Species/ strain	Duration/ Dose levels/ Chemical form	Effects (mg/kg bw/day)	NOAEL [1] (mg/kg bw/day)	LOAEL [1] (mg/kg bw/day)	Reference
Rat Long-Evans	3 days 0, 20, 100 mg Co/kg bw/day Cobalt chloride in diet	100: ↓Body weight (<20%) (♂) Saccharin and food aversion (♂)	20 (bw, neuro)	100 (bw, neuro)	Wellman et al. (1984) in ATSDR (2004)
Rat	12-16 days 0, 10.6 mg Co/kg bw/day Cobalt chloride in drinking water	10.6: ↓ Serum glucose levels in diabetic rats	10.6 (bw)	10.6 (metab)	Saker et al. (1998) in ATSDR (2004)
Rat Wistar	30 days 0, 4.96 mg Co/kg bw/day Cobalt chloride in drinking water	4.96: Alterations in sympathetically-induced contractility of vas deferens		4.96 (neuro)	Mutafova-Yambolieva et al. (1994) in ATSDR (2004)
Rat Wistar	30 days 0, 6.44 mg Co/kg bw/day Cobalt nitrate in drinking water	6.44: Alterations in cholinergic sensitivity		6.44 (neuro)	Vassilev et al. (1993) in ATSDR (2004)
Rat CFY	3 weeks 0, 12.4 mg Co/kg bw/day Cobalt chloride by gavage	12.4: ↓ Body weight (8%) (♂) Cardiac damage (multifocal myocytolysis with degeneration of myofibrilles)		12.4 (cardio, bw)	Morvai et al. (1993) in ATSDR (2004)
Rat Sprague-Dawley	4 weeks 0, 3.79 mg Co/kg bw/day Cobalt chloride in diet	3.79: ↓ Body weight gain (45-65%) (♂) Atrophy of the thymus (♂)		3.79 (bw, immun)	Chetty et al. (1979) in ATSDR (2004)
Rat, 6/group SD	6 weeks, 7 days/week 0, 0.6, 2.5, 10 mg Co/kg bw/day Cobalt chloride in gelatine capsules	10: ↑ Erythrocyte number (29%) 2.5: ↑ Erythrocyte number (17%) 0.6: No change in erythrocyte number	0.6 (haemato)	2.5 (haemato)	Stanley et al. (1947) in ATSDR (2004)
Rat	8 weeks 0, 4.2 mg Co/kg bw/day in diet	4.2: ↓ Body weight gain (33%)		4.2 (bw)	Clyne et al. (1988) in ATSDR (2004)
Rat	8 weeks 0, 26 mg Co/kg bw/day Cobalt sulphate in diet	26: Degenerative heart lesions		26 (cardio)	Grice et al. (1969) in ATSDR (2004)
Rat Sprague-Dawley	8 weeks 0, 8.4 mg Co/kg bw/day Cobalt sulphate	8.4: ↓ Body weight (>20%)	8.4 (haemato)	8.4 (bw)	Pehrsson et al. (1991) in ATSDR (2004)
Rat Sprague-Dawley	57 days 0, 20 mg Co/kg bw/day	20: ↑ Latency during retention testing (♂)		20 (neuro)	Bourg et al. (1985) in ATSDR (2004)

Species/ strain	Duration/ Dose levels/ Chemical form	Effects (mg/kg bw/day)	NOAEL [1] (mg/kg bw/day)	LOAEL [1] (mg/kg bw/day)	Reference
	Cobalt chloride in drinking water				
Rat	57 days 0, 20 mg Co/kg bw/day Cobalt chloride in drinking water	20: ↑ Reactivity		20 (neuro)	Bourg et al. (1985) in ATSDR (2004)
Rat	69 days 0, 5, 20 mg Co/kg bw/day in diet	20: Changes in schedule training, conditioned suppression and mixed schedule training tests	5 (neuro)	20 (neuro)	Nation et al. (1983) in ATSDR (2004)
Rat (20 ♂)	3 months 0, 30.2 mg Co/kg bw/day Cobalt chloride in drinking water	No morphological changes in gastrointestinal system, liver, and skeletal muscle No effect on bw 30.2: ↑ Lung weight (33%) (no morphological or histological changes) ↑ Heart weight (9.4%) ↑ Haematocrit (29%)	30.2 (gastro, skel, hepatic, renal)	30.2 (resp, cardio, haemato)	Domingo et al. (1984) in ATSDR (2004)
Rat	4 months 0, 18 mg Co/kg bw/day Cobalt chloride by gavage	No morphological or histological changes No morphological changes in gastrointestinal system and liver 18: Erythrocytosis Tubular necrosis	18 (resp, cardio, gastro, hepatic)	18 (haemato, renal)	Holly (1955) in ATSDR (2004)
Rat	5 months, 5 days/week 0, 10 mg Co/kg bw/day Cobalt chloride by gavage	No effect on bw 10: ↑ Haemoglobin ↑ Liver weight (17%) Necrosis of renal tubular lining cells	10 (bw)	10 (haemato, hepatic, renal)	Murdock (1959) in ATSDR (2004)
Rat Sprague-Dawley	24 weeks 0, 8.4 mg Co/kg bw/day Cobalt sulphate in diet	8.4: Left ventricular hypertrophy and impaired ventricular function (♂)		8.4 (cardio)	Haga et al. (1996) in ATSDR (2004)
Rat	24 weeks 0, 8.4 mg Co/kg bw/day Cobalt sulphate in the diet	8.4: ↓ Number of enzymes in cardiac tissues (manganese-superoxide dismutase, succinate-cytochrome c oxidase, NADH-cytochrome c reductase, cytochrome c oxidase (significant) ↓ Mitochondrial ATP production rate			Clyne et al. (2001) in ATSDR (2004)
Rat	7 months, 6 days/week 0, 0.05, 0.5, 2.5 mg Co/kg bw/day Cobalt chloride by gavage	No morphological changes liver 2.5: ↑ Erythrocyte number (persistent) ↑ Latent reflex (pronounced) 0.5:	0.05 (haemato, immune, neuro) 2.5 (hepatic)	0.5 (haemato, immune, neuro)	Krasovskii and Fridlyand (1971) in ATSDR (2004)

Species/ strain	Duration/ Dose levels/ Chemical form	Effects (mg/kg bw/day)	NOAEL [1] (mg/kg bw/day)	LOAEL [1] (mg/kg bw/day)	Reference
		↑ Erythrocyte number (trancient) ↑ Haemoglobin ↓ Phagocytic activity ↑ Latent reflex (mildly) 0.05: No change in erythrocyte number			
Mouse Parkes	45 days 0, 26 mg Co/kg bw/day Cobalt chloride in drinking water	26: Necrosis and inflammation of thyroid (♀)		26 (endocr)	Shrivastava et al. (1996) in ATSDR (2004)
Mouse Swiss-Webster	3 months 0, 76.4 mg Co/kg bw/day Cobalt chloride in drinking water		76.4 (haemato) (♂)		Bryan and Bright 1973 in ATSDR (2004)
Guinea pig	5 weeks 0, 20 mg Co/kg bw/day Cobalt sulphate in diet	No effect on bw 20: ↑ Mortality (20-25%) Cardiomyopathy	20 (bw)	20 (death, cardio)	Mohiuddin et al. (1970) in ATSDR (2004)
Dog	4 weeks, 7 days/week 0, 5 mg Co/kg bw/day in diet	5: Polycythemia		5 (haemato)	Brewer (1940)

↓: Reduced ♀ : Female
↑: Increased ♂ : Male
Bd wt = body weight Haemato = haematological
Cardio =cardiovascular Metab = metabolic
Endocr = endocrine Skel = musculoskeletal
Gastro = gastrointestinal Immun = immunological
Neuro = neurological SD=Sprague-Dawley

1) The NOAELs and LOAELs presented in this section are those stated in ATSDR (2004).

Rat, 8 weeks, cobalt chloride (Stanley et al. 1947 – quoted from ATSDR 2004)
The dose levels were 0, 0.62, 2.5 or 10 mg Co/kg bw/day in gelatine capsules. Blood counts and haemoglobin levels were examined at the beginning of the experiment and at 2-week intervals. At 2.5 mg Co/kg bw/day, an increase in erythrocyte number was observed, increasing up to a maximum of 17% above pre-treatment values on week 6. At 10 mg Co/kg bw/day, increased erythrocyte numbers reached 29% above pre-treatment values at 8 weeks of exposure. Statistical analyses of the group means were not provided, and the study provided only mean values of the measurements, precluding statistical analysis.

Rat, 7 months, cobalt choride (Krasovskii and Fridyland 1971 – quoted from ATSDR 2004)
The dose levels were 0, 0.05, 0.5 or 2.5 mg Co/kg bw/day by gavage. Numerical data were not presented and statistical significance was not reported.

Guinea pig, 5 weeks, cobalt sulphate (Mohiuddin et al. 1970 – quoted from ATSDR 2004)
The dose was 0, 20 mg Co/kg bw/day in the diet. The study was designed to simulate conditions leading to beer-cobalt cardiomyopathy in humans. The animals were given cobalt sulphate alone or in combination with ethanol. The cardiomyopathy was characterized by abnormal EKGs, increased heart weights, lesions involving the pericardium, myocardium, and endocardium, and disfigured mitochondria. Alcohol did not intensify the cardiac effects.

4.3.3 Dermal contact

No data have been located

4.4 Toxicity to reproduction

4.4.1 Inhalation

The inhalation studies on toxicity to reproduction are summarised in Table 4.4.1. The NOAELs and LOAELs presented in this section are those stated in ATSDR (2004).

4.4.2 Oral intake

The oral studies on toxicity to reproduction are summarised in Table 4.4.2 and supplementary information on the studies are given in the text. The NOAELs and LOAELs presented in this section are those stated in the criteria documents.

Rat (GD 1-21), mouse (GD 6-15), rabbits (GD 6-20), cobalt sulphate by gavage (Szakamary et al. 2001 – quoted from IARC 2006)
The dose levels were 0, 9.5, 19 or 38 mg Co/kg bw/day (rats), 0, 19 mg Co/kg bw/day (mice) and 0, 7.6, 38 or 76 mg Co/kg bw/day (rabbits) by gavage. According to IARC, the used doses were relatively high and produced maternal toxicity and the interpretation of the data should be considered with caution.

Rat 98 days, cobalt in diet (Mollenhauer et al. 1985 – quoted from IARC 2006).
Neither ATSDR nor IARC stated which cobalt compound was used in the study. According to IARC the dose level was 31.8 mg/kg bw/day in the diet; however, it was not stated if this concentration was expressed as cobalt or as the used cobalt

compound. Cobalt was not detected in testis and the degenerative changes in the testis were therefore, according to IARC, considered secondary to hypoxia due to blockage of veins and arteries by red blood cells and changes in permeability of the vasculature and seminiferous tubules.

<u>Mouse, 13 weeks, cobalt chloride (Pedigo et al. 1988 – quoted from IARC 2006)</u>
According to IARC the dose levels were 0.1-0.4 mg/l cobalt chloride in drinking water. The mice showed marked dose-related decreases in fertility, testicular weight and sperm concentration and increased circulating levels of testosterone.

4.4.3 Dermal contact

No data have been located.

4.5 Mutagenic and genotoxic effects

4.5.1 *In vitro* studies

<u>For cobalt chloride</u> negative results were obtained for induced reverse mutations in *Salmonella typhimurium* (Ogawa et al 1986, Wong 1988, Arlauskas et al. 1985, Tso and Fung 1981, Arlauskas et al. 1985, Mochizuki and Kada 1982 – all references quoted in IARC 2006).
In *Escherichia coli,* negative results were obtained for mutations and reverse mutations (Kada and Kanematsu 1978, Arlauskas et al. 1985, Leitao etal. 1993 – all references quoted in IARC 2006).
In *Bacillus subtilis,* negative results were obtained for reverse mutations (Inoue et al.1981 – quoted in IARC 2006).

In *Saccharomyces cerevisiae,* <u>cobalt chloride</u> showed negative results for erythromycin-resistant mutations (Putrament et al. 1977 – quoted in IARC 2006) and *ilv* gene mutations (Fukunaga et al. 1982, Singh 1983 – quoted in IARC 2006), whereas positive results were obtained for induced *trp* gene conversions (Fukunaga et al. 1982, Singh 1983, Kharab and Singh 1985) and petite p(-) mutations in mitochondrial DNA (Prazmo et al. 1975, Putrament et al. 1977, Egilsson et al. 1979, Kharab and Singh 1987, Lindegren et al. 1958 – all references quoted in IARC 2006).

In Chinese hamster V79 cells, <u>cobalt chloride</u> induced mutations at the *Hprt* locus (Miyaki et al. 1979, Hartwig et al. 1990 – quoted in IARC 2006) but not at the *8AG* (Yokoiyama et al. 1990 – quoted in IARC 2006) or the *Gpt* loci (however, in the same *Gpt* locus in a transgenic Chinese hamster cell line (G12), lower concentrations did induce gene mutations) (Kitahara et al. 1996 – quoted in IARC 2006).
In mouse lymphoma L5178Y cells, gene mutations were induced in the *Tk* locus (Amacher and Pailllet 1980 – quoted in IARC 2006).

In mammalian cells (Chinese hamster ovary cells, HeLa cells, mouse macrophage-like cells P388D$_1$), <u>cobalt chloride</u> induced DNA-protein cross-linkage, DNA strand breakage and sister chromatid exchange in most studies (Hamilton-Koch et al. 1986, Hartwig et al. 1990, Wedrychowski et al. 1986, Andersen 1983 – all references quoted in IARC 2006).

Table 4.4.1 Toxicity to reproduction, inhalation

Species/strain	Duration/ Exposure levels/ Chemical form	Effects (mg/m^3)	NOAEL [1] (mg/m^3 kg bw/day)	LOAEL [1] (mg//m^3 kg bw/day)	Reference
Rat F344/N 5/group/sex	16 days 0, 0.04, 0.19, 1.9, 19, 76 mg Co/m^3 Cobalt sulphate heptahydrate	19: Testicular atrophy, characterized by a decresed number of cells in the seminiferous tubules and atypical germinal epithelial cells in the epididymal ducts		11 (repro) (ATSDR)	NTP (1991)
Rat F344/N 10/group/sex	13 weeks 0, 0.11, 0.38, 1.1, 3.8, 11 mg Co/m^3 Cobalt sulphate heptahydrate	No statistically significant effects on sperm motility, sperm counts or incidence of abnormal sperm (♂) 11: Longer average estrous cycle (not significant) (♀)			NTP (1991)
Rat F344/N 50/group/sex	2 years 0, 0.11, 0.38, 1.1 mg Co/m^3 Cobalt sulphate heptahydrate	No histopathological changes in ovary, preputial gland, prostate gland, seminal vesicle, testes/epididymides, or uterus were reported.			NTP (1998))
Mouse B6C3F$_1$ 5/group/sex	16 days 0, 0.04, 0.19, 1.9, 19, 76 mg Co/m^3 Cobalt sulphate heptahydrate	No testicular atrophy (♂)			NTP (1991)
Mouse B6C3F$_1$ 10/group/sex	13 weeks 0, 0.11, 0.38, 1.1, 3.8, 11 mg Co/m^3 Cobalt sulphate heptahydrate	11: Testicular atrophy, characterized by loss of germinal epithelium in the seminiferous tubules. More severely affected testes also contained foci of mineralization. (♂) ↓ Testis and epididymal weight 0.38: ↓ Sperm motility (♂) ↑ number of abnormal sperm		0.63 (repro) (♂) (ATSDR)	NTP (1991)
Mouse B6C3F$_1$ 50/group/sex	2 years 0, 0.11, 0.38, 1.1 mg Co/m^3 Cobalt sulphate heptahydrate	No histopathological changes in ovary, preputial gland, prostate gland, seminal vesicle, testes/epididymides, or uterus were reported.			NTP (1998)

↓: Reduced   ♀ : Female
↑: Increased   ♂ : Male
Repro = reproduction

1) The NOAELs and LOAELs presented in this section are those stated in ATSDR (2004).

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Table 4.4.2 Toxicity to reproduction, oral administration

Species/strain	Duration/ Dose levels/ Chemical form	Effects (mg Co/kg bw/day)	NOAEL[1] (mg/kg bw/day)	LOAEL[1] (mg/kg bw/day)	Reference
Rat SD	GD 6-15 0, 24.8 mg Co/kg bw/day Cobalt chloride by gavage	24.8: No effect on foetal growth or survival	24.8 (develop)		Paternian et al. (1988) in ATSDR (2004)
Rat SD	GD 1-21 9.5, 19, 38 mg Co/kg bw/day Cobalt sulphate by gavage	No changes in foetal death rates, maternal body weigh gain, average litter size, or average foetal or placental weights 38: ↑ Retarded foetal body weights (dose-related trend) Maternal toxicity (↑ relative weights of liver, adrenals and spleen) 19: ↑ Visceral retardation (significant) 9.5: ↑ Skeletal retardation (significant)			Szakmary et al. (2001) in ATSDR 2004 and IARC (2006)
Rat Wistar (♀)	GD 14 - LD 21 0, 5.4, 10.9, 21.8 mg Co/kg bw/day Cobalt chloride	No teratogenic effects 5.4: Stunted growth (pups) ↓ Survival (pups) Maternal toxicity			Domingo et al. (1985b) in ATSDR (2004) and IARC (2006)
Rat	69 days 0, 5, 20 mg Co/kg bw/day Cobalt chloride in diet	20: Testicular atrophy (♂)	5 (repro)	20 (repro)	Nation et al. (1983) in ATSDR (2004)
Rat Sprague-Dawley	90 days 0, 30.2 mg Co/kg bw/day Cobalt chloride in drinking water	30.2: ↓ Testicular weight (26%) (♂)		30.2 (repro)	Domingo et al. (1984) in ATSDR (2004)
Rat Sprague-Dawley	98 days 0, 20 mg Co/kg bw/day Cobalt chloride in diet	20: Pronounced histological alteration of seminiferous tubules (♂)		20 (repro)	Corrier et al. (1985) in ATSDR (2004)
Rat SD	98 days, 7 days/week 0, 13.25 mg Co/kg bw/day In diet	13.25: Testicular degeneration		13.25 (repro)	Mollenhauer et al. (1985) in ATSDR (2004) (supplementary information in text)
Mouse	GD 8-12 0, 81.7 mg Co/kg bw/day Cobalt chloride by gavage	81.7: No effect on foetal growth or mortality	81.7 (develop)		Seidenberg (1986) in ATSDR (2004)

Species/strain	Duration/ Dose levels/ Chemical form	Effects (mg Co/kg bw/day)	NOAEL [1] (mg/kg bw/day)	LOAEL [1] (mg/kg bw/day)	Reference
Mouse C57BL	GD 6-15 0, 19 mg Co/kg bw/day Cobalt sulphate by gavage	No changes in litter size, post-implantation loss, or average foetal or placental weights 19: ↑ Retarded foetal body weights ↑ Skeletal retardation (significant) Maternal toxicity (↓ weight gain, non-significant)			Szakmary et al. (2001) in ATSDR (2004) and IARC (2006)
Mouse B6C3F1	10 weeks 0, 58.9 mg Co/kg bw/day Cobalt chloride in drinking water	58.9: ↓ Pregnant females, fertility and pups per litter (♂)		58.9 (repro)	Pedigo et al. (1993) in ATSDR (2004)
Mouse CD-1	13 weeks 0, 43.4 mg Co/kg bw/day Cobalt chloride in drinking water	43.4: Irreversible testicular degeneration (♂)		43.4 (repro)	Anderson et al. (1992) in ATSDR (2004)
Mouse CD-1	13 weeks 0, 43.4 mg Co/kg bw/day Cobalt chloride in drinking water	43.4: Testicular degeneration (♂)		43.4 (repro)	Anderson et al. (1993) in ATSDR (2004)
Mouse CD-1 (♂)	13 weeks 0, 23 mg Co/kg bw/day Cobalt chloride in drinking water	23: Reversible testicular degeneration (♂) Significantly stimulated testosterone (no dose-response)		23 (repro)	Pedigo et al. (1988) in ATSDR (2004)
Rabbit Nw Zealand	GD 6-20 0, 7.6, 38, 76 mg Co/kg bw/day Cobalt sulphate by gavage	38: Nearly complete maternal lethality and complete foetal loss 7.6: ↑ Foetal resorption ↑ Retarded foetal body weight ↑ Skeletal retardation (significant) Maternal toxicity (mortality and ↓ weight gain, significant)			Szakmary et al. (2001) in ATSDR (2004) and IARC (2006)

↓: Reduced ♀ : Female
↑: Increased ♂ Male
Repro = reproduction Develop = developmental
GD = gestation day LD = Lactation day

1) The NOAELs and LOAELs presented in this section are those stated in ATSDR (2004).

In cultured human cells (white blood cells, diploid fibroblasts, mononuclear leukocytes, lymphocytes), positive results were obtained for induction of DNA breakage and sister chromatid exchange (McLean et al. 1982, Hamilton-Koch et al. 1986, De Boeck et al. 1998, Andersen 1983 – all references quoted in IARC 2006); chromosomal aberrations were not observed in cultured human cells at the low concentrations used in the various assays (IARC 2006).
Cell transformation of C3H10T1/2 mouse fibroblast cells has been induced *in vitro* (Doran et al. 1998 – quoted in IARC 2006).

For cobalt nitrate negative results were obtained for induction of chromosome aberrations (numerical) in human diploid fibroblasts and mononuclear leucocytes (Paton and Allison 1972 – quoted in IARC 2006).

For cobalt sulphate a negative and a positive result were obtained for induced reverse mutations in *Salmonella typhimurium* (Zeiger et al. 1992 – quoted in IARC 2006).
Positive results were obtained for induction of chromosomal aberrations and aneuploidy in *Allium cepa* (plant cells) (Gori and Zucconi 1957 – quoted in IARC 2006), chemical changes in bases in purified calf thymus DNA and in isolated human chromatin in the presence of hydrogen peroxide (Nackerdien et al. 1991 – quoted in IARC 2006), and cytoskeletal perturbation of microtubules and microfilaments in mouse fibroblasts (Chou 1989 – quoted in IARC 2006).
Cell transformation of Syrian hamster embryo cells has been induced *in vitro* (Kerckaert et al. 1996 – quoted in IARC 2006).

4.5.2 *In vivo* studies

For cobalt chloride a negative result was obtained for gene mutations in a wing spot test mwh/TM3 in *Drosophila melanogaster* whereas cobalt chloride induced mitotic recombination in this test system (Ogawa et al. 1994 – quoted in IARC 2006).
In male BALB/c AnNCrj mice, micronucleus formation and enhanced micronucleus formation (compared to other mutagens) was observed in the bone marrow thirty hours after a single intraperitoneal injection of 12.4 or 22.3 mg Co/kg bw, but not at 6.19 mg/kg (Suzuki et al. 1993 – quoted in IARC 2006, ATSDR 2004).
Intraperitoneal injection of cobalt chloride induced aneuploidy (pseudodiploidy and hyperploidy) in bone marrow and testes of Syrian hamsters (Farah 1983 – quoted in IARC 2006).
Exposure of male Swiss mice to a single oral dose of 0, 4.96, 9.92, or 19.8 mg Co/kg bw resulted in significantly increased percentages of both chromosomal breaks and chromosomal aberrations in bone marrow cells, with significant linear trends toward increasing aberrations with increased exposure (Palit et al. 1991a, 1991b, 1991c, 1991d – quoted from ATSDR 2004).
In male and female F344 rats given an intraperitoneal injection of 3 or 6 mg Co/kg bw, increased levels of oxidatively-damaged DNA bases were noted in the liver, kidney, and to a lesser extent, the lung at two or 10 days after exposure (Kasprzak et al. 1994 – quoted from ATSDR 2004).

For cobalt nitrate positive results were obtained for induction of gene mutations, chromosomal deletion, and non disjunction or mitotic recombination in a SMART test in *Drosophila melanogaster* (Yeºilada 2001 – quoted in IARC 2006).

4.5.3 IARC conclusion

The IARC has concluded: "*The results of genotoxicity assays with a variety of cobalt salts demonstrate the mutagenic potential of these salts bout in vitro and in vivo*". The conclusion was based on the results of numerous genotoxicity studies *in vitro* and *in vivo*. (IARC 2006).

4.6 Carcinogenic effects

4.6.1 Inhalation

Rats and mice (50/group/sex) were exposed to 0, 0.3, 1.0 or 3.0 mg/m^3 (0, 0.11, 0.38 or 1.1 mg Co/m^3, as cobalt sulphate heptahydrate) for 2 years, 5 days/week, 6 hours/day. A significantly increased incidence of alveolar/bronchiolar neoplasms was noted in male rats exposed to 1.1 mg Co/m^3, in female rats exposed from 0.38 mg Co/m^3 and in mice of both sexes exposed to 1.1 mg Co/m^3. In addition, a marginal increase in the incidences of pheochromocytomas of the adrenal medulla (benign, complex or malignant) was observed in male and female rats exposed to 0.38 and 1.1 mg Co/m^3, respectively (NTP 1998).

4.6.2 Oral intake

No data have been located.

4.6.3 Dermal contact

No data have been located.

4.6.4 Update on the genotoxicity and carcinogenicity of cobalt compounds

A review of the studies published since the IARC assessment in 1991 (genotoxicity, experimental carcinogenesis, and epidemiology) has been published by Lison et al. (2001). The authors noted that two different mechanisms of genotoxicity (DNA breakage induced by cobalt metal and especially hard metal particles, and inhibition of DNA repair by cobalt(II) ions) contribute to the carcinogenic potential of cobalt compounds. The authors concluded that there is evidence that soluble cobalt(II) cations exert a genotoxic and carcinogenic activity *in vitro* and *in vivo* in experimental systems, but evidence in humans is lacking.

5 Regulations

5.1 Ambient air

Denmark (C-value):   Inorganic cobalt compounds in dust:
0.0005 mg/m^3 (MST 2002).

5.2 Drinking water

Denmark: -

WHO: -

5.3 Soil

Denmark: -

The Netherlands: Target value: 20 mg/kg dm (NL 1994).
Intervention value: 240 mg/kg dm (NL 1994).

5.4 Occupational Exposure Limits

Denmark: Inorganic cobalt compounds (as Co): 0.01 mg/m^3 (At 2007)

ACGIH: 0.02 mg/m^3 (ACGIH 2001)

5.5 Classification

Cobalt dichloride, cobalt sulphate, cobalt nitrate, cobalt carbonate, cobalt acetate: Carc. Cat. 2; R49, Muta. Cat. 3; R68, Repr. Cat. 2; R60, Xn; R22, R42/43 N; R50/53 (ECB 2009).

5.6 IARC

Cobalt sulphate and other soluble cobalt(II) salts are possibly carcinogenic to humans (Group 2B). There is sufficient evidence in experimental animals for the carcinogenicity of cobalt sulphate based on increased incidences of alveolar/bronchiolar neoplasms in rats and mice after inhalation. (IARC 2006).

5.7 EFSA

The Scientific Panel on Additives and Nutrient Sources added to Food (ANS) of EFSA has assessed the safety of cobalt(II) chloride hexahydrate added for nutritional purposes as a source of cobalt in food supplements. The ANS Panel concluded: *"Given the toxicological profile of cobalt(II) chloride hexahydrate, including genotoxicity and carcinogenicity, the proposed uses of cobalt(II) chloride hexahydrate*

added for nutritional purposes in food supplements as a source of cobalt are of safety concern." (EFSA 2009).

5.8 ATSDR

ATSDR derived an intermediate duration oral minimal risk level (MRL) of 0.01 mg/kg bw/day based on a LOAEL of 1 mg/kg bw/day for polycythemia in human volunteers (Davis and Fields 1958) and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for intraspecies variations) (ATSDR 2004).

6 Summary and evaluation

6.1 Description

Cobalt is a naturally occurring metal. It exists in the oxidation states 0, +2 and +3 and belongs to the group of transition elements of the periodic table together with iron and nickel. Cobalt usually exists under naturally conditions associated with other elements.

6.2 Environment

With an average concentration of 20-25 mg/kg, cobalt is the 33rd most abundant element in the earth's crust. The estimated natural content of cobalt in fossil fuels is 5 mg/kg in coal and 0.2 mg/kg in oil. Cobalt is naturally occurring in seawater and in some surface water and groundwater at low levels. Anthropogenic sources of cobalt release into water include disposal of cobalt-containing wastewater and atmospheric deposition from activities such as the burning of fossil fuels and smelting and refining of metals. In the US, measured levels in most drinking water samples were <1-2 µg/l although levels up to 107 µg/l were recorded. In Denmark, the concentration of cobalt was measured in a few samples in the area of Copenhagen. With the exception of one sample with a concentration of 20 µg Co/l, all were below the detection limit (MST 1986b).

Cobalt occurs naturally in soil. Anthropogenic sources of cobalt release into soil include activities such as the mining and processing of cobalt-bearing ores, the application of cobalt-containing sludge or phosphate fertilizers, the disposal of cobalt-containing wastes and atmospheric deposition from activities such as the burning of fossil fuels and smelting and refining of metals. In the US, the average concentration of cobalt measured in soils was 7.2 mg/kg. Most soils contain 1-40 mg Co/kg. In Denmark, the concentrations of cobalt measured at 44 farmlands were in the range 0.4–5 mg Co/kg (dry weight) with an average concentration of 2.1 mg/kg (MST 1986a).

In Sweden, the levels of cobalt in 50 different food items ranged from <1-860 µg Co/kg. In Denmark, the median concentration of cobalt was 1.51 µg Co/kg in onions and 4.6 µg Co/kg in peas.

Available data indicate that cobalt is not taken up appreciably by plants and does not bio magnify up the food chain.

6.3 Human exposure

Cobalt is a component of vitamin B_{12}. Vitamin B_{12} occurs almost entirely in food of animal origin and constitutes only a very small fraction of cobalt intake. For adults the Recommended Dietary Allowance (RDA) for vitamin B_{12} is 2.4 µg/day corresponding to 0.1 µg of cobalt.

Using the highest reported concentration of cobalt in Danish ground water of 20 µg/l (MST 1986b), and the consumption rate of 0.08 l/kg bw/day (for children

1-10 years old), the intake from drinking water would be 1.6 µg Co/kg bw/day (assuming no dilution of groundwater). For an adult (body weight of 70 kg), the daily exposure of cobalt from drinking water would be 112 µg.

The estimated average daily intake of cobalt from the diet was reported to be 11 µg/day (range 4-15 µg/day) in Canada and 7.5 (adults) and 7.3 (children) µg/day in France.

Using the highest measured average cobalt concentration of 48 ng/m^3 recorded at the site of a nickel refinery and assuming the inhalation rate as 0.5 m³/kg bw/day (for children 1-5 years old), the exposure to cobalt from air would be 24 ng Co/kg bw/day. For an adult (body weight of 70 kg), the daily exposure of cobalt from air would be 1.68 µg.

6.4 Toxicokinetics

In humans, the gastrointestinal absorption of cobalt varied between 18-97% of the given dose based on the type and dose of cobalt compound given and the nutritional status of the subjects. Iron deficiency was shown to increase absorption of cobalt from the gastrointestinal tract (31-71% in iron deficient individuals, 18-44% in controls) whereas simultaneous administration of cobalt and iron reduced the amount of cobalt absorbed. Absorbed cobalt is transported throughout the body with the highest cobalt concentration found in the liver, followed by the kidney, and is excreted via the urine. The total body burden of cobalt was estimated to be 1.1-1.5 mg. About 0.11 mg cobalt was found in the liver.

In several oral studies in rats, cobalt chloride was absorbed in the range of 13-34%. In rats and guinea pigs, the absorption of cobalt from the gastrointestinal tract was 3- to 15-fold greater in younger animals (1-60 days of age) than in adult animals (200 days of age). Reported faecal elimination levels in rats of cobalt chloride ranged from 70-83% of the oral administered dose, with urinary excretion accounting for the majority of the remainder of the dose.

In pregnant rats, oral exposure to cobalt sulphate showed a dose-dependent increase in cobalt levels in foetal blood and amniotic fluid. In lactating dairy cows, 0.26 and 0.012% of an oral dose of cobalt chloride was recovered in the urine and milk, respectively, by day 70 post-exposure.

6.5 Human toxicity

6.5.1 Single dose toxicity

In a case report of a 6-year-old boy, who ingested approximately 1.7 mg of cobalt chloride, neutropenia by 7 hours post-exposure was reported.

6.5.2 Irritation and sensitisation

Cobalt-induced dermatitis is well documented in the literature, and the studies indicate that cobalt is a skin sensitizer. In cobalt-sensitized people challenged orally with 1 mg cobalt (as cobalt sulphate) given in tablet form once per week for 3 weeks (0.014 mg Co/kg bw/day), an observed flaring of eczema was considered to be a positive allergic response to cobalt.

6.5.3 Repeated dose toxicity

In beer drinkers who ingested an average of 0.04-0.14 mg Co/kg/day for a period of years (approximately 8-30 pints of beer each day), cardiomyopathy and deaths were observed. This beer-cobalt cardiomyopathy appeared to be similar to alcoholic cardiomyopathy and beriberi (disease of the nervous system caused by lack of vitamin B_1), but the onset of beer-cobalt cardiomyopathy was very abrupt. The cardiomyopathy may also have been due to the fact that the beer-drinkers had protein-poor diets and may have had prior cardiac damage from alcohol abuse.

Seventy-eight pregnant women were treated with 0.5-0.6 mg Co/kg/day (as cobalt chloride, alone or combined with iron) for 90 days, to prevent the decrease in haematocrit and haemoglobin levels commonly found during pregnancy. The treatment did not prevent the reduction in haematocrit and haemoglobin levels, however, a small percentage of those treated complained of gastric intolerance. Liver function tests were found to be normal.

Six apparently normal men, ages 20-47, were exposed to a daily dose of cobalt chloride (2% solution) for up to 22 days. Five of the six received 150 mg $CoCl_2$/day (68 mg Co/day) for the entire exposure period, while the sixth was started on 120 mg $CoCl_2$/day (54 mg Co/day) and later increased to 150 mg/day. All six subjects developed polycythemia with increases in red blood cell numbers of approximately 16-20% above pre-treatment levels. Polycythemic erythrocyte counts returned to normal 9-15 days after cessation of cobalt administration. Haemoglobin levels were increased 6-11% over pre-treatment values. In five of the six subjects, reticulocyte levels were elevated, reaching at least twice the pre-experiment values. Thrombocyte and total leukocyte counts did not deviate significantly from pre-treatment values.

In anephric patients (with resulting anaemia), treated with 0.16-1.0 mg Co/kg/day daily (as cobalt chloride) for 3-32 weeks, increased levels of erythrocytes were observed. This increase resulted in a decreased need for blood transfusions.

In several cases where cobalt was used therapeutically for anaemia, effects on the thyroid, including enlargement, microscopic alterations, hyperplasia, and an increased firmness, were reported.

6.5.4 Toxicity to reproduction

In pregnant women, treated with up to 0.6 mg Co/kg/day (as cobalt chloride) for 90 days to raise haematocrit and haemoglobin levels, no developmental effects on the foetuses were observed. However, examination of the foetuses was limited to the reporting of obvious birth defects, and exposure only occurred in the final trimester.

6.5.5 Mutagenic and genotoxic effects

No human data regarding mutagenic and genotoxic effects following exposure to cobalt compounds have been found.

6.5.6 Carcinogenic effects

In a survey assessing the correlation between cancer mortality and trace metals in water supplies throughout the United States (10 basins, 1-19 µg Co/l), no correlation was found between cancer mortality and the level of cobalt in the water.

6.6 Animal toxicity

6.6.1 Single dose toxicity

In mice, reported oral LD_{50}-values were 89.3 mg Co/kg bw for cobalt chloride and 123 mg Co/kg bw for cobalt sulphate. In rats, reported oral LD_{50}-values were 42.4, 161.1 and 190 mg Co/kg bw for cobalt chloride, 161 and 194 mg Co/kg bw for cobalt sulphate and 149 mg Co/kg bw for cobalt carbonate.

6.6.2 Irritation and sensitisation

No relevant animal data regarding irritation or sensitisation have been located.

6.6.3 Repeated dose toxicity

The toxicity of cobalt compounds following repeated exposure was studied in rat, mouse, rabbit, guinea pig, and dog using the oral route (dietary, drinking water, gavage), in studies with durations ranging from 3 days to 7 months.

Respiratory effects have been observed in rats at dose levels from 30.2 mg Co/kg bw/day (increased lung weight – cobalt chloride in drinking water for 3 months).

Cardiovascular effects have been observed in rats at dose levels from 12.4 mg Co/kg bw/day (multifocal myocytolysis with degeneration of myofibrilles – cobalt chloride by gavage for 3 weeks) and in guinea pigs at 20 mg Co/kg bw/day (cardiomyopathy – cobalt sulphate in diet for 5 weeks).

Haematological effects have been observed in rats at dose levels from 0.05 mg Co/kg bw/day (increased red blood cells and haemoglobin – cobalt chloride by gavage for 7 months) and in dogs at 5 mg Co/kg bw/day (polycythemia – in diet for 4 weeks).

Hepatic effects have been observed in rats at 10 mg Co/kg bw/day (increased weight (17%) – cobalt chloride by gavage for 5 months).

Immunological effects have been observed in rats from 0.5 mg Co/kg bw/day (decreased phagocytic ability – cobalt chloride by gavage for 7 months).

Neurotoxicity has been observed in rats from 0.5 mg Co/kg bw/day (mildly increased latent reflex – cobalt chloride by gavage for 7 months).

6.6.4 Toxicity to reproduction

Following oral administration (dietary, drinking water, gavage), developmental effects have been observed in rats at dose levels from 5.4 mg Co/kg bw/day (stunted growth and decreased survival of pups – cobalt chloride on GD 14 - LD 21), in mice from 19 mg Co/kg bw/day (increased retarded foetal body weights –

cobalt sulphate on GD 1-20) and in rabbits from 7.6 mg Co/kg bw/day (increased mortality and foetal resorption and retarded foetal body weight – cobalt sulphate on GD 1-20). In studies with developmental effects maternal toxicity (decreased weight gain, mortality or increased weight of liver, adrenals and spleen) was also present. Effects in reproductive organs have been observed in rats at dose levels from 13.25 mg Co/kg bw/day (testicular degeneration – cobalt in diet for 98 days) and in mice at dose levels from 23 mg Co/kg bw/day (reversible testicular degeneration and stimulated serum testosterone – cobalt chloride in drinking water for 13 weeks).

6.6.5 Mutagenic and genotoxic effects

Cobalt(II) chloride was generally non-mutagenic in bacteria (*Salmonella typhimurium, Escherichia coli, Bacillus subtilis*) and yeast (*Saccharomyces cerevisiae*).
For cobalt(II) sulphate a negative and a positive result were obtained for reverse mutations in *Salmonella typhimurium*.

Exposure to cobalt(II) chloride resulted in mutations in Chinese hamster V79 cells at the *Hprt* locus but not at the *8AG* or *Gpt* loci; however, a positive response was observed at the *Gpt* locus in a transgenic Chinese hamster cell line (G12). Gene mutations were also induced at the *Tk* locus in mouse lymphoma L5178Y cells. Clastogenic effects were observed in mammalian cells, including DNA breakage and sister chromatid exchange in human white blood cells, diploid fibroblasts, mononuclear leukocytes and lymphocytes, and DNA-protein cross-linkage, DNA strand breakage and sister chromatid exchange in Chinese hamster ovary cells, HeLa cells, mouse macrophage-like cells. Chromosomal aberrations were not observed in cultured human cells.
Cell transformation of C3H10T1/2 mouse fibroblast cells has been induced *in vitro*.

Exposure to cobalt(II) sulphate resulted in induction of chromosomal aberrations and aneuploidy in *Allium cepa* (plant cells), chemical changes in bases in purified calf thymus DNA and in isolated human chromatin in the presence of hydrogen peroxide, cytoskeletal perturbation of microtubules and microfilaments in mouse fibroblasts, and cell transformation of Syrian hamster embryo cells has been induced *in vitro*.

For cobalt(II) nitrate, negative results were obtained for induction of chromosome aberrations (numerical) in human diploid fibroblasts and mononuclear leucocytes.

In *Drosophila melanogaster*, cobalt(II) chloride showed a negative result for gene mutations in a wing spot test whereas it induced mitotic recombination in this test system. For cobalt(II) nitrate positive results were obtained for induction of gene mutations, chromosomal deletion, and non disjunction or mitotic recombination in a SMART test in *Drosophila melanogaster*.

In vivo, cobalt(II) chloride administered by intraperitoneal injection induced aneuploidy in the bone marrow and testes of Syrian hamsters, and enhanced micronuclei formation in the bone marrow in male BALB/c mice, and enhanced the micronuclei formation frequencies induced by other mutagens. Oral exposure to male mice resulted in both chromosomal breaks and chromosomal aberrations in bone marrow cells.
Increased levels of oxidatively-damaged DNA bases were noted in the liver, kidney, and to a lesser extent, the lung in rats given an intraperitoneal injection.

The IARC has (in 2006) concluded: "*The results of genotoxicity assays with a variety of cobalt salts demonstrate the mutagenic potential of these salts both in vitro and in vivo*". The conclusion was based on the results of numerous genotoxicity studies *in vitro* and *in vivo*.

6.6.6 Carcinogenic effects

No oral carcinogenicity studies with cobalt compounds in animals have been located.

Following inhalation of cobalt sulphate heptahydrate a significantly increased incidence of alveolar/bronchiolar neoplasms was observed in male rats exposed to 1.1 mg Co/m^3, in female rats exposed to 0.38 mg Co/m^3 and in mice of both sexes exposed to 1.1 mg Co/m^3.

A recent review concluded that there is evidence that soluble cobalt(II) cations exert a genotoxic and carcinogenic activity *in vitro* and *in vivo* in experimental systems, but evidence in humans is lacking.

6.7 Evaluation

The general population is primarily <u>exposed</u> to cobalt from food and drinking water.

In humans, the gastrointestinal <u>absorption</u> of cobalt varied between 18-97% of a given dose based on the type and dose of cobalt compound and the nutritional status of the subjects. Absorbed cobalt is transported throughout the body with the highest concentration found in the liver and kidney. Absorbed cobalt is excreted via the urine. Animal studies indicate that transfer of cobalt through the placenta of exposed mothers can take place.

The <u>acute toxicity</u> of cobalt compounds in experimental <u>animals</u> is high with reported oral LD_{50}-values ranging from 42.4 to 194 mg Co/kg bw in rats and from 89.3 to 123 mg Co/kg bw in mice. Signs of acute cobalt toxicity were polycythemia, hyperaemia of the liver, renal injury, hypothermia, reduction in spontaneous activity, ataxia, diarrhoea and salivation.

A flaring of eczema was considered to be a positive <u>allergic response</u> to cobalt in cobalt-sensitized people challenged orally with 1 mg cobalt (as cobalt sulphate) given in tablet form once per week for 3 weeks (0.014 mg Co/kg bw/day).

<u>Repeated exposure</u> to cobalt compounds in humans and/or animals resulted in respiratory, cardiovascular, gastrointestinal, haematological, hepatic, renal, endocrine, dermal, ocular, hypothermic and body weight effects.

A consistent finding in humans following oral exposure to cobalt appears to be polycythemia. This effect has been observed in both normal subjects (Davis and Fields 1958) exposed to approximately 1 mg Co/kg bw/day (as cobalt chloride) and in anephric patients (Duckham and Lee 1976b, Taylor et al. 1977) exposed to 0.16-1 mg Co/kg bw/day (as cobalt chloride) in treatment of anaemia. However, treatment of pregnant women (Holly 1955) with 0.6 mg Co/kg bw/day (as cobalt chloride) did not prevent the reduction in haematocrit and haemoglobin levels often found during pregnancy.

Significantly increased erythrocyte, haematocrit, and haemoglobin levels were found in <u>animals</u> treated orally with cobalt chloride as a <u>single dose</u> of 161 mg

cobalt/kg or with repeated exposure (3 weeks to 7 months) at dose levels from 0.5 mg/kg/day. In an 8-week study in rats orally exposed to cobalt chloride (Stanley et al. 1947) a dose- and time-related increase in erythrocyte number was observed. Increased erythrocyte number is not necessarily considered an adverse effect, however, at 2.5 mg Co/kg bw/day the erythrocyte number increased up to a maximum of 17% above pre-treatment values, which can be assumed to be an effect level. From this study a NOAEL of 0.6 mg Co/kg bw/day is considered. In rats exposed to cobalt chloride for 7 months (Krasovskii and Fridyland 1971), a transient increase in erythrocyte number was observed at 0.5 mg Co/kg bw/day, while this increase was persistent at 2.5 mg Co/kg bw/day. There was no information on the magnitude of the increases and thus no NOAEL is considered from this study.

Human data on reproductive and developmental toxicity is very limited. One study was located in which no obvious birth defects were observed on foetuses of mothers exposed to 0.6 mg Co/kg bw/day (as cobalt chloride) for 90 days in the final trimester. In rats developmental effects were observed at dose levels from 5.4 mg Co/kg bw/day (as cobalt chloride) on GD 14 - LD 21 (Domingo et al. 1985b), in mice from 19 mg Co/kg bw/day (as cobalt sulphate) on GD 1-20 (Szakmary et al. 2001) and in rabbits from 7.6 mg Co/kg bw/day (as cobalt sulphate) on GD 1-20 (Szakmary et al. 2001), however, maternal toxicity (decreased weight gain, mortality or increased weight of liver, adrenals and spleen) was also present at these dose levels. Testicular degeneration was observed in both rats and mice at dose levels from 13.25 mg Co/kg bw/day (cobalt in diet for 98 days) and 23 mg Co/kg bw/day (reversible testicular degeneration and stimulated serum testosterone – cobalt chloride in drinking water for 13 weeks), respectively. Because the data on reproductive toxicity are very limited and because the observed developmental effects possibly were due to maternal toxicity, no firm conclusion can be drawn regarding reproductive and developmental toxicity.

The genotoxicity of cobalt compounds was investigated in multiple *in vitro* and *in vivo* tests. Cobalt(II) compounds were generally non-mutagenic in bacteria (*Salmonella typhimurium*, *Escherichia coli*) and yeast. In contrast to the results seen in bacteria and yeast, cobalt compounds were generally found to be mutagenic and genotoxic in a wide range of mammalian *in vitro* assays. In addition, results from *in vivo* studies showed that cobalt(II) chloride induced aneuploidy in the bone marrow and testes of Syrian hamsters, micronuclei formation and chromosomal breaks and chromosomal aberrations in the bone marrow of mice, and increased levels of oxidatively-damaged DNA bases were noted in the liver, kidney, and to a lesser extent, the lung in rats. Overall, the available data indicate a genotoxic potential of cobalt(II) compounds.
The IARC has (in 2006) concluded: *"The results of genotoxicity assays with a variety of cobalt salts demonstrate the mutagenic potential of these salts both in vitro and in vivo"*. The conclusion was based on the results of numerous genotoxicity studies *in vitro* and *in vivo*.

No oral data were located regarding carcinogenicity following exposure to cobalt compounds.
In rats and mice, a significantly increased incidence of alveolar/bronchiolar neoplasms was observed following inhalation of cobalt sulphate heptahydrate. Increased mortality rates resulting from lung cancer were observed following occupational exposure to cobalt, either as a mixture of cobalt compounds or as hard metal. It should be noted that the local lung tumours observed following inhalation exposure are of no relevance for an evaluation of systemic carcinogenicity following oral exposure to cobalt compounds.
No conclusion can be drawn regarding a carcinogenic potential following oral exposure to cobalt compounds based on the available data; however, a carcinogenic

potential cannot be excluded as the available genotoxicity data indicate a genotoxic potential of cobalt(II) compounds.

A recent review concluded that there is evidence that soluble cobalt(II) cations exert a genotoxic and carcinogenic activity *in vitro* and *in vivo* in experimental systems, but evidence in humans is lacking.

6.7.1 Critical effect and NOAEL

Human and animal data indicate that polycythemia is the critical effect following oral repeated exposure to soluble cobalt compounds.

In humans, an increase in erythrocyte number up to 20% above pre-treatment values was observed in normal subjects exposed to approximately 1 mg Co/kg bw/day (as cobalt chloride) (Davis and Fields 1958).

In rats, the erythrocyte number increased up to a maximum of 17% above pre-treatment values on week 6 following administration of 2.5 mg Co/kg bw/day (as cobalt chloride in gelatine capsules) (Stanley et al. 1947). A NO(A)EL for polycythemia of 0.6 mg Co/kg bw/day is considered for rats based on this study. (Stanley et al. 1947).

In the 13-week NTP inhalation study in rats (NTP 1991), polycythemia (significant increases in erythrocytes, in the mean haemoglobin concentration, and in the haematocrit value) was seen from 1.1 mg Co/m^3 for male rats and from 3.8 mg Co/m^3 for female rats (as cobalt sulphate heptahydrate); no consistent significant haematological effects were seen in a similar study in mice. Haematological analyses were not part of the 2-year NTP inhalation studies in rats and mice (NTP 1998). Cobalt sulphate heptahydrate is absorbed following inhalation at least in rats as the 13-week inhalation study showed that the amount of cobalt excreted in the urine of rats exposed to 0.11 mg Co/m^3 (the lowest concentration in the study) was approximately 10 times that excreted by controls. However, no data are available regarding the absorbed fraction of cobalt following inhalation neither in rats nor in humans. In addition, the critical effect following inhalation is severe necrotising injury to the respiratory tract (LOAEC of 0.11 mg Co/m^3), a condition which possibly may result in a higher absorption of cobalt from the respiratory tract. Therefore, the inhalation data are not considered further for the purpose of estimating a quality criterion for cobalt in drinking water.

A health-based quality criterion in drinking water for repeated exposure to soluble inorganic cobalt salts will be estimated based on the LOAEL of 1 mg Co/kg bw/day for polycythemia from the human voluntary study with cobalt chloride (Davis and Fields 1958).
As an alternative approach, the quality criterion will also be estimated based on the NO(A)EL of 0.6 mg Co/kg bw/day for polycythemia from the rat study with cobalt chloride (Stanley et al. 1947).

7 TDI and quality criteria

7.1 TDI

The TDI is calculated based on the LOAEL of 1 mg Co/kg bw/day for polycythemia from the human voluntary study with cobalt chloride (Davis and Fields 1958):

$$\text{TDI} = \frac{\text{LOAEL}}{\text{UF}_I * \text{UF}_{II} * \text{UF}_{III}} = \frac{1 \text{ mg Co/kg bw/day}}{1 * 10 * 300} = 0.33 \text{ µg Co/kg bw/day}$$

The uncertainty factor UF_I accounting for interspecies variability is set to 1 as human data are the basis for the TDI. The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 300 taking into account that a LOAEL instead of a NOAEL is the basis for the TDI, that data on oral repeated toxicity and reproductive and developmental toxicity in general are very old and inadequate and because reproductive effects were observed in the 13-week inhalation study in mice, that no oral data were located regarding carcinogenicity, and that the available genotoxicity data demonstrate a genotoxic potential of cobalt(II) compounds both *in vitro* and *in vivo*.

7.2 Allocation

The general population is primarily exposed to cobalt from food and drinking water.

Using the highest reported concentration of cobalt in Danish ground water of 20 µg/l (MST 1986b), and the consumption rate of 0.08 l/kg bw/day (for children 1-10 years old), the intake from drinking water would be 1.6 µg Co/kg bw/day (assuming no dilution of groundwater). For an adult, assuming a 90th percentile for the intake of drinking water of 2.3 litre/day, the daily exposure to cobalt from drinking water would be 46 µg (about 0.7 µg Co/kg bw/day assuming an adult body weight of 70 kg). It should be noted, however, that in all the other water samples analysed, the concentration of cobalt was below the detection limit (detection limit not stated in the report). Therefore, the intake of cobalt from drinking water is considered as being a really worst case estimate and not representative for the intake of cobalt from drinking water in the general population.

The estimated average daily intake of cobalt from the diet was reported to be 11 µg/day (range 4-15 µg/day, 0.06-0.2 µg Co/kg bw/day assuming an adult body weight of 70 kg) in Canada and 7.5 µg/day (adults, 0.1 µg Co/kg bw/day) and 7.3 µg/day (children, 0.6 µg Co/kg bw/day assuming a body weight for small children of 13 kg) in France.

Based on these data, the contribution of cobalt from food is considered as being at the same order of magnitude as the contribution of cobalt from the drinking water. Therefore, only 50% of the TDI is allocated to ingestion of drinking water.

7.3 Quality criterion in drinking water

The quality criterion in drinking water QC_{dw} is calculated based on the TDI of 0.33 µg Co/kg bw/day and assuming a daily ingestion of 0.03 l/kg bw of drinking water for children 1-10 years old:

$$QC_{dw} = \frac{TDI * Y}{ingestion_{dw}} = \frac{0.33 \text{ µg Co/kg bw/day} * 0.5}{0.03 \text{ l/kg bw/day}}$$

$$= 5.5 \text{ µg Co/l}$$

7.3.1 Quality criterion in drinking water

5 µg Co/l

8 References

ACGIH (2001). 2001 TVLs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices.

ATSDR (2004). Toxicological Profile for cobalt. U.S. Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

At (2007). Grænseværdier for stoffer og materialer. Arbejdstilsynets At-vejledning C.0.1, august 2007.

ChemIDplus Advanced. http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp

Davis and Fields (1958). Experimental production of polycythemia in humans by administration of cobalt chloride. Proc Soc Exp Biol Med **99**:493-495.

ECB (2009). ClassLab Database. http://ecb.jrc.ec.europa.eu/classification-labelling/search-classlab/

EFSA (2006). Tolerable upper intake levels for vitamins and minerals. Scientific committee on food. Scientific panel on dietetic products, nutrition and allergies. European food safety authority. February 2006.

EFSA (2009). Scientific Opinion: Assessment of the safety of cobalt(II) chloride hexahydrate added for nutritional purposes as a source of cobalt in food supplements and the bioavailability of cobalt from this source. Statement of the Scientific Panel on Additives and Nutrient Sources added to Food (ANS), Adopted on 28 April 2009. The EFSA Journal (2009) 1066, 1-8.

EU (2001). Integrated pollution prevention and control (IPPC). Reference document on best available techniques in the non ferrous metals industries. European commission. December 2001.

IARC (2006). Cobalt in hard metals and cobalt sulphate, gallium arsenide, indium phsphide and vanadium pentoxide. In: IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 86, 38-155.

IARC (1991). Cobalt and cobalt compounds. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 52. Lyon: International agency for research on cancer, 1991, Vol. 52, 362-487.

INRA (2004). The 1st French total diet study – mycotoxins, minerals and trace elements. Institut national de la recherché agronomique 2004.

IUCLID (2000). Cobalt sulphate. In: International Uniform Chemical Information Database. European Commission, ECB, JRC, Ispra.

Lison D, De Boeck M, Verougstraete V and Kirsch-Volders M (2001). Update on the genotoxicity and carcinogenicity of cobalt compounds. Occup Environ Med 58, 619-625.

MST (1986a). Forbrug og forurening med Arsen, chrom, cobalt og nikkel i Danmark. Arbejdsrapport fra Miljøstyrelsen. Nr.2 1986.

(MST 1986b). Human eksposition og sundhedsmæssig vurdering af arsen, chrom, cobalt og nikkel i Danmark. Arbejdsrapport fra Miljøstyrelsen. Nr.3 1986.

MST (2009). Personal communication.

MST (2002). B-værdivejledningen. Vejledning Nr. 2 2002, Miljøstyrelsen, Miljøministeriet.

MM (2005).Bekendtgørelse om listen over farlige stoffer. Bekendtgørelse nr. 923 af 28. september 2005. Miljøstyrelsen, Miljøministeriet.

NL (1994). Environmental quality objectives in the Netherlands. Risk assessment and environmental quality division directorate for chemicals, external safety and radiation protection. Ministry of housing, spatial planning and the environment 1994.

NTP (1991). NTP report on the toxicity studies of cobalt sulphate heptahydrate in F344/N rats and B6C3F$_1$ mice (inhalation study):NTP TOX 5, NIH publication no. 91-3124. January 1991.

NTP (1998). NTP technical report on the toxicology and carcinogenesis studies of cobalt sulphate heptahydrate in F344/N rats and B6C3F$_1$ mice (inhalation study):NTP TR 471, NIH publication no. 98-3961. August 1998.

Weast RC, ed. 1985. CRC Handbook of chemistry and physics. 66th ed.

WHO (2004). Vitamin B$_{12}$. In: Vitamins and mineral requirements in human nutrition. Second edition. World Health Organization and Food and Agriculture Organization of the United Nations, 279-288.

Cobalt (II), inorganic and soluble salts

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to cobalt (II), inorganic and soluble salts. This resulted in 2010 in the present report which includes estimation of a quality criterion in drinking water for the mentioned substances.